



**MULTI-CENTER, OPEN LABEL, RANDOMIZED TRIAL
COMPARING SINGLE VERSUS DOUBLE UMBILICAL
CORD BLOOD (UCB) TRANSPLANTATION IN
PEDIATRIC PATIENTS WITH HIGH RISK
LEUKEMIA AND MYELODYSPLASIA**

**BMT CTN PROTOCOL 0501
VERSION 6.0**

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PROTOCOL SYNOPSIS - BMT CTN PROTOCOL #0501**Multi-center, Open Label, Randomized Trial Comparing Single Versus Double Umbilical Cord Blood (UCB) Transplantation in Pediatric Patients with Leukemia and Myelodysplasia**

Study Chairperson:	John E. Wagner, M.D.
Study Co-Chair:	Joanne Kurtzberg, M.D.
Primary Objective:	The primary objective is to determine the efficacy of using two UCB units versus one UCB unit. The primary endpoint is one-year survival.
Secondary Objectives:	Patients randomized to the two study arms will be compared for the following endpoints: disease-free survival, incidences of neutrophil and platelet engraftment, chimerism, acute graft-versus-host disease (GVHD), chronic GVHD, transplant-related mortality, infections, immune reconstitution, and relapse.
Study Design:	This study is a Phase III, randomized, open-label, multi-center, prospective study of single UCB transplantation vs. double UCB transplantation in pediatric patients with hematologic malignancies.
Accrual Objective:	The target sample size is 110 patients per study arm (total of 220 patients).
Accrual Period:	The estimated accrual period is five years to enroll the targeted sample size.
Eligibility Criteria:	<p>Patients 1-21 years of age with a diagnosis of hematological malignancy and with two partially HLA-matched UCB units. Units must be HLA-matched at 3 of 6 HLA-A and B (intermediate resolution molecular typing) and DRB1 (high resolution molecular typing) with each other and 4 of 6 with the recipient. Two appropriately HLA-matched units must be available such that one unit delivers a pre-cryopreserved, nucleated cell dose of at least 2.5×10^7 per kilogram and the second unit at least 1.5×10^7 per kilogram.</p> <p>Patients will be randomized no more than 14 days prior to initiation of conditioning. UCB units will be shipped prior to initiation of conditioning.</p>
Treatment Description:	<p>The preparative regimen will consist of:</p> <ul style="list-style-type: none">- Fludarabine: 25 mg/m²/day IV on Day -10, -9 and -8.- Total Body Irradiation (TBI): 165 cGy twice daily on Day -7, -6, -5 and -4.- Cyclophosphamide: 60 mg/kg/day x 2 on Day -3 and -2.- Rest on Day -1.- Day 0 will be the day of the UCB transplant.

- The GVHD prophylaxis regimen will be mycophenolate mofetil (MMF) 15 mg/kg IV TID Day –3 to Day + 45 and cyclosporine A (CSA) to maintain level 200-400 ng/mL beginning on Day –3.

Study Duration:

Patients will be followed for at least 24 months post-transplant.

TREATMENT SCHEMA

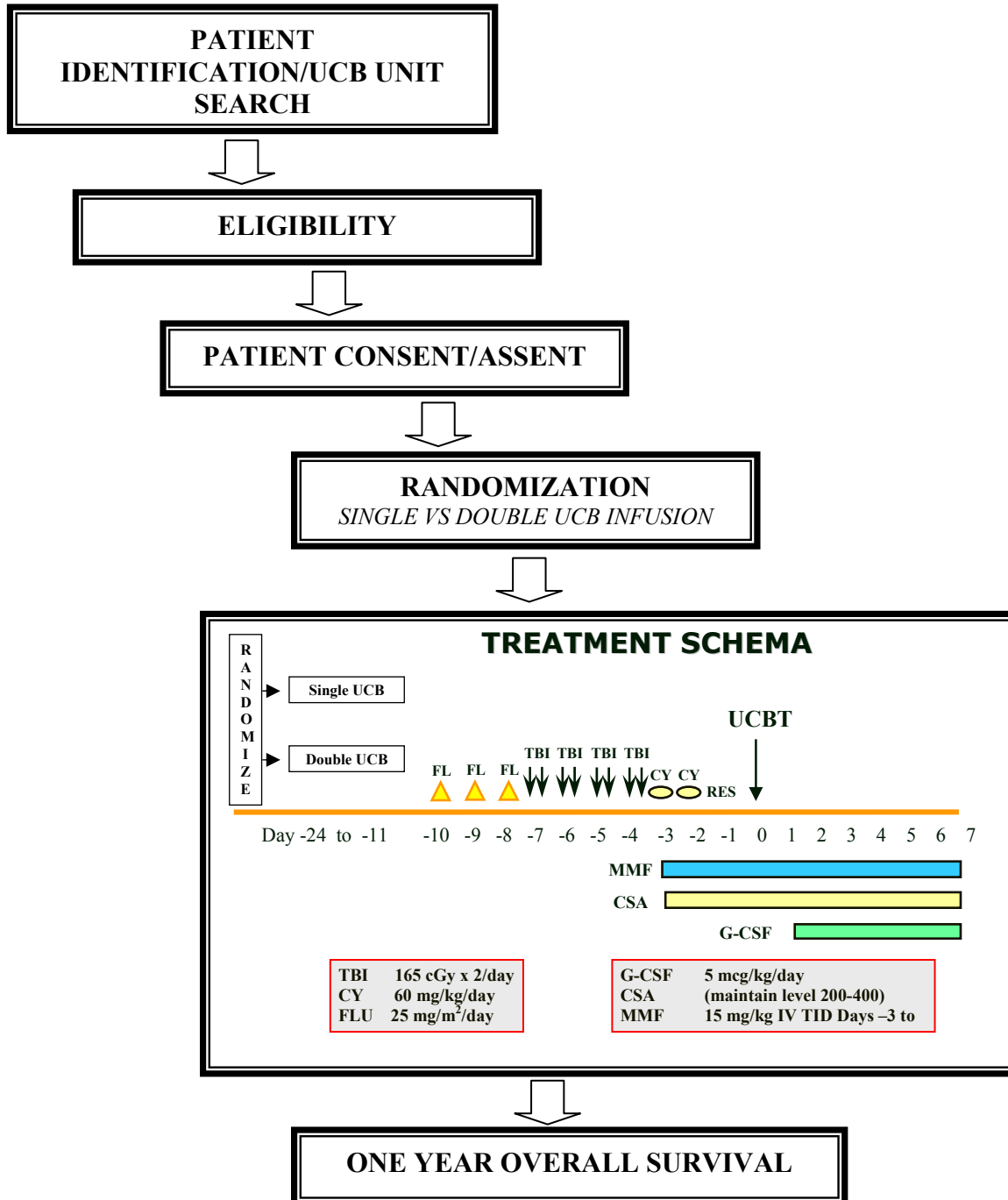


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CHAPTER 1

1. BACKGROUND AND RATIONALE

1.1. Overview

In nearly every large single center or registry analysis of outcomes after umbilical cord blood (UCB) transplantation, cell dose is identified as an important factor influencing the incidence and rate of hematopoietic recovery, risk of transplant-related mortality and probability of survival. Pilot data suggest that infusion of two partially HLA-matched UCB units, which always augments the graft cell dose, is safe and may improve neutrophil recovery and survival. To determine whether the infusion of two UCB units enhances survival, a multi-center, open-label, randomized trial is proposed. As adequate single UCB units can be identified for > 80% of pediatric recipients (in contrast to < 30% for adults), this study will be open only to pediatric patients. The population will be restricted to patients with high-risk hematologic malignancy, the most common indication of UCB transplantation in children.

1.2. Background

Human UCB contains sufficient numbers of hematopoietic stem cells (HSC) for transplantation as evidenced by durable hematopoietic and immune reconstitution of UCB cell derived donor cells after myeloablative therapy. A recent survey by the Institute of Medicine found that more than 180,000 UCB units have been banked and more than 6,000 unrelated donor UCB transplantations have been performed. UCB transplants offer several advantages over adults bone marrow or peripheral blood stem cell transplants, including:

1. Rapid availability;
2. Absence of risk to the mother or infant donor;
3. Reduced incidence of some blood-borne infectious disease agents (e.g., Epstein-Barr virus [EBV], cytomegalovirus [CMV]);
4. Reduced donor attrition;
5. Reduced risk of severe acute graft versus host disease (GVHD) in the setting of donor-recipient HLA mismatch (as compared to recipients of unrelated donor marrow and peripheral blood).

1.2.1. Unrelated Donor UCB Transplantation: Clinical Results

The first UCB transplantation was performed by Gluckman et al. in 1988 in a child with Fanconi anemia¹. Subsequently, reports documented the feasibility and efficacy of HLA-mismatched related and unrelated UCBT. By 1993, the first repository of unrelated donor UCB was established in New York^{2,3}. Currently, private and publicly funded UCB banks worldwide store an estimated 180,000 cryopreserved HLA-A, B, and DRB1 typed units⁴. Several findings emerge from review of the literature.

Engraftment. The incidence of neutrophil recovery after single UCB transplantation ranges from 65-92% in larger series^{4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17}. Findings consistently reported include the following: 1) Engraftment after UCB transplantation is significantly slower and less complete than after bone marrow transplantation^{6, 18, 19}. 2) Time to neutrophil and platelet recovery is cell dose dependent with more rapid recovery in those receiving higher cell doses. In a report by Gluckman et al.¹⁴, a graft nucleated cell dose $> 3.7 \times 10^7/\text{kg}$ was associated with shorter time to neutrophil recovery (25 vs. 35 days). Similarly, Rubinstein et al.^{7, 16} demonstrated that a step-wise increase in graft nucleated cell dose was associated with progressively shortened time to neutrophil recovery. Further, this study showed graft CD34+ cell dose predicted speed of recovery. 3) There is a threshold cell dose required for consistent engraftment. Rubinstein et al.¹⁶ suggested that the threshold was a cryopreserved nucleated cell dose $\geq 2.5 \times 10^7/\text{kg}$; while Wagner et al.⁵ observed a threshold infused CD34+ cell dose of $\geq 1.7 \times 10^5/\text{kg}$ ⁵. Other prognostic factors are also reported but are not as consistently observed as the association with cell dose. As one example, Rubinstein et al.^{7, 16} observed a relationship between HLA match and neutrophil recovery (23 days for HLA-matched versus 28 days for HLA mismatched grafts, $p=0.0027$); this association was not observed by Gluckman et al.¹⁴ and Wagner et al.⁵.

GVHD. The incidence of acute GVHD reported in larger series ranges from 33-44% and 11-22% for grades II-IV and III-IV acute GVHD, respectively^{5, 6, 7, 14}. The incidence of chronic GVHD ranges from 0-25%^{5, 6, 7, 14}. These results are particularly notable since most UCB donor-recipient pairs are 1-2 HLA-antigen mismatched. However, most recipients of UCB transplants are young and younger age is also associated with lower rates of GVHD. It is notable that most studies demonstrate no or weak associations between HLA match and occurrence of GVHD. Although few in number, Rubinstein et al.¹⁶ did report a significantly lower rate of acute GVHD in recipients of HLA-matched grafts with no further increase observed in those with increasing HLA disparity (1 vs. 2 vs. 3 antigen mismatches). Associations between HLA-match and chronic GVHD are not reported.

Survival. The probability of survival after single UCB transplantation ranges from 18-78% in larger series^{5, 6, 7, 14}. This wide range can be explained, in part, by marked differences in patient characteristics among studies. However, nearly all studies demonstrate a significant relationship between UCB cell dose and survival. The association between HLA match and survival is more controversial, which may, in part, be explained by limited patient numbers and recipient age. For example, Locatelli et al.¹⁵ reported outcomes of UCB transplantation for pediatric acute leukemia from the Eurocord registry. In multivariate analysis, the number of HLA-mismatches did not influence survival. Similarly Laughlin et al.⁸ in a study of 68 adult recipients of 0-3 antigen HLA-mismatched UCB transplants also found no association between degree of HLA-mismatch and overall survival. In contrast, in two series by Rubinstein et al. (562 patients and subsequently updated for 862 patients) and Wagner et al., a significant association between HLA-mismatch and survival was observed^{5, 7, 16}. Further, data comparing the results of 492 unrelated donor bone marrow transplants and 508 UCB transplants in patients younger than 16 years from the Center for International Blood and Marrow Transplant Research (CIBMTR) and the New York Blood Center indicates a significant association between degree of HLA disparity and overall and

leukemia-free survival. In the latter study, the 3-year probability of leukemia-free survival was highest after HLA-matched UCB transplantation (60%); probabilities were similar after HLA-matched bone marrow transplants and UCB transplants mismatched at 1-locus with cell dose $> 3.0 \times 10^7/\text{kg}$ (40% and 41% respectively). Probabilities were lower with 1-antigen mismatched bone marrow, 1-antigen mismatched UCB with cell doses $\leq 3.0 \times 10^7/\text{kg}$ and UCB grafts mismatched at 2-loci (30%, 36% and 33% respectively). Importantly, the data demonstrate an impact of cell dose within each mismatch category (one or two antigen mismatch) in recipients of UCB. For those with a one-antigen mismatched UCB graft, recipients of units containing higher cell doses ($> 3.0 \times 10^7/\text{kg}$) had a probability of survival that was similar to that observed after HLA-matched BM. Among those with a two-antigen mismatched UCB graft, higher cell doses were associated with improved survival but were still inferior to that observed with better HLA-matched UCB units.

Together these data suggest that graft selection should be principally based on HLA match for those units with an acceptable cell dose ($\geq 2.5 \times 10^7/\text{kg}$).

1.2.2. Cord Blood Transplantation Study (COBLT) Study

Recent results of COBLT, a multi-institutional, prospective NIH-sponsored trial of unrelated donor UCB transplantation have further advanced the field of UCB transplantation¹⁹. In this Phase II study, 191 pediatric patients diagnosed with a hematologic malignancy [(median age = 7.7 years) (range: 1-18)]; [(median weight = 25.9 kg) (range: 7.5 – 118.4 kg)] were transplanted with one UCB unit. All patients received total body irradiation (TBI), cyclophosphamide and antithymocyte globulin (ATG) for pre-transplant conditioning and post-transplant cyclosporine and methylprednisone for GVHD prophylaxis. Sixty-one percent were males, 51% CMV seropositive and 28% of ethnic minority backgrounds. The median cell dose delivered as measured by the pre-cryopreservation cell count was 5.2×10^7 cells/kg and 1.5×10^5 CD34 cells/kg. Donor selection was based on low resolution DNA-based HLA typing at Class I A and B and high resolution DNA-based typing at Class II DRB1. Retrospectively, donor/recipient pairs were fully typed for Class I A, B, C and Class II DRB1 by high resolution typing to determine the impact of high resolution on engraftment, GVHD, relapse and survival. The primary endpoint of the study was 180-day survival and secondary endpoints included disease-free-survival, incidence of neutrophil and platelet engraftment, incidence of acute and chronic GVHD, infection burden and incidence of relapse.

Engraftment. The cumulative incidence of neutrophil engraftment, defined as achieving an ANC of 500/mcL by Day +42 with $> 90\%$ donor chimerism, was 75% (95% confidence interval [CI] 69-81%). The cumulative incidence of platelet engraftment, defined as maintaining a platelet count of $> 50 \times 10^9 /\text{L}$ without transfusion support, by Day 180 was 50% (95% CI 42-59%) as shown below:

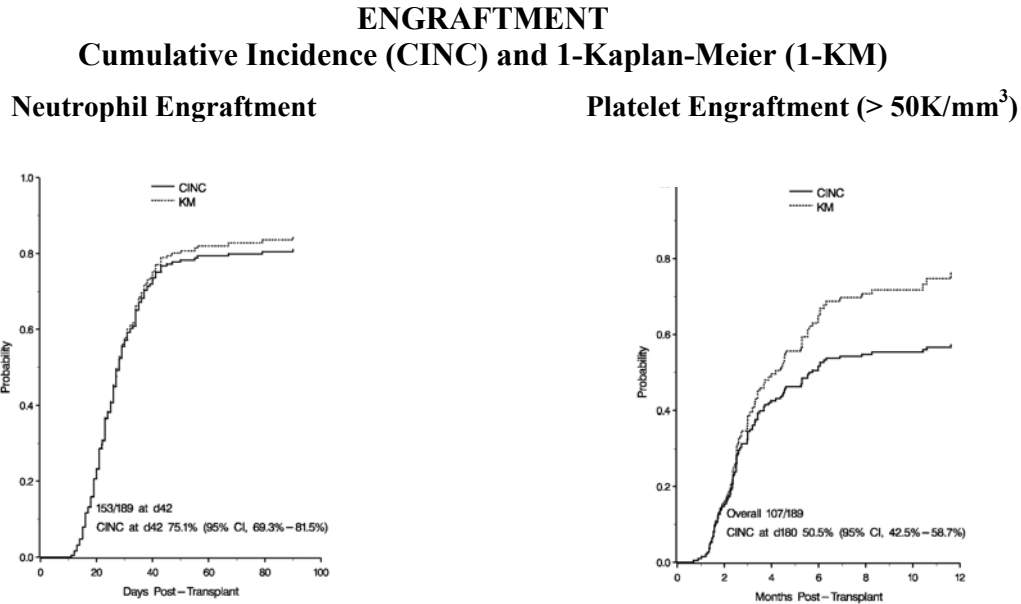


Figure 1.2.2a — Hematopoietic recovery in the COBLT Study

In multivariate analysis, the pre-cryopreservation cell dose ($P = 0.05$) and gestational age of the UCB donor ($P = 0.04$) were significant predictors of engraftment. HLA matching, low resolution or high resolution, was not associated with engraftment.

Graft-versus-Host Disease. The cumulative incidence of acute grades III/IV GVHD at Day +100 was 19% (95% CI 12-24%). The incidence of chronic GVHD at 1 year was 20% (95% CI 15-26%) with 70% of the chronic GVHD classified as limited disease. In multivariate analysis, HLA matching was associated with the incidence of GVHD ($P = 0.007$).

Disease-free Survival (DFS). Most of the children enrolled on this study had high risk (beyond first remission) hematologic malignancies. Their cumulative incidence of relapse at one year was 19% (95% CI 14-25%).

Overall 95/191 patients died on study, with 41% of deaths from relapse, 19% from graft failure and 18% from GVHD (50% of those dying from GVHD had infection as a secondary cause of death).

Overall survival at 180 days and one year was 67% (95% CI 61-74%) and 57% (95% CI 50-64%), respectively with a median follow-up of 24 months. In multivariate analysis, factors associated with survival included early engraftment ($P < 0.001$), recipient CMV serostatus negative ($P = 0.002$) and recipient gender male ($P = 0.01$).

Total nucleated cell dose significantly impacted engraftment and survival, but CD34 dose did not correlate with either suggesting that CD34 may not be the ideal surrogate marker for stem cell dose in UCB transplantation.

Survival by Cell Dose

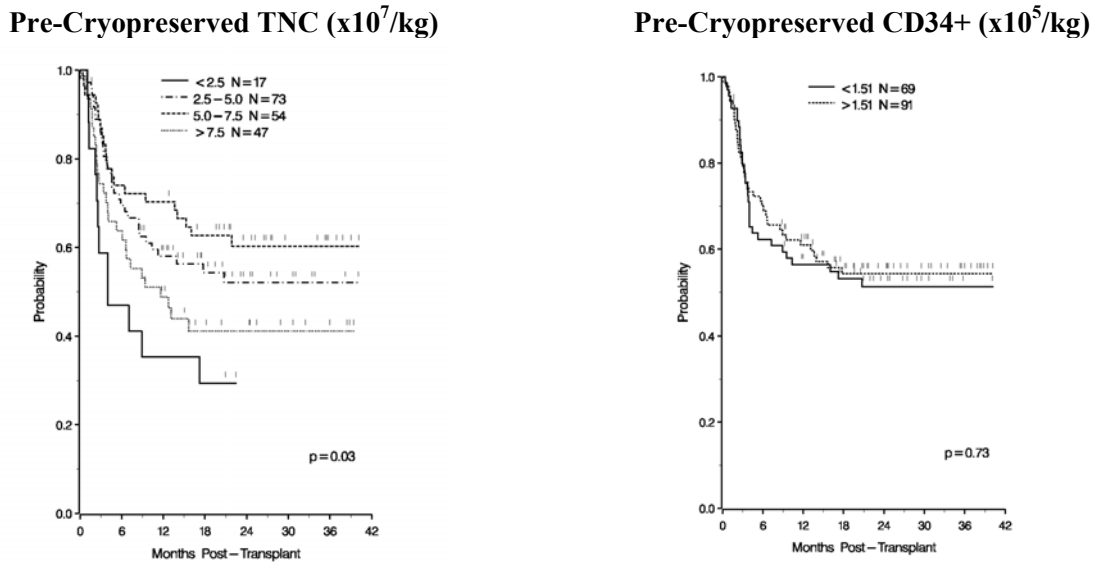


Figure 1.2.2b Overall Survival by Cell Dose in the COBLT Study

The COBLT study provided much insight into the logistics of establishing and accessing UCB banks, selecting appropriate units for transplantation and performing UCB successfully in a variety of conditions, particularly in children. The main clinical lessons learned were 1) that outcomes were sufficiently good to perform unrelated UCB transplantation as a reasonable alternative for children with hematologic malignancies considered appropriate indications for transplantation but no HLA-identical sibling donor; and, 2) that the major transplant-related causes of death were the slow rate and low incidence of engraftment and the risk of GVHD.

Immune Reconstitution Studies. The pace and quality of immune reconstitution after unrelated donor cord blood transplantation is an important predictor of overall outcomes. Multiple factors may influence immune reconstitution including, but not limited to, the cell dose and quality of the donor graft, HLA-matching, GVHD, age, preparative regimen and infections. Previous studies in recipients of unrelated donor cord blood grafts have shown that immune reconstitution, as measured by CD4 counts, PHA responses and levels of TRECs, is more rapid in children (1 year) than adults (3 years)²⁰. In the COBLT Study, pediatric patients between 1-18 years of age, enrolled on the pediatric malignancy strata were longitudinally evaluated over a 3-year period for the presence of T cells capable of recognizing herpes virus specific antigens. Approximately 43% of patients developed a positive T lymphocyte proliferative response to one or more herpes viruses and that group had a lower probability of post-transplant leukemic relapse. The presence of herpes virus reactive T cells did not correlate with immunophenotypic T lymphocyte reconstitution, graft cell dose or the presence of acute or chronic GVHD. In the current study, these observations will undergo additional study with specific emphasis on the contribution of donor-derived naive T cells to immune reconstitution. Since the COBLT study has shown

poorer correlations with more traditional measures of immune reconstitution (e.g., CD4, mitogen responses), this study will test interventions that directly challenge the developing immune system in the early post-transplant period. Accordingly, children will be immunized with tetanus vaccine at 100 days, 6, 12 and 24 months post-transplant. Peripheral blood will be monitored for the development of T cells capable of recognizing tetanus and PRP antigens at 6, 12, and 24 months. In addition, PRP and tetanus titers will be monitored in children after completing therapy with IVIG. TREC analysis and T cell responses to HSV, CMV, and VZV will also be analyzed to extend and confirm the COBLT observations.

One of the hypotheses of the current protocol is that two cords will facilitate immune reconstitution more efficiently than one cord. The studies proposed will provide the data to answer this question. If naive T cells contribute to durable immune reconstitution and if transplantation of two cords accelerate this process, both TRECs and T cell responses to antigens should recover earlier in the patients receiving the double cord transplants.

1.2.3. Double UCB Transplantation

1.2.3.1. Double UCB transplantation after myeloablative preparative therapy

It is clear that cell dose is a critical determinant of hematopoietic recovery and survival after single unrelated donor UCB transplantation⁶. However, unlike bone marrow or peripheral blood stem cell transplantation where large numbers of cells may safely be harvested, the number of cells obtained from a single UCB unit is limited. Since the number of UCB cells needed to safely transplant a patient is calculated on the basis of the recipient's body weight, adolescents and adults (typical weight 70–90 kg) require larger numbers of UCB cells than children (typical weight 10-20 kg), as noted above. The limitation of cell dose is a major obstacle in the application of UCBT to adolescents and adults.

Various methods of augmenting UCB cell dose have been considered^{21, 22}. One strategy is to infuse two UCB units. This strategy has been piloted at the University of Minnesota primarily in adult patients who had two UCB units that were partially HLA-matched with the recipient (4-6/6 HLA match) and with each other. The hypothesis was the increased cell dose would enhance engraftment. In a Phase I-II study, 23 consecutive patients (median age 24 years [range: 13-53]; weight 73 kg [48-120]; 57% male; 61% CMV positive) were transplanted with two UCB units. All patients received cyclophosphamide 120 mg/kg, fludarabine 75 mg/m² and TBI 1320 cGy pre-transplant and cyclosporine, mycophenolate mofetil (MMF) and filgrastim (G-CSF) after UCB infusion.

Engraftment. The incidence of sustained donor engraftment was 100% at a median of 23 days (range 15-41) post-transplant. All patients had complete donor chimerism and there were no secondary graft failures. By Day 180, the incidence of platelets > 50 x 10⁹/L was 71% (95% CI: 47-95%). These data demonstrate the safety of double UCB infusion in terms of engraftment, a previous concern because of the theoretical possibility of bi-directional immunological rejection.

Chimerism. In this series, 16 (76%) recipients had persistence of only one UCB unit by Day 21. While the remaining five patients (24%) had evidence of both units at Day 21 (median total donor chimerism 91% [range 64-100%]), one unit predominated (median 74% [range 42-85%]) versus 20% [range 15-40%]). Skewed engraftment progressed such that evidence of ‘double chimerism’ was observed in only two patients at Day 60, and in none by Day 100 (n=17). The relative percent viability, order of infusion, ABO match, gender match, infused cell dose and HLA match of the UCB units did not predict which unit would predominate.

GVHD and Transplant-related Mortality (TRM). Incidences of grade II-IV and III-IV acute GVHD were 65% (95% CI 42-88) and 17% (95% CI 2-32), respectively. Of the three patients with grade III-IV acute GVHD, one had involvement of skin only, one of skin and gut, and one of skin and liver. All responded to immunosuppression. Five patients have had chronic GVHD (all extensive) for a cumulative incidence of 23% (95% CI 6-40%). Six month TRM was 22% (95% CI 5-39%).

Disease-free Survival (DFS). With a median follow-up of 10 months (range: 3.5 months-2.5 years), the probability of DFS at 1 year is 57% (95% CI 35-79%). For those in remission at the time of transplantation (n = 15), DFS was 72% (95% CI 49-95%) versus 25% (95% CI 0-64%), respectively (P=0.04) (Figure 1.2.3.1). Causes of death were GVHD/infection (n=3), GVHD/organ failure (n=2), hemorrhage (n=1), and relapse (n=3).

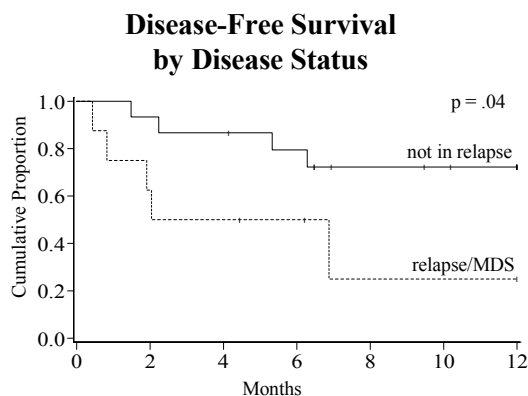


Figure 1.2.3.1 – Disease-free Survival by Disease Status in University of Minnesota Phase II Trial

Summary. These results indicate that co-infusion of two UCB units is safe and may improve upon the rate of engraftment anticipated after transplantation with an available single UCB unit.

1.2.3.2. Rationale for the proposed study

Engraftment is a major obstacle to the success of cord blood transplantation in children with malignancies, despite the fact that they receive relatively high doses of nucleated cells from a

single cord blood graft. Primary graft failure occurred in approximately 20% of patients enrolled on the COBLT study despite the fact that the median cell dose delivered was $5 \times 10^7/\text{kg}$. Pilot studies of double cord transplantation in adults at the University of Minnesota demonstrate an improved rate of engraftment ($> 95\%$) with much lower cell doses administered overall. The mechanisms underlying the effect on engraftment are currently unknown. They could be secondary to a facilitating function provided by the second UCB unit, a difference in the preparative regimen (e.g., substitution of fludarabine for ATG) or GVHD preparative regimen (e.g., substitution of Cellcept for steroids) or another yet to be discovered phenomenon. Nonetheless, the superior engraftment with the Minnesota double cord protocol leads to the hypothesis that double cords may also be beneficial in children and that the mechanism underlying this benefit is not simply related to incremental increases in cell dose, but instead is related to more complex interactions between the donors and the host. Furthermore, we expect that increased rates of engraftment will result in improved rates of overall survival. Effects on rates of GVHD, early and late TRM, and leukemic relapse (the leading cause of death in the COBLT study) are unknown but will be examined. The number of T lymphocytes will significantly increase with the use of two units. Additionally, the requirement for two units will increase the likelihood of infusing at least one that is 2 HLA antigen mismatched unit. Consequently, it is possible that the risk of acute and chronic GVHD will be greater in subjects randomized to two UCB units. Furthermore, with two cord blood donors, each donor could reject the other, leaving the patient without engrafting donor cells. While early studies of double cord blood transplantation do not show a risk of either of these problems, these potential complications will be monitored carefully during this study to be sure that they do not occur when a larger group of patients are studied.

Adult patients are excluded from this study since fewer than a third will have an available unit with the minimum cell dose of $2.5 \times 10^7/\text{kg}$ and thus would not be eligible for randomization. Further immune reconstitution is biologically different in children as compared to adults and this is an important endpoint of the study.

Thus, this study will test whether the addition of a second cord blood unit to a conventional cord blood transplant is beneficial. We will specifically study whether the double cord transplant improves rates of overall survival and engraftment, without increasing rates of GVHD, while decreasing rates of leukemic relapse.

CHAPTER 2

2. STUDY DESIGN

2.1. Primary Hypothesis

This is a multi-center, open-label, randomized clinical study to determine the benefit of double UCB transplantation on survival. The central hypothesis is that infusion of additional hematopoietic stem and progenitor cells from a partially HLA-matched second UCB unit will improve survival after UCB transplantation in children with high risk hematologic malignancies.

2.2. Secondary Hypotheses

Infusion of a second UCB unit will lower relapse rates, enhance hematopoietic recovery, reduce transplant-related mortality and lead to faster immune reconstitution compared to infusion of a single UCB unit. Acute and chronic graft-versus-host disease (GVHD) will not be increased.

2.3. Inclusion Criteria

2.3.1. Patient and Donor Eligibility Criteria

Patient and donor criteria include:

1. Patients must be 1-21 years of age.
2. Patients must have two partially HLA-matched UCB units. Units must be HLA-matched minimally at 4 of 6 HLA-A and B (at intermediate resolution by molecular typing) and DRB1 (at high resolution by molecular typing) loci with the patient, and the units must be HLA-matched at 3 of 6 HLA- A, B, DRB1 loci with each other (using same resolution of molecular typing as indicated above). Two appropriately HLA-matched units must be available such that one unit delivers a pre-cryopreserved nucleated cell dose of at least 2.5×10^7 per kilogram and the second unit at least 1.5×10^7 per kilogram.
3. Acute myelogenous leukemia (AML) at the following stages:
 - High risk first complete remission (CR1), defined as:
 - a. Having preceding myelodysplasia (MDS)
 - b. High risk cytogenetics (High-risk cytogenetics: del (5q) –5, -7, abn (3q), t (6;9) complex karyotype (≥ 5 abnormalities), the presence of a high FLT3 ITD-AR (> 0.4))
 - c. Requiring > 1 cycle chemotherapy to obtain CR;
 - d. FAB M6
 - Second or greater CR.
 - First relapse with $< 25\%$ blasts in bone marrow.

- Morphologic complete remission with incomplete blood count recovery (CRi).
4. Patients with therapy-related AML whose prior malignancy has been in remission for at least 12 months. If the remission is less than 12 months, Medical Monitor or Protocol Chair approval is required.
 5. Acute lymphocytic leukemia (ALL) at the following stages:
 - High risk first remission, defined as:
 - a. Ph+ ALL; or,
 - b. MLL rearrangement with slow early response [defined as having M2 (5-25% blasts) or M3 (>25% blasts on bone marrow examination on Day 14 of induction therapy)]; or,
 - c. Hypodiploidy (< 44 chromosomes or DNA index < 0.81); or,
 - d. End of induction M3 bone marrow; or,
 - e. End of induction M2 with M2-3 at Day 42.
 - f. Evidence of minimal residual disease (MRD). If a patient's only high risk criterion is MRD, approval by a protocol chair or protocol officer is required for enrollment.
 - High risk second remission, defined as:
 - a. Ph+ ALL; or,
 - b. Bone marrow relapse < 36 months from induction; or,
 - c. T-lineage relapse at any time; or,
 - d. Very early isolated CNS relapse (6 months from diagnosis); or,
 - e. Slow reinduction (M2-3 at Day 28) after relapse at any time.
 - f. Evidence of minimal residual disease (MRD). If a patient's only high risk criterion is MRD, approval by a protocol chair or protocol officer is required for enrollment.
 - Any third or subsequent CR.
 6. NK cell lymphoblastic leukemia in any CR:
 7. Biphenotypic or undifferentiated leukemia in any CR or if in 1st relapse must have < 25% blasts in BM.
 8. MDS at any stage.
 9. Chronic myelogenous leukemia (CML) in chronic or accelerated phase.
 10. All patients with evidence of CNS leukemia must be treated and be in CNS CR to be eligible for study.
 11. Patients \geq 16 years old must have a Karnofsky score \geq 70% and patients < 16 years old must have a Lansky score \geq 70%.

12. Signed informed consent.

13. Patients with adequate physical function as measured by:

- a. Cardiac: Left ventricular ejection fraction at rest must be $> 40\%$, or shortening fraction $> 26\%$
- b. Hepatic: Bilirubin ≤ 2.5 mg/dL; and ALT, AST and Alkaline Phosphatase ≤ 5 x ULN
- c. Renal: Serum creatinine within normal range for age, or if serum creatinine outside normal range for age, then renal function (creatinine clearance or GFR) > 70 mL/min/1.73 m².
- d. Pulmonary: DLCO, FEV1, FVC (diffusion capacity) $> 50\%$ of predicted (corrected for hemoglobin); if unable to perform pulmonary function tests, then O₂ saturation $> 92\%$ of room air.

2.4. Exclusion Criteria

1. Pregnant (β -positive HCG) or breastfeeding.
2. Evidence of HIV infection or HIV positive serology.
3. Current uncontrolled bacterial, viral or fungal infection (currently taking medication and progression of clinical symptoms).
4. Autologous transplant < 12 months prior to enrollment.
5. Prior autologous transplant for the disease for which the UCB transplant will be performed.
6. Prior allogeneic hematopoietic stem cell transplant.
7. Active malignancy other than the one for which the UCB transplant is being performed within 12 months of enrollment
8. Inability to receive TBI.
9. Requirement of supplemental oxygen.
10. HLA-matched related donor able to donate.

2.5. Graft Selection

All patients must have two suitable UCB units for transplantation to be eligible for randomization. Based on prior analyses, a cell dose of 2.5×10^7 nucleated cells/kg is required for recipients of single units. Therefore, the following selection algorithm will be used.

- Unit 1 will be the best available HLA-matched (with the patient) UCB unit with a nucleated cell dose $\geq 2.5 \times 10^7$ /kg except as noted below.
- Unit 2 will be the next best HLA-matched UCB with a nucleated cell dose $\geq 1.5 \times 10^7$ /kg that is at least 3 of 6 HLA-A, B and DRB1 HLA loci with Unit 1.

General Comments:

- Unit selection is based on cryopreserved nucleated cell (NC) dose & HLA-A, B, DRB1 matching using intermediate resolution A and B and high resolution DRB1 typing. Each unit must be at least 4/6 HLA-matched with the patient.
- CD34+ cell dose will not be used for unit selection unless 2 units of equal HLA-match are available from the same cord blood bank. Then the unit with the larger CD34+ dose should be selected.
- A UCB unit that is 5/6 matched but homozygous at the locus of mismatch should be chosen over a 5/6 unit with bidirectional mismatch even if the latter unit is larger (has more cells). This also applies to 4/6 units. This is only applicable to choosing units within a given match level.
- If the patient is randomized to receive a single UCB unit, choose the best HLA-matched unit from all units with a dose $2.5 \times 10^7/\text{kg}$ (this may not be the same unit chosen to be Unit 1 for a double cord blood transplant since the selection of Unit 1 may be influenced (within a given match level) by the requirement for inter-Unit HLA-matching).
- Within an HLA match level, the unit containing the greatest number of cells will be chosen. If there are two units of equivalent cell dose ($\pm 0.5 \times 10^7/\text{kg}$) within a match level, choose the unit with match by higher resolution molecular typing, if known).

2.6. Treatment Plan

All patients will receive the same preparative therapy as shown in Table 2.6.

TABLE 2.6 – TREATMENT PLAN

DAY	TREATMENT
-10	Fludarabine 25 mg/m ² IV over 1 hour
-9	Fludarabine 25 mg/m ² IV over 1 hour
-8	Fludarabine 25 mg/m ² IV over 1 hour
-7	TBI (165 cGy) x 2
-6	TBI (165 cGy) x 2
-5	TBI (165 cGy) x 2
-4	TBI (165 cGy) x 2
-3	Cyclophosphamide 60 mg/kg IV
-2	Cyclophosphamide 60 mg/kg IV
-1	Rest
0	UCB infusion

2.6.1. Study Drugs

2.6.1.1. Fludarabine

Fludarabine 25 mg/m²/day will be administered over 30-60 minutes intravenous infusion on Days –10 through –8. Fludarabine will not be dose adjusted for body weight.

2.6.1.2. Cyclophosphamide

Cyclophosphamide 60 mg/kg/day will be administered as a 2 hour intravenous infusion with a high volume fluid flush on Days –3 and –2. Cyclophosphamide dose adjustments for ideal body weight are recommended but not required. Doses and schedule for uroprotective agents (i.e., mesna) should follow local institutional guidelines. For patients weighing more than 125% of their IBW, cyclophosphamide will be dosed based on the adjusted ideal body weight (AIBW).

The following are dose adjustment formulas:

Ideal Body Weight (IBW) Formulas:

Patients Over 18 Years

Males IBW = 50 kg + 2.3 kg/inch over 5 feet

Females IBW = 45.5 kg + 2.3 kg/inch over 5 feet

Patients 1 to 18 Years of Age

Less than 60 inches

IBW = (ht² x 1.65)/1000 where ht = cm, IBW = kg

More than 60 inches

Males IBW = 39.0 + [2.27 x (ht - 60)] where ht = inches, IBW = kg

Females IBW = 42.2 + [2.27 x (ht - 60)] where ht = inches, IBW = kg

Adjusted Ideal Body Weight Formula:

$$\text{AIBW} = \text{IBW} + [(0.25) \times (\text{ABW} - \text{IBW})]$$

2.6.1.3. Radiotherapy

Patients may be treated either in the AP/PA position and/or in the right and left lateral position. Compensators or blocks may be used to compensate for the thinner parts of the anatomy (neck, head, lower legs, and feet).

Total dose will be 1320 cGy in 8 fractions over 4 days. Dose will be prescribed at the level of the umbilicus at midplane.

The dose along the central axis of the patient should be kept to within 10% of the prescription dose. Compensators, bolus, or transmission blocks may be placed in an effort to accomplish this.

To compensate for decreased attenuation through the lungs, partial compensators may be used to prevent the lung dose from exceeding the prescription point dose. No adjustments are made for lower lung density. The estimated lung dose is calculated by measuring the off-axis thickness in the mid-lung area:

- If the patient is treated with AP and PA fields, the lungs may be partially blocked with 50% transmission blocks such that the lung receives an estimated minimum of 675 cGy. With the use of 50% transmission blocks, an anterior and posterior electron chestwall boosts, calculated to D90, where electron energy is selected to place the D90 at the pleural surface, must be employed. 300 cGy per fraction for a total of two fractions will be given to both the anterior and posterior chestwall. Regardless of the partial blocking used, the lung may receive an estimated maximum of the prescription dose (1320 cGy).
- If the patient is treated with right and left lateral fields, separations are taken with the arms placed along the axis of the thoracic cavity, and the tissue deficit calculated (without lung correction). Since the effective thickness at the level of the mid-mediastinum is often greater than the thickness at the umbilicus, this may be all the compensation that is necessary. However, if additional tissue deficit is calculated, lung compensators may also be placed such that the estimated lung dose is between a minimum of 1000 cGy and a maximum of 1320 cGy. (The minimum lung dose allowed with this technique is somewhat higher than the right left lateral technique since, by default, some of the mediastinum and spine will also be under the compensator.)

A total of 8 fractions are given over 4 days (Days -7, -6, -5, and -4). The two fractions are given at a minimum of 6 hours apart from beam on to beam on.

The TBI will be delivered from either a linear accelerator or cobalt source at a dose rate of between 4 and 26 cGy/minute using energies of between 1 and 25 MV.

The skin dose should be at least 90% of the prescribed dose. If a higher energy beam (> 4 MV) is used for the TBI treatments, a beam spoiler should be used to accomplish this or thermoluminescent dosimetry data submitted showing that the skin dose is \geq 90% of the prescribed dose.

Testicular boosts should be used for all males with ALL (and according to institutional practice for other diseases). The testicular boost is given in at least a single 400 cGy fraction with either electrons prescribed to Dmax or photons prescribed to the midplane of the scrotum. If electrons are used, the energy for the testicular boost depends on the thickness of the testicles and is chosen so that the D90 corresponds to the posterior surface of the scrotum.

2.6.2. Immunosuppressive Therapies

All patients will receive GVHD prophylaxis with two drugs as follows:

2.6.2.1. Cyclosporine A (CSA)

CSA will be administered beginning on Day –3 and doses will be adjusted to maintain a level of 200-400 ng/mL by TDX method (or 100-250 ng/mL by Tandem MS or equivalent level for other CSA testing methods). CSA can be administered per institutional practice.

Dose adjustments will be made on the basis of toxicity and low CSA levels with a trough level of < 200 ng/mL. Once the patient can tolerate oral medications and has a normal gastro-intestinal transit time, CSA will be converted to an oral form at 2-3x the current IV dose. CSA dosing will be monitored and altered as clinically appropriate.

Patients will receive CSA until Day +180. If no GVHD, the dose will be tapered 10% per week beginning on Day 100.

2.6.2.2. Mycophenolate mofetil (MMF)

MMF will be given at a dose of 1 gram IV q 8 hours if > 50 kg or 15 mg/kg IV q 8 hours if < 50 kg beginning the morning of Day –3. (If renal failure and GFR < 25 mL/min do not exceed dose of 1 gm q 8 hours. No dose adjustment for liver disease.) MMF should be given IV until patient can tolerate oral medications and has a normal gastro-intestinal transit time. Tablets or suspension may be used to achieve calculated doses. MMF will be dosed based on the patients actual body weight.

MMF should be continued for 45 days *or* 7 days after engraftment, whichever day is later, if acute GVHD has not occurred. If GVHD occurs before Day 45, continue MMF and treat according to institutional protocols. Methylprednisolone, 2 mg/kg divided q12H IV, is suggested as initial therapy. If no response after 7 days, treat with second line agents per institutional guidelines.

If the patient has active acute GVHD requiring systemic therapy, treat according to institutional guidelines.

2.6.3. UCB Thaw and Infusion

In both the COBLT study and the double cord studies at the University of Minnesota, cord blood units were thawed and washed in Dextran 40 + 5% albumin as described by Rubinstein et al²³. This approach is the recommended method of thawing for patients transplanted on this study. This method results in the lowest exposure to DMSO and control of the volume of the transplant product(s) for the recipient. It is important that young and smaller children not be exposed to excessive amounts of DMSO, free hemoglobin or volume when undergoing transplantation with 1 or 2 cord blood units.

Over the last few years, some centers have elected to thaw, dilute and infuse cord blood cells without a wash. The rationale for dilution with the hypertonic solution without a wash is to minimize cell loss due to aggregation and/or to avoid the small risk of loss of product bag integrity during the wash centrifugation. Both dilution alone or dilution and wash are acceptable practices as long as the transplant recipient does not receive too excessive volume or DMSO with the transplant. These are concerns in small children receiving two cord blood units.

There are two types of UCB products that are currently banked: red cell and plasma depleted or plasma depleted only. The later products have a larger amount of lysed red blood cells in the product and thus there is a greater risk of hemolytic reaction during infusion. Since the majority of red cells in the product are lysed during thaw, the presence or absence of ABO incompatibility is not significant. It is important that the cellular therapy laboratory and clinical team review the type of unit(s) that are being used and the thaw plans prior to the day of transplant.

Guidelines for thawing UCB(s):

	Final product*	Dilution only	Dilution and wash
DMSO concentration	<3% final volume	acceptable	acceptable
	>3% final volume	not recommended	recommended
Red cell lysate	<2 ml RBC volume/kg	acceptable	acceptable
	> 2 ml RBC volume/kg	not recommended	recommended
Final volume of infusate	<20 ml/kg	acceptable	acceptable
	>20 ml/kg	not recommended	recommended

*Note: since both units are infused in close proximity the calculations should be based on the sum of both units.

2.6.3.1. UCB unit thaw

The cord blood should be thawed, diluted with or without wash per validated institutional or supplying cord blood bank procedures with the exception that bedside thawing and direct infusion is not allowed. Bedside thaws are not recommended because of the inability to rescue the product if there is loss of integrity of the UCB bag on thaw at the bedside and because of the instability of the cells in 10% DMSO post thaw.

All transplant centers/cellular therapy laboratories must be familiar with thawing of cord blood units. They must have validated procedures and maintain competency in the thaw process (see Appendix F for sample thawing procedures). The cord blood unit must be thawed in a qualified laboratory by trained personnel. Generally the cryopreserved unit is removed from the protective cassette, placed in a ziplock bag and thawed rapidly in a 37° C waterbath. The ziplock bag allows for recovery of cells if the cryopreservation bag cracks or leaks during the thawing process, a rare but possible event. Once the contents of the bag reach a slushy consistency, the cells can be diluted in dextran/albumin, a hypertonic solution that buffers against the intracellular hypertonicity created by DMSO. Cell suspensions can subsequently be washed to remove DMSO, free hemoglobin and other cellular debris allowing for resuspension in a volume appropriate for the size of the patient to be transplanted. The full procedure is detailed in Appendix F.

2.6.3.2. UCB infusion on Day 0

Under no circumstances is the cord blood to be irradiated. There must be no in-line leukocyte filter used with product infusion nor any medications or fluids infused in the same line with the cord blood (i.e., no piggyback fluids). Vital signs should be monitored before beginning the infusion and periodically during administration. Infusion should begin within 2 hours of washing. The infusion should take no longer than 1 hour. Pre-medications and hydration prior to cord blood infusion will be administered per institutional procedure. Diphenhydramine, epinephrine, and hydrocortisone should be available at the bedside for emergency use if infusion reactions occur. Oxygen with nasal prongs for standby use should be present in the room. The two units for double UCB transplantation are infused one after the other.

2.6.4. Supportive Care

Patients will receive transfusions, infection prophylaxis and nutritional support according to institutional guidelines. Infection prophylaxis should include, but is not limited to, agents or strategies (e.g., PCR screening and preemptive therapy) to prevent herpes simplex, cytomegalovirus (CMV), *Pneumocystis carinii*, and fungal infections.

Transfusion thresholds for blood product support will be consistent with BMT CTN MOP and standard institutional guidelines. All blood products will be irradiated.

Additional details are provided in Appendix E.

2.6.5. Immunizations

Tetanus immunizations will be done at Day 100, 6 months, 12 months and 24 months post-transplant. Other immunizations will be according to institutional practice.

2.6.6. Growth Factors

G-CSF will be given beginning on Day 1 at a dose of 5 mcg/kg/day (rounding to the nearest vial dose is allowed), until absolute neutrophil count (ANC) is $\geq 2,000/\text{mm}^3$ for three consecutive days. G-CSF should then be titrated to maintain ANC $> 1,000/\text{mm}^3$. G-CSF may be given by IV or subcutaneously.

2.6.7. Intravenous Immune Globulin (IVIG)

Bi-weekly IVIG infusions (200-500 mg/kg/dose) are recommended for immunoprophylaxis through Day 100 to maintain IgG levels in the normal range for age. Alternatively, if IVIG is not given on a regular schedule, IgG levels should be monitored and IVIG infusions given to maintain IgG level in the normal range for age.

2.6.8. Risks and Toxicities

Cyclophosphamide:

Cyclophosphamide side effects include: nausea/vomiting, cardiomyopathy, skin rash, mucositis, stomatitis, sterility, diarrhea, hemorrhagic cystitis, fluid weight gain/edema, alopecia and hemolytic/anemia.

Fludarabine:

- a. Neurotoxicity: Agitation or confusion, blurred vision, loss of hearing, peripheral neuropathy or weakness have been reported. Severe neurologic effects, including blindness, coma, and death are seen in 36% of patients treated with doses approximately four times greater than recommended; severe CNS toxicity is rarely seen with doses in the recommended range for nontransplant therapy of hematologic malignancies. Effect of chronic use on the CNS is unknown, although patients have received recommended doses for up to 15 courses. The dose used in this study is approximately 1.5 times the usual one-course dose given in non-transplant settings. Doses and schedules such as those used in this study have been used in adult and pediatric patients and increased neurotoxicity has not been observed.
- b. Anemia: Life-threatening and sometimes fatal autoimmune hemolytic anemia has been reported after one or more cycles of therapy in patients with or without a previous history of autoimmune hemolytic anemia or a positive Coombs' test and who may or may not be in remission; no mechanisms for development of this complication have been identified. Corticosteroids may or may not be effective in controlling these episodes. The majority of patients re-challenged developed a recurrence of the hemolytic process.
- c. Cardiovascular: Deep venous thrombosis, phlebitis, transient ischemic attack, and aneurysm (1%) are reported.
- d. Fever: 60% of patients develop fever.

- e. Skin Rash: 15% of patients develop a skin rash which may be pruritic.
- f. Digestive: Gastrointestinal side effects include: nausea/vomiting (36%), diarrhea (15%), stomatitis (9%), anorexia (7%), GI bleeding and esophagitis (3%), mucositis (2%), liver failure, abnormal liver function test, constipation, dysphagia (1%) and mouth sores.
- g. Some other effects are: Chills (11%), peripheral edema (8%), myalgia (4%), osteoporosis (2%), pancytopenia, arthralgia (1%), dysuria (4%), urinary tract infection and hematuria (2%); renal failure, abnormal renal function test, and proteinuria (1%); and, very rarely, hemorrhagic cystitis and pulmonary toxicity.

Total Body Irradiation:

TBI can cause: nausea and vomiting, diarrhea, parotitis (rapid onset within 24-48 hours, usually self-limited), generalized mild erythema, hyperpigmentation, fever, mucositis, and alopecia.

Late effects include: possible growth retardation, vertebral deformities, cataracts, probable increased risk of secondary malignant neoplasms, sterility, nephropathy, interstitial pneumonitis and veno-occlusive disease.

UCB Graft Infusion:

Potential toxicities associated with the infusion include DMSO toxicity and side effects from red cells. These may include changes in heart rate or rhythm, changes in blood pressure, fever, chills, sweats, nausea/vomiting, diarrhea, abdominal cramping, headache, allergic reaction, presence of DMSO taste and odor, hemoglobinuria, and acute renal failure. Due to the washing and processing steps, these toxicities are unlikely.

Cyclosporine A:

Cyclosporine may cause: nephrotoxicity, seizures, hypertension, hirsutism, thrombotic microangiopathy, electrolyte imbalances, paresthesias/neuropathy, gingival hyperplasia, transient-blindness, and hepatic and renal dysfunction.

Mycophenolate Mofetil:

Side effects include: pancytopenia, nausea, vomiting, diarrhea, hypertension, headache, dizziness, insomnia, hyperglycemia, electrolyte imbalances, rash, and leg cramps/bone pain.

Growth Factor or G-CSF (Filgrastim, Neupogen):

G-CSF may cause: Bone pain, insomnia, headaches, dyspnea, body aches, rash, fever, splenomegaly allergic reaction, fatigue, edema and nausea/vomiting.

Graft Failure:

Based on historical data, there could be a 20% chance of graft failure. Contingency plans are recommended and include obtaining marrow from a haploidentical relative, supportive care or acquisition of another compatible UCB unit.

CHAPTER 3

3. STUDY DESIGN

3.1. Primary Endpoint

The primary endpoint is one-year overall survival from the time of randomization. The event analyzed is death from any cause.

3.2. Secondary Endpoints

3.2.1. Neutrophil Engraftment

Neutrophil engraftment is defined as achieving an ANC $\geq 500/\text{mm}^3$ for three consecutive measurements on different days. The first of the three days will be designated the day of neutrophil engraftment. It will be measured at Days 42, 100, and 180. Patients surviving at least 14 days will be evaluable for this endpoint.

3.2.1.1. Autologous recovery

Autologous recovery is defined as an ANC $\geq 500/\text{mm}^3$ for three consecutive measurements on different days with $> 50\%$ host chimerism. This endpoint will be assessed at Days 42, 100 and 180.

3.2.1.2. Primary graft failure

Primary graft failure is defined as lack of neutrophil engraftment by 42 days in patients surviving a minimum of 14 days.

3.2.1.3. Secondary graft failure

Secondary graft failure is defined as initial neutrophil engraftment followed by subsequent decline in the ANC to $< 500/\text{mm}^3$ for three consecutive measurements on different days, unresponsive to growth factor therapy.

3.2.1.4. Platelet engraftment of $> 20,000$ and $50,000/\text{mm}^3$

Platelet engraftment is defined as the first day of a minimum of three consecutive measurements on different days such that the patient has achieved a platelet count $> 20,000/\text{mm}^3$ and $> 50,000/\text{mm}^3$ with no platelet transfusions in the preceding seven days. The first day of the three measurements will be designated the day of platelet engraftment.

Platelet engraftment will be assessed at Days 100, 180 and 365.

3.2.2. Chimerism

Incidence of chimerism (e.g., engraftment of both UCB units) will be assessed at Days 28, 42, 60, 100, 180 and 365.

3.2.3. Acute Graft-versus-Host Disease

Incidences of grade II – IV and III – IV acute GVHD at Day 100 will be graded according to the BMT CTN Manual of Procedures (MOP). This will be assessed at Days 100, 180 and 365.

3.2.4. Chronic Graft-versus-Host Disease

Incidences of chronic GVHD will be scored according to the BMT CTN MOP. This will be assessed at Days 100, 180, 365 and 730.

3.2.5. Disease-free Survival

Disease-free survival is defined as the minimum time interval of the times to relapse/recurrence, to death or to last follow-up.

3.2.6. Transplant-related Mortality (TRM)

Incidence of transplant-related mortality will be estimated at Day 100. An event for this endpoint is death in continuous CR. Relapse is a competing risk.

3.2.7. Infections

Microbiologically documented infections will be reported by anatomic site, date of onset, organism and resolution, if any. For definitions, see the BMT CTN MOP. Patients will be followed for infection for 2 years post-transplant.

3.2.8. Immune Reconstitution

Formal comparison of the rate and completeness of immune recovery will be compared in between treatment arms. This will be measured as indicated in Appendix C. It will be assessed at Day 100, 6 months, 1 year and 2 years post-transplant.

Quantitative recovery of T cells and subsets, B cells and NK cells, immunoglobulin synthesis and functional T and B cell generation and antigen driven responses will be correlated with post-transplant infections and with malignant relapse in the two randomized cohorts. Given the hypothesis that recipients of two UCB units will have faster and more complete hematologic and immune reconstitution, it is anticipated that their rates of infection will be lower. The incidence of bacteremia; fungal infections and reactivation of CMV (adjusted for pre-HCT CMV serologic status) will be compared between the two arms within the first 100 days and for fungi and CMV

between Day 100 and 365 post-HCT. These infection incidences will be correlated with the prospectively collected parameters of immune recovery.

Similarly, it is hypothesized that recipients of two UCB units will have enhanced immunologic competence and thus superior protection against malignant relapse within the first year. The diagnosis and disease-stage adjusted risk of relapse will be compared within the 2 randomized cohorts and correlated with recovery of T cells, CD4+ T cells and NK cells after HCT.

3.2.9. Relapse and Residual Disease

Relapse of Malignancy – Testing for recurrent malignancy in the blood, marrow or other sites will be used to assess relapse after transplantation. For the purpose of this study, relapse is defined by either morphological or cytogenetic evidence of AML, ALL, CML, or MDS consistent with pre-transplant features.

Minimal Residual Disease – Minimal residual disease is defined by the sole evidence of malignant cells by flow cytometry, or fluorescent in situ hybridization (FISH), or Southern blot, or Western blot, or polymerase chain reaction (PCR), or other techniques, in absence of morphological or cytogenetic evidence of disease in blood or marrow. Since the frequency of testing for minimal residual disease is highly variable among centers, and the sensitivity is highly variable among laboratory techniques, evidence of minimal residual disease will not be sufficient to meet the definition of relapse in the context of this study. Data on tapering immunosuppression, administering chemotherapy or biological agents to attempt reducing the tumor load will be captured in the case report forms.

Acute Leukemia – Relapse will be diagnosed when there is:

1. The reappearance of leukemia blast cells in the peripheral blood; or,
2. > 5% blasts in the marrow, not attributable to another cause (e.g., bone marrow regeneration); or,
3. If there are no circulating blasts, but the marrow contains 5-20% blasts, a repeat bone marrow examination \geq one week later demonstrating > 5% blasts is necessary to meet this criterion for relapse; or,
4. The appearance of new dysplastic changes within the bone marrow; or,
5. The development of extramedullary leukemia or leukemic cells in the cerebral spinal fluid.

Chronic Myelogenous Leukemia (CML) –

Hematologic relapse will be diagnosed when:

1. Immature hematopoietic cells are persistently documented in the peripheral blood; or,
2. There is myeloid hyperplasia in the bone marrow in the presence of cytogenetic relapse.

Cytogenetic relapse will be diagnosed when:

1. 50% of the metaphases exhibit the characteristic 9;22 translocation with at least ten metaphases analyzed; or,
2. At least one metaphase exhibits the 9;22 translocation on each of two separate consecutive examinations at least one month apart, regardless of number of metaphases analyzed.

Myelodysplastic (MDS) and Myeloproliferative Syndromes - Relapse will be diagnosed when there is:

1. Reappearance of pre-transplant morphologic abnormalities, detected in two consecutive bone marrow specimens taken at least one month apart; or,
2. Evidence of satisfying above criteria for evolution into acute leukemia; or,
3. Reappearance of pre-transplant cytogenetic abnormalities in at least 50% of metaphases with at least ten metaphases examined; or,
4. Reappearance of pre-transplant a cytogenetic abnormality in at least one metaphase on each of two separate consecutive examinations at least one month apart, regardless of the number of metaphases analyzed.

Relapse will be defined to occur in the absence of the evidence above if specific therapy, such as use of interferon or second transplant, is initiated for relapse reversal.

3.3. Other Definitions

3.3.1. Complete Remission for Acute Leukemias Pre-transplant

Complete remission prior to entering the study will be defined as all of the following according to the revised recommendations of the international working group:

- A bone marrow aspirate containing spicules with < 5% blasts with a count of at least 200 nucleated cells and no Auer rods seen. If spicules are absent in the aspirate, a bone marrow biopsy should confirm that < 5% blasts are present.
- ANC > 1,000/mm³ and platelet count > 100,000/mm³.
- No extramedullary leukemia.
- No blasts in peripheral blood.

3.3.2. Morphologic Complete Remission with Incomplete Blood Count Recovery (CRi)

- A bone marrow aspirate containing spicules with < 5% blasts with a count of at least 200 nucleated cells and no Auer rods seen. If spicules are absent in the aspirate, a bone marrow biopsy should confirm that < 5% blasts are present.
- No extramedullary leukemia.
- No blasts in peripheral blood.

3.3.3. CML Stages

Chronic phase is defined as:

- Stable, not hematologic remission: blasts present in marrow and/or peripheral blood, but disease does not qualify as accelerated or blast phase
- Hematological remission: no blast cells or precursor cells in the blood or marrow
- Partial cytogenetic remission: Ph+ metaphases >0% but < 35%
- Complete cytogenetic remission: absence of Ph+ metaphases

Accelerated phase is defined as:

- WBC difficult to control ($> 50 \times 10^9/L$ despite therapy)
- Rapid doubling of WBC (< 5 days)
- Anemia or thrombocytopenia unresponsive to standard treatment
- Persistent thrombocytosis ($> 1000 \times 10^9/L$)
- Cytogenetic abnormalities in addition to Ph+
- Increasing splenomegaly
- Marrow fibrosis

CHAPTER 4

4. PATIENT ENROLLMENT AND EVALUATION

4.1. Enrollment Procedures

4.1.1. Screening and Eligibility Procedures

Patients will be registered using the BMT CTN Electronic Data Capture System (AdvantageEDCSM). The Children's Oncology Group (COG) should follow instructions in Section 4.1.2 **prior** to following the instructions below. The following procedures should be followed:

1. Prior to initiation of conditioning regimen, but no more than 14 days prior to initiation of conditioning regimen, an authorized user at the transplant center enters the patient demographics and Segment A of the Enrollment Form in AdvantageEDC. The eligibility screening (Segment A) includes a question confirming that the patient (or legal guardian) signed the informed consent.
2. Prior to initiation of the conditioning regimen, the selected cord blood unit must be at the transplant center. In the event that the patient has been randomized to receive two units, at least one of the units must be at the transplant center. However, it is strongly recommended that both units are present at the transplant center in this case.
3. If the patient is eligible, a study number is generated and a treatment assignment is displayed.
4. A visit schedule based on treatment start date is displayed for printing and is referred to as 'Segment A Follow-up.'

4.1.2. COG Patient Registration Procedures

Prior to enrollment on study, all patients must have been registered via the Remote Data Entry (RDE) system into the COG Cancer Registry (Diagnosis/Registry). The patient registration application is available 24 hours a day, 7 days a week. The assigned COG patient identification number will be used to identify the patient in all future interactions with the COG. If you have problems with registration, please refer to the online help in the eRDE area of the COG website.

4.2. Study Monitoring

4.2.1. Follow-up Schedule

The follow-up schedule for scheduled study visits is outlined in Table 4.2.1. A detailed description of each of the forms and the procedures required for forms completion and submission can be found in the Data Management Handbook and User's Guide.

TABLE 4.2.1: FOLLOW-UP SCHEDULE

Study Visit	Target Day Post-Transplant
1 week	7 ± 2 days
2 week	14 ± 2 days
3 week	21 ± 2 days
4 week	28 ± 2 days
5 week	35 ± 2 days
6 week	42 ± 2 days
7 week	49 ± 2 days
8 week	56 ± 2 days
60 days	60 ± 2 days
9 week	63 ± 2 days
10 week	70 ± 2 days
11 week	77 ± 2 days
12 week	84 ± 2 days
13 week	91 ± 2 days
14 week	98 ± 2 days
100 day	100 ± 2 days
6 month	180 ± 28 days
12 month	365 ± 28 days
24 month	730 ± 28 days

Criteria for Forms Submission: Criteria for timeliness of submission for all study forms are detailed in the Data Management Handbook and User’s Guide. Forms that are not entered into AdvantageEDC within the specified time will be considered delinquent. A missing form will continue to be requested either until the form is entered into the AdvantageEDC and integrated into the Data Coordinating Center’s (DCC) master database, or until an exception is granted and entered into the Missing Form Exception File, as detailed in the Data Management Handbook.

Reporting Patient Deaths: Recipient death information must be entered into AdvantageEDC within 24 hours of knowledge of the patient’s death. If the cause of death is unknown at that time, it need not be recorded at that time. However, once the cause of death is determined, the form must be updated in AdvantageEDC.

CIBMTR Data Reporting: Centers participating in BMT CTN trials must register pre and post-transplant outcomes on all consecutive hematopoietic stem cell transplants done at their institution during their time of participation to the Center for International Blood and Marrow Transplant Research (CIBMTR). Registration is done using procedures and forms of the Stem Cell Transplant Outcomes Database (SCTOD). (Note: Federal legislation requires submission of

these forms for all US allotransplant recipients.) Enrollment of BMT CTN #0403 must be indicated on the SCTOD pre-transplant registration form, if applicable. Additionally, CIBMTR pre- and post-transplant Report Forms must also be submitted for all patients enrolled on this trial. CIBMTR forms will be submitted directly to the CIBMTR at the times specified on the Form Submission Schedule.

Weekly GVHD Monitoring: GVHD should be monitored in accordance with BMT CTN guidelines as specified in the Manual of Procedures. Patients should be assessed weekly until Day 100 post-transplant for GVHD. After Day 100 patients will be assessed at each follow-up visit (Day 180, 365 and 730) for the presence of GVHD.

4.2.2. Adverse Event Reporting

Unexpected, grade 3-5 adverse events (AE) will be reported through an expedited AE reporting system via AdvantageEDC. Unexpected, grade 4-5 AEs must be reported within 24 hours of knowledge of the event. Unexpected, grade 3 AEs must be reported within three business days of knowledge of the event. Expected AEs will be reported using NCI's Common Terminology Criteria for Adverse Events (CTCAE) Version 3.0 at regular intervals as defined on the Form Submission Schedule.

4.2.3. Patient Assessments

Table 4.2.3 summarizes patient clinical assessments over the course of the study.

4.2.3.1. Pre-transplant evaluations

The following observations are considered standard evaluations for transplant eligibility and should be determined < 4 weeks before initiation of conditioning therapy.

1. History, physical examination, height and weight.
2. Karnofsky/Lansky performance status.
3. CBC with differential and platelet count, serum creatinine, bilirubin, alkaline phosphatase, ALT, and AST.
4. CMV antibody test, hepatitis panel (HepA Ab, HepB Sab, HepB Sag, HepB Core Ab, HepC Ab), herpes simplex, syphilis, HIV and HTLV1 I/II antibody, and varicella zoster virus.
5. High resolution HLA typing, if not already performed.
6. EKG.
7. Left ventricular ejection fraction or shortening fraction.
8. DLCO, FEV1, and FVC or O₂ saturation.
9. Bone marrow aspirates for pathology and cytogenetics and/or biopsy.
10. β -HCG serum pregnancy test for females of childbearing potential.

11. Chest x-ray.
12. Heparinized blood for pre-transplant chimerism assay.
13. Total nucleated cell count and viability of the infused product on Day 0. A CD34+ count of the infused product is strongly recommended.

4.2.3.2. Post-transplant evaluations

The following evaluations are considered standard evaluations for transplant recipients:

1. History and physical exam to assess GVHD and other morbidity weekly until Day 100 post-transplant, then at six months, one year and then yearly until two years post-transplant. GVHD evaluation and grading to be in keeping with BMT CTN MOP.
2. CBC at least three times a week from Day 0 until ANC > 500 mm³ for 3 days after nadir reached. Thereafter CBC twice per week until Day 28, then weekly until 12 weeks, then six months, one year and then yearly until two years post-transplant.
3. Creatinine, bilirubin, alkaline phosphatase, ALT, AST, twice a week until Day 28 (or four weeks) and then weekly until 12 weeks, and then at six months, one year and two years post-transplant.
4. Heparinized blood for immune reconstitution at Day 100, 6 months, 1 year and 2 years post-transplant. Note that some of the immune reconstitution studies being performed for this study are not standard of care as described in Appendix C.
5. Heparinized blood for post-transplant chimerism assay collected at Day 28, 42, 60, 100, 180 and 365.
6. Bone marrow aspirate at Day 21 if WBC < 500. If there is an insufficient number of cells for chimerism assay on Day 21, repeat on Day 28.
7. Tetanus immunization at Day 100, 6 months and 1 year.
8. Toxicity assessments at Day 28, 56, 100, 6 months and 1 year.

TABLE 4.2.3: SUMMARY OF PATIENT CLINICAL ASSESSMENTS

Study Assessments/ Testing	Baseline	Days Post-Transplant																	
		7	14	21	28	35	42	49	56	60	63	70	77	84	91	100	180	365	730
History, physical exam, weight, height, and Karnofsky/Lansky performance status	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X
CBC ¹ , differential, platelet count, and blood chemistries ²	X	X	X	X	X	X	X	X	X		X	X	X	X			X	X	X
Infectious disease titers ³	X																		
EKG, LVEF, or shortening fraction	X																		
DLCO, FEV1 and FVC or O ₂ saturation	X																		
Bone marrow aspirate for pathology and cytogenetics ⁴ and/or biopsy	X			X															
Chest X-ray	X																		
β-HCG serum pregnancy test (females only)	X																		
Blood samples for immune reconstitution assays ⁵																X	X	X	X
GVHD and other morbidity assessments ⁶		X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X
Toxicity assessments	X				X				X							X	X	X	
Chimerism ⁷	X				X		X			X						X	X	X	
Tetanus Immunization																X	X	X	

Notes:

- ¹ CBC performed at least three times a week from Day 0 until ANC >500 mcL for three days after nadir. CBC performed twice weekly until Day 28. CBC performed weekly after Day 28 until 12 weeks post-transplant.
- ² A standard chemistry panel to include: creatinine, ALT, AST, bilirubin, alkaline phosphatase, sodium, potassium, chloride, CO₂ and magnesium should be performed twice weekly until Day 28, then weekly until 12 weeks post-transplant. \
- ³ Infectious disease titers include: CMV, Hepatitis panel (HepA, Ab, HepB SAb, HepB SAg, HepB Core Ab, HepC Ab), herpes simplex virus, syphilis, HIV and HTLV I/II antibody, and varicella zoster.
- ⁴ Bone marrow biopsy and aspirates to pathology and aspirate for cytogenetics. Flow cytometry required on aspirate. Day 21 bone marrow aspirate only if WBC < 500.
- ⁵ See Appendix C for details. If results for tetanus response are abnormal, repeat at 15 months for T cell blastogenesis (i.e., not antibody titers).
- ⁶ GVHD and other morbidity assessments performed weekly until Day 100 post-transplant, and then at Day 180, 365 and 730.
- ⁷ Chimerism will be measured by RFLP or microsatellite. On Day 28 chimerism tests will be performed on total, lymphoid and myeloid fractions. At all other timepoints only total chimerism is required.

CHAPTER 5

5. STATISTICAL CONSIDERATIONS

5.1. Study Design

This is a Phase III, multi-center, open label, randomized clinical study to determine the benefit of double UCB unit infusion on survival after UCB transplantation in children with high-risk leukemia or MDS. The central hypothesis is that the infusion of additional hematopoietic stem and progenitor cells from a partially HLA-matched second UCB unit will improve survival.

5.1.1. Accrual

The target enrollment is 220 patients. It is estimated that five years of accrual will be necessary to enroll the targeted sample size. Both Core and Affiliate Centers will enroll patients on this study. Accrual will be reported by race, ethnicity, and gender.

5.1.2. Randomization

All patients will be randomized within 14 days prior to the initiation of conditioning therapy. Randomization will be performed in a 1:1 ratio using random block sizes for the two arms. Randomization will be stratified by transplant center size (each transplant center expected to enroll more than 10 patients over the course of the study will have its own stratum and transplant centers expected to enroll fewer than 10 patients will be considered together in a single stratum) and age (patients ≤ 10 years of age will be in one stratum and patients > 10 years of age will be in another stratum).

5.1.3. Primary Endpoint

The primary endpoint is one-year survival. The primary analysis will be performed using the intent-to-treat principle so that all randomized patients will be included in the analysis.

5.1.4. Primary Hypothesis

The primary null hypothesis of the study is that there is no difference in overall survival after UCB transplantation using two versus one UCB units.

$$\begin{aligned} H_0: \quad & OS_d(t) = OS_s(t) \text{ for all } t \\ H_a: \quad & OS_d(t) \neq OS_s(t) \text{ for at least one } t \end{aligned}$$

5.2. Sample Size and Power Calculations

Overall survival will be compared between double and single UCB unit transplant arms using the log-rank test. The final analysis will be performed after all patients have been followed for at

least one year. The sample size of 110 patients per group is sufficient to maintain type I error of 5% across all planned interim analyses (see below) while providing > 86% statistical power for a two-sided test to detect an increase in the proportion surviving at one year from 0.57 in the standard therapy arm to 0.77 in the experimental arm.

5.3. Interim Analysis and Stopping Guidelines

Interim analyses for efficacy will be conducted at times coincident with regularly scheduled meetings of the NHLBI-appointed Data and Safety Monitoring Board (DSMB) at approximately one-year intervals. Monitoring of key safety endpoints will be conducted monthly, and if rates significantly exceed pre-set thresholds, the NHLBI will be notified in order that the DSMB can be advised. Policies and composition of the DSMB are described in the BMT CTN's MOP.

5.3.1. Interim Analysis for Efficacy

At the time of each interim analysis, the log rank test statistic will be computed, and if the null hypothesis is rejected, the DSMB will be consulted to discuss the continuation of the trial. In order to preserve the over-all type I error rate at 5%, the critical values used for the interim analysis will be inflated over 3.84, the value that would be used if no repeated testing were used. Equivalently, the nominal p-value at which an observed difference is declared significant will be reduced below 0.05. The actual critical values and nominal p-values will be computed using statistical methods for group sequential testing with O'Brien Fleming boundaries.

As an example, Table 5.3.1a shows the critical values and nominal p-values for tests conducted at 1, 2, 3, 4, and 6 years after the study opens to enrollment.

TABLE 5.3.1A CRITICAL VALUES AND NOMINAL P-VALUES

Calendar Time (year)	Number of Events	Information Time	Critical Value	Nominal P-value	Cumulative Type I Error
1	10	0.13	37.2930	0.00000	0.00000
2	24	0.33	13.9196	0.00019	0.00019
3	39	0.53	8.2426	0.00409	0.00416
4	53	0.73	5.7917	0.01610	0.01741
5	68	0.93	4.4576	0.03475	0.04023
6	73	1.00	4.3422	0.03718	0.05000

In practice, the rate of accrual or timing of DSMB meetings may not be as anticipated. To permit necessary flexibility in scheduling interim analyses, the critical values will be recomputed to correspond to the actual available statistical information using the “use-function” approach of Lan and DeMets.

Operating Characteristics of the Design

Under the assumption that time to death has a piece-wise exponential distribution, the statistical power to reject the null hypothesis of equal one year survival is shown below under a variety of scenarios.

TABLE 5.3.1B – POWER TO REJECT THE NULL HYPOTHESIS UNDER VARIOUS SCENARIOS

N per Arm	Proportion Surviving Disease-Free at One Year		Power at Interim and Final Analyses By Year of Scheduled Analysis						Overall Power
	Standard	Experiment	1	2	3	4	5	6	
80	0.57	0.57	0.0000	0.0001	0.0033	0.0131	0.0230	0.0092	0.0487
80	0.57	0.77	0.0000	0.0121	0.1736	0.2911	0.2355	0.0525	0.7648
95	0.57	0.57	0.0000	0.0001	0.0037	0.0136	0.0204	0.0086	0.0464
95	0.57	0.77	0.0000	0.0152	0.2249	0.3223	0.2223	0.0492	0.8339
110	0.57	0.57	0.0000	0.0005	0.0046	0.0123	0.0231	0.0102	0.0507
110	0.57	0.77	0.0000	0.0232	0.2591	0.3485	0.2130	0.0406	0.8844

* from simulation with 10,000 replications, assuming piecewise exponential time to failure

5.4. Safety Stopping Guidelines

Monitoring of key safety endpoints (TRM, primary graft failure) will be conducted monthly. The stopping guidelines serve as a trigger for consultation with the DSMB for additional review, and are not formal “stopping rules” that would mandate automatic closure of study enrollment.

The rate of TRM at 100 days post-transplant will be monitored. Monitoring will be performed monthly until enrollment is closed. Each month, the null hypothesis that the 100-day TRM rate is less than or equal to 30% is tested. Based on published reports of single UCB transplants, TRM of 30% at Day 100 was considered acceptable. An extension of the sequential probability ratio test (SPRT) will be used to monitor TRM. A description of the SPRT is provided below.

The SPRT can be represented graphically. At each monthly interim analysis, the total time on study is plotted against the total number of patients experiencing TRM. The continuation region of the SPRT is defined by two parallel lines. Only the lower boundary will be used for monitoring to protect against excessive 100-day TRM. If the graph falls below the lower boundary, the SPRT rejects the null hypothesis, and concludes that there are more events than predicted by the observed time on study. Otherwise, the SPRT continues until enrollment reaches the target goal.

This procedure assumes an exponential distribution for the time until TRM during the first 100 days post-transplant, but censors follow-up time after 100 days post-transplant. Only events that occur on or before the patient has been followed for 100 days are counted. Total time on study is

computed as time from initiation of conditioning regimen to event, or to 100 days post-transplant, whichever comes first, summed for all patients who initiate conditioning regimen therapy.

The usual measures of performance of an SPRT are the error probabilities α and β of rejecting H_0 when $\theta = \theta_0$ and of accepting H_1 when $\theta = \theta_1$, respectively, and the expected sample size $E(N|\theta_i)$. The test to be used in this protocol was developed from the following SPRTs:

- A SPRT contrasting 30% versus 40% 100-day rate of TRM results in a common slope of 0.638 and the intercepts are -3.801 and 3.182 with $\alpha=0.1$ and $\beta=0.15$.
- A SPRT contrasting 20% versus 30% 42-day rate of graft failure results in a common slope of 0.446 and the intercepts are -1.892 and 1.584 with $\alpha=0.1$ and $\beta=0.15$.

Note that since the test uses only the lower boundary, and is truncated by a finite sample size, both the size and power of the test will be lower than nominal levels.

The actual operating characteristics of the truncated test, shown in Table 5.4.1, were determined in a simulation study that assumed uniform accrual of 110 individuals per treatment arm over a five-year time period, and exponential time to failure after initiation of conditioning therapy. Since 100,000 replications were used, the estimates have two digits of precision.

TABLE 5.4.1: OPERATING CHARACTERISTICS OF SEQUENTIAL TESTING PROCEDURE FROM A SIMULATION STUDY WITH 100,000 REPLICATIONS FOR TRANSPLANT-RELATED MORTALITY (TRM)

True 100-Day Rate	30%	32%	35%	38%	40%
Probability Reject Null	0.07	0.13	0.30	0.53	0.68
Mean Month Stopped	58.1	56.3	51.9	45.3	40.1
Mean # Endpoints in 100 Days	31	32	32	31	29
Mean # Patients Enrolled	107	103	95	83	74

The testing procedure for TRM rejects the null hypothesis in favor of the alternative 7% of the time when the true TRM rate is 30%, and 68% of the time when the rate is 40%. This corresponds to a type I error rate of $\alpha = 0.07$ and a type II error rate of $\beta = 0.32$. When the true TRM rate is 40%, on average, the DSMB will be consulted 40.1 months after opening, when 29 events have been observed in 74 patients.

Graft failure by Day 42 will be followed by testing the null hypothesis that the Day 42 post-transplant rate of graft failures is less than or equal to 20%. Review of the CIBMTR data for patients undergoing single unit cord blood transplantation (N=397) shows that Day 42 ANC

recovery was 86% and 79% after 6 of 6 and < 6 of 6 HLA-matched transplants, respectively. Similarly, on the COBLT study, the pediatric patients with malignant diseases (N=191) had an engraftment rate of 75 % (95% CI 70-81%). All patients who survived at least 14 days post-transplant were evaluated for primary graft failure. Thus, patients who survived 15 – 41 days post-transplant without ANC recovery were considered graft failures. The same criteria will be used on this study. Table 5.4.2 shows the operating characteristics for graft failure determined in a simulation study.

TABLE 5.4.2: OPERATING CHARACTERISTICS OF SEQUENTIAL TESTING PROCEDURE FROM A SIMULATION STUDY WITH 100,000 REPLICATIONS FOR GRAFT FAILURE

True 42-Day Rate	20%	22%	25%	28%	30%
Probability Reject Null	0.07	0.15	0.36	0.61	0.76
Mean Month Stopped	57.7	55.4	49.5	41.3	35.5
Mean # Endpoints in 100 Days	21	22	22	21	19
Mean # Patients Enrolled	106	102	91	76	65

The testing procedure for graft failure rejects the null hypothesis in favor of the alternative 7% of the time when the true 42-day graft failure rate is 20% and 76% of the time when the rate is 30%. This corresponds to a type I error rate of $\alpha = 0.07$ and a type II error rate of $\beta = 0.24$. When the true 42-day graft failure rate is 30%, on average, the DSMB will be consulted 35.5 months after opening, when 19 graft failures have been observed in 65 patients.

5.5. Analysis Plan

5.5.1. Analysis of the Primary Endpoint

The primary analysis will consist of estimating the one-year overall survival (from day of randomization) probability. All registered patients will be considered for this analysis. The primary null hypothesis of the study is that there is no difference in overall survival between double and single UCBT arms. The alternative hypothesis is that survival differs between double and single UCBT strategies.

The primary outcome will be assessed in a final analysis to be performed after the last enrolled patient has been followed for one year post-transplant. The one-year overall survival probability and confidence interval will be calculated. The event is death from any cause. Patients alive at the time of the last observation are censored at the time of the last observation. Overall survival will be compared between treatment arms using a log-rank test, and the survival curves will be estimated using the Kaplan Meier product limit estimator.

A secondary analysis will be performed using only patients who were transplanted. Multi-variate analysis using the Cox proportional hazards model will also be performed.

5.5.2. Analysis of Secondary Endpoints

A number of secondary endpoints will be examined to compare the patient's disease status over time between the two treatment arms.

- **Disease-free Survival (DFS):** One-year DFS distribution will be estimated by the Kaplan-Meier product limit estimator. The two treatment arms will be compared using the log-rank test. A Cox proportional hazard model will be fit to control for important prognostic variables. All patients will be followed for a minimum of two years post-transplant for mortality.
- **Neutrophil Engraftment:** To assess the incidence of neutrophil engraftment from day of transplant, a cumulative incidence curve will be computed along with a 95% confidence interval. Death will be considered as a competing risk. A log-rank test will be used to compare the two treatment arms.
- **Platelet Engraftment:** To assess the incidence of platelet engraftment from day of transplant, a cumulative incidence curve will be computed along with a 95% confidence interval. Death will be considered as a competing risk. A log-rank test will be used to compare the two treatment arms.
- **Chimerism:** The proportion of patients with chimerism at each timepoint will be estimated along with a 95% confidence interval.
- **Acute GVHD:** To assess the incidence and severity of grades II-IV acute GVHD from day of transplant. The first day of acute GVHD onset at a certain grade will be used to calculate a cumulative incidence curve for that acute GVHD grade. An overall cumulative incidence curve will be computed along with a 95% confidence interval at 100 days post-transplant with death considered as a competing risk. A log-rank test will be used to compare the two treatment arms.
- **Chronic GVHD:** To assess the incidence and severity of chronic GVHD from day of transplant, rates of chronic GVHD will be compared between the two groups using a log-rank test and the cumulative incidence curves will be computed along with a 95% confidence interval at two years post-transplant. Death will be considered as a competing risk.
- **Transplant Related Mortality (TRM):** TRM is death occurring in patients in continuous complete remission. The TRM distribution will be estimated by the cumulative incidence curve, with relapse considered a competing risk. A log-rank test will be used to compare the two treatment arms.

- **Infections:** The incidence of definite and probable viral, fungal and bacterial infections will be tabulated for each patient according to criteria in the BMT CTN Manual of Procedures. Infections will be classified by organism, site and severity. Infection burden will be compared between treatment arms.
- **Relapse:** To assess the incidence of relapse from day of transplant, the cumulative incidence of relapse, treating death as a competing risk, will be compared between the two groups using a log-rank test.
- **Immune Reconstitution:** Descriptive statistics will be computed for all the immune reconstitution assays. Treatment arms will be compared using chi-square tests, t-tests or non-parametric tests as appropriate for the assay.

5.6. Subgroup Analyses

Subgroup analyses will be performed on the following subgroups defined by age (≤ 10 years and > 10 years), donor-recipient HLA-match (5 or 6 of 6 and 4 of 6), ethnicity, and gender.

5.7. Data and Safety Monitoring Plan

All entered patients will be included in the safety analysis.

All reported serious treatment-related adverse events will be carefully examined with respect to the severity and relationship to study treatment. The reporting of serious adverse event will be consistent with standard BMT CTN procedures. Adverse events will be graded according to the NCI Common Terminology Criteria for Adverse Events (CTCAE), Version 3.0. The incidence for each reported study group associated adverse experience delineated in Section 4.2 will be presented for each group.

APPENDIX A
HUMAN SUBJECTS

APPENDIX A

HUMAN SUBJECTS

1. Subject Consent

A conference will be held with the patient and family to discuss this study and alternative treatments available for the treatment of the underlying disease. The Principal Investigator or another designated physician will conduct the conference. All potential risks associated with the use of fludarabine, total body irradiation (TBI) or cyclophosphamide should be discussed as objectively as possible.

The consent document should be reviewed with the patient and family prior to proceeding to transplantation

Informed consent from the patient will be obtained using a form approved by the Institutional Review Board of the institution enrolling the patient.

2. Confidentiality

Confidentiality will be maintained by individual names being masked and assigned a patient identifier code. The code relating the patient's identity with the ID code will be kept separately at the center. The ID code will be transmitted to the BMT CTN Data Coordinating Center upon enrollment.

3. Participation of Women and Minorities and Other Populations

Women and ethnic minorities and other populations will be included in this study. Accrual of women and minorities at each center will be monitored to determine whether their rates of enrollment are reflective of the distribution of potentially eligible women and minorities expected from data reported to the CIBMTR. Centers will be notified if their rates differ significantly from those expected and asked to develop appropriate recruitment strategies.

Since UCB transplantation allows for greater HLA disparity between recipient and donor, it is anticipated that this study will recruit more minority patients than a study using other hematopoietic cell sources (e.g., bone marrow or peripheral blood stem cells). It is expected that number of minority patients on this study will be more than accrued on studies using adult bone marrow or PBSC donors. In 2005, the National Marrow Donor Program (NMDP) reported 27% of patients receiving cord blood transplants were non-Caucasian, while only 12% of patients receiving bone marrow or PBSC transplants were non-Caucasians.

4. Ethical Considerations

Patients are referred to the Transplant Center for consideration of hematopoietic cell transplantation. While there will be every effort to seek out and include females and minority patients, the patient population is dependent upon the referral pattern and the ability to identify suitable UCB units. Female and minority patients are eligible for all aspects of the study and their participation will be actively encouraged.

APPENDIX B
CONSENT FORMS

Informed Consent to Participate in Research

MULTI-CENTER, OPEN LABEL, RANDOMIZED TRIAL COMPARING SINGLE VERSUS DOUBLE UMBILICAL CORD BLOOD TRANSPLANTATION IN PEDIATRIC PATIENTS WITH HIGH RISK LEUKEMIA AND MYELODYSPLASIA

This is a clinical trial, which is a research study to answer specific medical questions. The information from this study will help future patients. The Study doctor (the person in charge of the research) will explain the clinical trial to you and your child. Clinical trials include only people who choose to take part. Please take your time to make your decision about allowing your child to take part. You may discuss your decision with family and friends. You and your child can also discuss this with your child's health care team. If you or your child have any questions, you and your child can ask the Study doctor for more explanation.

Your child is being asked to take part in this study by your child's doctor because your child has leukemia or myelodysplastic syndrome (MDS) that has failed other treatment or your child's leukemia or MDS is not likely to respond to other treatment. These diseases can be treated and sometimes cured with very high doses of chemotherapy and radiation therapy given to kill leukemia or MDS cells. However, this treatment also harms normal cells in the bone marrow. The bone marrow is the body's "blood factory." It makes the cells that circulate in the blood, including: red blood cells (which carry oxygen), white blood cells (which fight infection), and platelets (which prevent bleeding). The bone marrow can be fixed by giving "hematopoietic or blood stem cells" donated by someone else. This is called a hematopoietic stem cell transplant. Blood stem cells are the "parent cells" of the bone marrow that produce all blood cells. For a transplant to be successful, the donor blood stem cells must have a tissue type that is completely or closely matched to the patient's tissue type. Genetic markers on the surface of cells make up our tissue type. These genetic markers are like a "finger print" and help our immune system to determine which cells belong to the body and which do not. For patients needing a transplant who do not have a family donor who is a match (has the same tissue type), blood stem cells from unrelated donors can be used.

Blood stem cells are found in bone marrow and in umbilical cord blood. Umbilical cord blood is the blood left over in the placenta (afterbirth) after a baby is born. Usually this blood is thrown out with the placenta. Over the past 15 years, we have learned how to collect and freeze cord blood cells to be used for transplants at a later time. A cord blood unit is the cord blood cells collected and stored from a single placenta. Cord blood units have been used for more than 6,500 transplants performed around the world. **The purpose of this study is to determine whether giving two units of cord blood to a patient is better than giving one cord blood unit.**

Before you decide whether or not to have your child join the study, please read the information below. Feel free to ask questions to understand your child's rights and protections. It is your choice and that of your child to take part in this study.

Sponsor and source of funding:

This study is sponsored by the National Institutes of Health (NIH), which gives financial support through the Blood and Marrow Transplant Clinical Trials Network (BMT CTN). The NIH is a government funded research program and the BMT CTN is a not-for-profit group of transplant programs conducting research on hematopoietic stem cell transplant to benefit future patients.

If you decide to allow your child to take part in this research study:

- You or your child's insurance company will pay for all medical bills for your child's treatment
- You or your child will not incur additional cost for any research lab tests that are part of this study (i.e., lab tests that are not part of routine care)
- You or your child will not be paid to participate in this study
- Your child will face the same risks and benefits as any other transplant patient

If you decide not to allow your child to participate in this study, your child's doctor will discuss other treatment options with you.

What other choices does my child have if my child does not take part in the study?

Your child's other choices may include:

- Treatment with other drugs
- Experimental treatment with drugs or cells
- A transplant with umbilical cord blood that is not part of this study
- A bone marrow transplant from a tissue-type mismatched related donor or from a tissue-type matched or mismatched adult unrelated donor
- No therapy to try to control your child's leukemia but treatment to make sure your child remains comfortable for the remainder of his or her life

Please talk to your child's doctor about your child's treatment choices before you decide to allow your child to take part in this study.

Why is this study being done?

More than 6,500 umbilical cord blood transplants have been done worldwide, mostly in children with leukemia. One important factor affecting the success of a cord blood transplant is the number of cells in the cord blood unit given divided by the recipient's weight (cell dose). Patients who receive a high cell dose ($> 2.5 \times 10^7$ cells/kilogram) have better marrow recovery and a higher rate of survival than those who receive a lower cell dose.

In an attempt to make umbilical cord blood transplantation possible for bigger children, adolescents and adults, researchers have tried giving two cord blood units on the same day for their transplant, one after the other. The data from more than 150 "double cord blood" transplants in adults suggest that the "double cord blood" transplants may allow bone marrow

recovery and survival in patients who do not have a single cord blood unit with enough cells for successful transplantation. It is not known whether giving two units will improve bone marrow recovery or survival over a single unit with a high number of cells.

What the doctors need to know from this study:

Your child's doctor and others would like to know whether giving two umbilical cord blood units will result in faster bone marrow recovery and improved survival compared to giving only one unit of umbilical cord blood that has an acceptable cell dose for your child.

How many children will take part in the study?

Two hundred and twenty (220) patients will take part in this study. Half (110) will receive two umbilical cord blood units and the other half (110) will receive a single umbilical cord blood unit. To be part of the study your child must:

- Be between 1-21 years old
- Have leukemia or MDS
- Have two suitable umbilical cord blood units available
- Have your informed consent
- Give assent if your child is old enough to understand the risks and benefits and sign an assent document

What will happen if your child takes part in this research study? Before enrolling on study:

Your child will have the following examinations, tests or procedures to determine whether your child can be included in the study. These examinations, tests or procedures are required as part of regular pre-transplant care and may be done even if you decide not to allow your child to participate in this study:

- Medical history
- Physical examination
- Blood and bone marrow tests including a bone marrow biopsy to evaluate your child's leukemia
- Other tests such as a spinal tap to evaluate your child's leukemia
- Blood tests to determine whether your child has had prior illness such as HIV, hepatitis and other viral illness
- Heart function tests
- Lung function tests
- Kidney function tests
- If your child is a teenager and female, a blood pregnancy test will be performed to make sure that your child is not pregnant. If your child is pregnant, your child will not be able to take part in this study. The study treatment could be harmful to the fetus.

Randomization:

If your child is enrolled on this study, your child will be randomized to receive either one or two umbilical cord blood units. Randomization means that your child is assigned to a group by chance. This is done using a special computer program. Your child has a 50/50 chance of being in either group. Neither you nor your doctor can choose the group. If your child is assigned to

receive a single cord blood unit, that unit will contain enough cells for bone marrow recovery and survival.

Pre-transplant:

If your child does not have a central intravenous (Hickman) line, your child's doctor will ensure that your child has a central intravenous line prior to transplant. All drugs that may be required during your transplant will be given through the central intravenous line. Blood required for any tests will also be collected from the central intravenous line in an effort to minimize pain. Starting 10 days prior to transplant your child will receive chemotherapy and total body irradiation (TBI). This is called the preparative regimen because it prepares the body to receive the donor (cord blood) cells. The preparative regimen kills leukemia/MDS cells. It also kills cells in the body that would reject the donor cells. The chemotherapy and TBI will also damage your child's normal bone marrow cells. The transplanted cord blood will replace the damaged cells.

The drugs used as chemotherapy are fludarabine and cyclophosphamide (often called by its brand name, Cytoxan). Fludarabine will be given intravenously (through your child's central line) once a day for the first 3 days. Next, TBI will be given twice a day for 4 days. Then cyclophosphamide will be given intravenously (through your child's central line) once a day for 2 days. This will be followed by a "rest day" when your child will not receive chemotherapy or radiation.

Three days before the transplant, and continuing in the weeks after the transplant, your child will receive drugs to allow the umbilical cord blood stem cells to grow up inside your child's body. These drugs decrease the chance of a complication known as graft-versus-host disease (GVHD). GVHD results when the cord blood cells recognize your child's body as foreign and attack it. Your child will receive two standard drugs to prevent GVHD. These drugs are called cyclosporine (also called Gengraf or Neoral) and mycophenolate mofetil (also called MMF or Cellcept).

Transplant day:

On the day of transplant your child will receive either one or two umbilical cord blood units, given intravenously through the central intravenous line. Whether your child receives one or two umbilical cord blood units is decided at the time of randomization. The date of transplant is referred to as Day 0.

Post-transplant follow-up and care:

To speed the recovery of blood cells as much as possible your child will receive granulocyte-colony-stimulating factor (G-CSF or Neupogen). G-CSF is a hormone that tells the bone marrow to make white blood cells. Your child will start receiving growth factor the day after transplant. Your child will continue to receive it daily until his or her white blood cell count recovers.

After your child's transplant, your child will be watched very closely. He or she will have a physical examination and blood tests at least twice weekly. Additional blood tests and bone

marrow tests will be done if your child's doctor thinks it is indicated to take care of your child. Samples of blood (up to 75 ml or 5 tablespoons) and bone marrow (up to 15 ml or 1 tablespoon) will be drawn to evaluate how the new marrow is functioning. The blood tests will be done on Days 28, 42, 60, 100, 180, 1 year, and 2 years after transplantation (Day 0 is the day of transplant and all days are counted from the day of transplant, for example: Day 28 is the 28th day after transplant). If necessary, bone marrow tests will also be done. These examinations and tests are part of regular care after transplant and may be done even if you do not allow your child to join the study and may be done more frequently than described here if necessary for your child's care.

As part of the study your child will have blood samples drawn to evaluate the function of his or her immune system at Day 100, Day 180, 1 and 2 years after transplant. As part of the study, your child will receive tetanus vaccinations at Day 100, 6 months and 12 months. Tetanus re-vaccinations are done routinely after transplantation, since immunity from vaccinations given before the transplant is lost. In this study, your child will be vaccinated earlier than transplant patients are usually vaccinated after their transplant because we want to evaluate how the immune system is functioning and to determine whether vaccination protection can be effective sooner than generally believed. There is no additional risk to receiving this vaccine other than the risk of a tetanus injection.

Your child will be discharged from the hospital when your child's doctor feels he/she is ready. At first, your child will need to visit the bone marrow transplant clinic several times a week for check ups. Eventually, the visits will be less frequent. Your child's doctor will likely want to see your child at 6 months, 1 year, and 2 years after the transplant as part of your child's care after a transplant. In some cases, it may be necessary for your child to visit the transplant clinic more frequently and your child's doctor will determine this. Blood tests other than those mentioned above may also be necessary. Your child's doctor will make this decision.

Follow up for your child's transplant will last as long as your child requires care related to the transplant and afterwards for any of the late complications that may occur from chemotherapy or transplant. We encourage patients who have had a transplant to be followed either by their transplant doctor or another doctor who is familiar with late complications that may arise from transplantation. We would like to keep track of your child's medical condition for the rest of your child's life. We will do this by contacting you (or your child) and the doctor providing your child's regular medical care by phone or mail once a year. Checking on your child's condition every year helps us look at the long-term effects of the study and transplantation in general. Many transplant centers include this type of long-term follow-up as part of their regular medical care. However, it is not necessary for you to agree to follow-up of your child for longer than 2 years in order to participate in this study. If you would like to learn more about long-term care, please discuss it with your child's doctor.

How long will your child be in the study?

Your child will be in the study for 2 years. Your child's doctor (the doctor taking care of your child during his/her transplant or your child's oncologist or your child's primary care doctor), however, will follow your child indefinitely and provide us with information as described above.

Please notify your child's transplant doctor if you move or change your child's primary care doctor so that we will be able to obtain all the information requested.

Can your child stop being in the study?

You can decide to stop your child's participation at any time. Tell your child's doctor if you or your child are thinking about stopping or decide to stop. The doctor will tell you and your child how to stop safely. It is important to tell your child's doctor if you or your child are thinking about stopping so any risks from the medications can be evaluated. Another reason to tell your child's doctor is to discuss what follow-up care and testing could be most helpful for your child.

If you decide to withdraw your child, or your child's doctor withdraws your child from the study, we will ask your permission to use all the information about your child that has already been collected as part of the study and to continue to allow your child's doctor to tell us about your progress until at least two years post-transplant. You can choose to grant or not to grant this permission.

Can the doctor who is the Principal Investigator withdraw your child (you) from this study?

Your child can be taken off the study (with or without your consent) for any of the following reasons:

- Your child needs a medical treatment not allowed in this study
- The investigator decides that continuing in the study would be harmful to your child
- Your child becomes pregnant and the study treatment could be harmful to the fetus
- The study is cancelled by the Food and Drug Administration (FDA) or the National Institutes of Health (NIH)

What are the risks of being in this study?

So far, none of the studies using two cord blood units for transplantation show risks that are different from using a single cord blood unit for transplant. However, it is possible that there are new and unknown risks from receiving two cord blood units instead of one. For example, because the double cord transplant gives more donor cells, the frequency or severity of GVHD might be increased. Also, with two cord blood donors, each donor's cells could react against the other, rather than make new healthy bone marrow for the patient. While early studies of double cord blood transplants do not show a risk of either of these problems, we will be monitoring patients carefully during this study to be sure that they do not occur when a larger group of patients are treated.

If your child develops GVHD, your child's doctor will treat the GVHD with the best available treatment. If your child's bone marrow does not grow back as expected, your child's doctor will discuss it with the Principal Investigators of this study and the BMT CTN. We would try to identify another cord blood unit to be used for a second transplant if that is felt to be a good treatment.

Chemotherapy drugs and total body irradiation (TBI) used in this study:

The standard approach for treating patients with leukemia and/or MDS is to give high doses of cyclophosphamide (Cytosan) and TBI, followed by a transplant using stem cells from donor bone marrow or cord blood. This treatment can cure patients who have these diseases. However, this treatment can also cause short and long-term side effects which can be uncomfortable, and in some cases, dangerous, life threatening, or even fatal (see RISKS AND TOXICITIES RELATED TO STANDARD TRANSPLANT PROCEDURES below). In this study, in addition to TBI and cyclophosphamide, fludarabine is also given in an attempt to improve recovery after cord blood transplants. All patients receiving transplants must also receive GVHD prophylaxis (i.e., drugs to prevent GVHD). The drugs used to prevent GVHD are cyclosporine (CSA) and mycophenolate mofetil (MMF). These drugs are usually well tolerated, but they can cause serious side effects. The most common and most important of these side effects are listed below. Since this combination of treatments is relatively new, there may be additional unexpected side effects.

Everyone taking part in the study will be carefully monitored for side effects. Side effects can be mild or very serious. Your child's doctor and the health care team will give you medications to help lessen the risk of side effects, and make your child more comfortable if they occur. In most cases, the side effects are temporary and reversible. In some cases, side effects may be serious, last a long time or never go away. You should talk to your child's doctor about any side effects that your child has while taking part in this study. A group of experts will carefully watch the side effects experienced by patients on this study. If unexpected, dangerous complications are reported, and the experts determine that these side effects might occur in other patients on the study, you and your doctor will be notified and the study closed. Your child will continue to receive all the care related to his/her transplant.

There are other potential complications that all patients undergoing a transplant face, regardless of whether or not they participate in the study. Be sure to ask your doctor to discuss those with you.

Are there benefits to taking part in the study?

This research study is to determine whether giving two umbilical cord blood units results in faster bone marrow recovery and improved survival compared to giving only one umbilical cord blood unit. At this point doctors do not know whether two umbilical cord blood units are better than one umbilical cord blood unit. The information obtained from this study will help doctors treat future patients with leukemia who require an umbilical cord blood transplant. If one group does better than the other group, and your child is randomly assigned to that group, your child may benefit from being in the study. Beyond that, there are no other specific benefits for your child from taking part in this study.

POTENTIAL SIDE EFFECTS OF STUDY DRUGS

Cyclophosphamide

<p>Likely <i>("Likely" refers to a side effect that is expected to occur in more than 20% of patients.)</i></p>	<p>Less Likely <i>("Less likely" refers to a side effect that is expected to occur in 20% or fewer patients.)</i></p>	<p>Rare, but Serious <i>(These possible risks have been reported in rare occurrences, typically less than 2% of patients. They may be serious if they occur.)</i></p>
<ul style="list-style-type: none"> • Decreased white blood cell count with increased risk of infection • Temporary hair loss • Nausea • Vomiting • Loss of appetite • Sores in mouth or on lips • Diarrhea • Stopping of menstrual periods in women • Decreased sperm production in men 	<ul style="list-style-type: none"> • Decreased platelet count (mild) with increased risk of bleeding • Blood in urine • Temporary darkening of nail beds • Acne • Temporary tiredness • Damage to the fetus if your child becomes pregnant while taking cyclophosphamide 	<ul style="list-style-type: none"> • Scarring of lung tissue, with cough and shortness of breath • Severe heart muscle injury and death

Fludarabine

<p>Likely <i>("Likely" refers to a side effect that is expected to occur in more than 20% of patients.)</i></p>	<p>Less Likely <i>("Less likely" refers to a side effect that is expected to occur in 20% or fewer patients.)</i></p>	<p>Rare, but Serious <i>(These possible risks have been reported in rare occurrences, typically less than 2% of patients. They may be serious if they occur.)</i></p>
<ul style="list-style-type: none"> • Decreased white blood cell count with increased risk of infection • Decreased platelet count with increased risk of bleeding • Tiredness • Nausea • Vomiting 	<ul style="list-style-type: none"> • Pneumonia • Diarrhea 	<ul style="list-style-type: none"> • Numbness and tingling in hands and/or feet related to irritation of nerves of the hand and/or feet • Changes in vision • Agitation/nervousness • Confusion • Cough • Difficulty breathing • Weakness • Severe brain injury and death

Cyclosporine

<p>Likely <i>(“Likely” refers to a side effect that is expected to occur in more than 20% of patients.)</i></p>	<p>Less Likely <i>(“Less likely” refers to a side effect that is expected to occur in 20% or fewer patients.)</i></p>	<p>Rare, but Serious <i>(These possible risks have been reported in rare occurrences, typically less than 2% of patients. They may be serious if they occur.)</i></p>
<ul style="list-style-type: none"> • High blood pressure • Kidney problems • Headaches • Nausea • Vomiting • Stomach pain or indigestion • Swelling of the hands or feet. 	<ul style="list-style-type: none"> • Tremors • Increased hair growth 	<ul style="list-style-type: none"> • Muscle cramps • Numbness and tingling of the hands or feet • Seizure

G-CSF

<p>Likely <i>(“Likely” refers to a side effect that is expected to occur in more than 20% of patients.)</i></p>	<p>Less Likely <i>(“Less likely” refers to a side effect that is expected to occur in 20% or fewer patients.)</i></p>	<p>Rare, but Serious <i>(These possible risks have been reported in rare occurrences, typically less than 2% of patients. They may be serious if they occur.)</i></p>
	<ul style="list-style-type: none"> • Local irritation (skin) at injection site • Ache or pain inside the bones, increased levels of liver enzymes and uric acid in the blood, low number of platelets in the blood 	<ul style="list-style-type: none"> • Allergic reaction, low fever • Enlargement or rupture of the spleen • Worsening of pre-existing skin rashes • Temporary hair loss • Inflammation of a blood vessel in the skin

Mycophenolate Mofetil

<p>Likely <i>(“Likely” refers to a side effect that is expected to occur in more than 20% of patients.)</i></p>	<p>Less Likely <i>(“Less likely” refers to a side effect that is expected to occur in 20% or fewer patients.)</i></p>	<p>Rare, but Serious <i>(These possible risks have been reported in rare occurrences, typically less than 2% of patients. They may be serious if they occur.)</i></p>
<ul style="list-style-type: none"> • Diarrhea • Stomach pain • Upset stomach • Vomiting • Difficulty falling asleep or staying asleep 	<ul style="list-style-type: none"> • Pain, especially in the back, muscles, or joints • Constipation 	<ul style="list-style-type: none"> • Swelling of the hands, feet, ankles, or lower legs • Difficulty breathing • Shaking hands that you cannot control • Unusual bruising or bleeding • Headache • Fast heartbeat • Excessive tiredness • Dizziness • Pale skin • Weakness • Blood in stools • Bloody vomit • Loose, floppy muscles • White patches in mouth or throat • Swelling of gums • Vision changes • Rash • Low blood counts • Damage to unborn baby • Limited effectiveness of birth control • Progressive Multifocal Leukoencephalopathy

Total body Irradiation (TBI):

<p>Likely <i>(“Likely” refers to a side effect that is expected to occur in more than 20% of patients.)</i></p>	<p>Less Likely <i>(“Less likely” refers to a side effect that is expected to occur in 20% or fewer patients.)</i></p>	<p>Rare, but Serious <i>(These possible risks have been reported in rare occurrences, typically less than 2% of patients. They may be serious if they occur.)</i></p>
<ul style="list-style-type: none"> • Diarrhea • Nausea • Stomach cramps • Vomiting (throwing up) • Painful swelling of the salivary glands under the ears for a few days • Short-term hair loss • Anemia • Infection • Bleeding • Cataracts • Sterility (inability to have children) • Slow growth • Hormone problems (such as thyroid disease or diabetes) • Mouth sores 	<ul style="list-style-type: none"> • Lung inflammation • Pneumonia • Redness of the skin • Serious liver problems 	<ul style="list-style-type: none"> • Risk of developing other cancers in the future • Difficulty swallowing • Back problems • Kidney problems • Learning problems

Most of the problems described above that we anticipate to be common are temporary and treatable.

RISKS AND TOXICITIES RELATED TO STANDARD TRANSPLANT PROCEDURES

Risks of Cord Blood Transplantation

The following problems may occur as a result of the transplantation of umbilical cord blood. These are risks that would be present whether such a transplant was done as part of a study or not:

1. **Slow Recovery of Blood Counts.** The red blood cells, white blood cells and platelets can be slow to recover after umbilical cord blood transplantation. Until your child’s blood counts recover, he or she will need blood and platelet transfusions, and will be at

risk for bleeding and infections. Although infections can be treated with drugs, they can be very dangerous or fatal. To speed the recovery of the white cells as much as possible your child will receive growth factor, a hormone that tells the bone marrow to make white blood cells. This drug is very safe but can cause fever, bone pain, feeling tired and, very rarely, allergic reactions.

2. **Graft Failure.** The umbilical cord blood stem cells (the “graft”) may fail to grow inside your child’s body. Past experience suggests that there can be up to a 20% chance for failure to graft. If graft failure occurs, this will result in low blood counts for a long period of time and can be fatal. Should this happen, your child will not receive additional stem cells from the same cord blood donor. However, your child may be able to receive a second transplant with stem cells from another person (e.g., a different umbilical cord blood donor or an adult donor).
3. **Graft-versus-host Disease (GVHD).** This condition results from the umbilical cord blood cells recognizing your child’s body as foreign and attacking it. You are more likely to get GVHD if you receive a mismatched (tissue type) than a matched cord blood unit. In most cases, GVHD can be successfully treated. Sometimes GVHD is severe or difficult to treat and may lead to death. Your child will be watched closely for this complication and given treatment to prevent and treat it.

There are two forms of GVHD: acute GVHD (occurs in the first 3 months after transplant) and chronic GVHD (after the first 3 months). Acute GVHD may produce skin rash, nausea, vomiting, diarrhea, abdominal pain, abnormalities of liver function and an increased risk of infection. Chronic GVHD may produce skin rashes, hair loss, thickened skin, dry eyes, dry mouth, liver disease, weight loss, diarrhea and an increased risk of infection. To confirm the diagnosis of acute or chronic GVHD, your child may be asked to have a skin biopsy (i.e., taking a small sample of tissue to look at under the microscope) and possibly a gut biopsy and rarely a liver biopsy.

4. **Genetic Disease within the Cord Blood Cells.** It is possible that certain genetic diseases (for example thalassemia or immunodeficiency) may be passed through the umbilical cord blood stem cells. While these diseases are very rare, each umbilical cord blood can only be tested for a few of the many possible genetic diseases. To reduce this possibility, cord blood is not collected from babies that have genetic diseases running in their family.
5. **Incorrect Labeling of the UCB.** Though rare, it is possible that incorrect labeling of an umbilical cord blood unit could occur so that your child receives the wrong unit. To avoid this, umbilical cord blood unit is re-typed to ensure that the tissue type of the donor and your child are as previously reported (i.e., when the donor unit is confirmed). If the umbilical cord blood unit does not have an attached segment for us to re-type, there are several ways the unit labeling can be confirmed.

6. **Other Complications.** Other complications that can result from the transplantation procedure not specifically related to one specific drug or the cord blood stem cells or this study include:
- A. **Damage to the vital organs in your body.** This could result in problems in any body organ, such as, heart, lungs, liver, gut, kidneys and bladder, brain etc. The lungs and the liver are particularly vulnerable. Some patients will experience severe lung problems due to infections and/or due to a reaction of the lungs to the chemotherapy and radiation. Some patients can suffer veno-occlusive disease of the liver (VOD). This complication results from high doses of chemotherapy and/or radiation. Patients with VOD become jaundiced (yellowish skin), have liver function abnormalities, abdominal swelling, and abdominal pain. Although many patients recover completely, these complications may cause permanent damage or even death.
 - B. **Serious infections.** Full and complete recovery of your child's immune system may take many months following the initial recovery of your child's cell counts. During this time, there is an increased risk of infections. Your child will be prescribed certain medications to reduce the chance of those infections. However, preventative treatments are not always effective. If your child has an infection, he/she may have to stay in the hospital longer or be re-hospitalized after transplant. Although most infections can be successfully treated, some infections are fatal.
 - C. **Recurrence of disease.** Your child's leukemia or MDS may come back even if the transplant is initially successful.
 - D. **Risk to the unborn.** The treatments in this study have NOT been proven to be safe at any stage of pregnancy. Therefore, if your child is pregnant or nursing, your child is not eligible for this study. Women who have the potential of becoming pregnant must use some form of effective birth control while receiving chemotherapy, TBI and GVHD prophylaxis. Effective birth control is defined as the following: 1) refraining from all acts of vaginal intercourse (ABSTINENCE); 2) consistent use of birth control pills; 3) injectable birth control methods (Depo-Provera, Norplant); 4) tubal sterilization or male partner who has undergone a vasectomy; 5) placement of an IUD (intrauterine device); and, 6) use, with every act of intercourse, of a diaphragm with contraceptive jelly and/or condoms with contraceptive foam.
 - E. **Sterility and future childbearing potential for men and women.** Chemotherapy and/or irradiation may affect your child's ability to have children. Male patients are likely to become sterile (unable to produce sperm) and should discuss with their doctor regarding sperm banking prior to transplantation. Female patients who have attained puberty may find that their menstrual cycle becomes irregular or stops permanently. However, this DOES NOT MEAN THAT YOUR CHILD CANNOT BECOME PREGNANT, and your child must use some effective method of birth control (if she has a sexual partner) during transplant and afterwards until she is off GVHD prophylaxis. Damage to reproductive tissue may result in infertility (inability

to have children). It is not known if the damage could result in birth defects. You and your child should discuss these risks and options in detail with your child's doctor before entering this study.

- F. **Central venous catheter.** Central venous catheters are intravenous (IV) lines that are placed under the skin and in a large vein in the chest and which may remain in place for many months. Central venous catheters are used to draw blood and administer fluids and medicines. There is considerable experience with central venous catheter use. The most common complications associated with central venous catheters are blood clots in the catheter and an infection where the catheter was inserted which can sometimes lead to a generalized infection in the blood. Clotting may require the catheter to be removed or treatment with a fibrinolytic agent (medicines that dissolve blood clots). Sometimes if a blood clot occurs, the catheter may need to be replaced. Infections will be treated with drugs; sometimes, removal of the infected catheter is required and a new catheter will be placed. There is also a small risk of puncturing the lung at the time the catheter is put in. If this occurs, a temporary chest tube may be placed in the lung to re-inflate it. There are no long-term effects once the lung puncture has repaired.

What are the costs of taking part in this study?

Most of what happens in this study is standard care; it will be billed to you or your child's insurer in the usual way. Standard costs include those of your child's hospitalization, doctor's visits, standard laboratory tests, the radiation therapy, the drugs, and the cost of the umbilical cord blood unit(s). There will be no charge for research tests. In the event that this research activity results in an injury, treatment will be available, including first-aid, emergency treatment and follow-up care as needed. Care for such injuries will be billed in the ordinary manner, to your child's insurance company. If you or your child thinks that your child has suffered a research-related injury, let the study doctors know right away.

What happens if your child is injured because of participation in this study?

It is important that you tell your child's doctor, _____ [investigator's name], if you or your child feel that your child has been injured because of taking part in this study. You can tell the doctor in person or call him/her at _____ [telephone number]. Your child will receive medical treatment if injured as a result of taking part in this study. You or your child's insurance will be charged for this treatment.

What are your child's rights if your child takes part in this study?

You may choose to allow your child to either take part or to not take part in the study. If you decide to allow your child to take part in this study, your child may leave the study at any time. No matter what decision is made, there will be no penalty and your child will not lose any of his or her regular benefits. If your child leaves the study, he/she can still get medical care from your child's doctor and transplant center. We will tell you and your child about new information or changes in the study that may affect your child's health or your child's willingness to continue in the study. In the case of injury resulting from this study, your child does not lose any legal rights to seek payment by signing this form.

Who can answer your (and your child's) questions about the study?

You and your child can talk to your child's doctor about any questions or concerns about this study. Contact your child's doctor _____ [name(s)] at _____ [telephone number].

For questions about your child's rights while taking part in this study, call the _____ [name of center] Institutional Review Board (a group of people who review the research to protect your child's rights) at _____ (telephone number).

Will your child's medical information be kept private?

We will do our best to make sure that the personal information in your child's medical record be kept private. However, we cannot guarantee total privacy. Your child's personal information may be given out if required by law. If information from this study is published or presented at scientific meetings, your child's name and other personal information will not be used.

Organizations that may look at and/or copy your medical records for research, quality assurance, and data analysis include:

- Members of the Blood and Marrow Transplant Clinical Trials Network, which is conducting this study
- The EMMES Corporation, a research organization that is helping to coordinate this study
- The National Marrow Donor Program and the Center for International Blood and Marrow Transplant Research, organizations involved in research on blood and marrow transplantation and in the coordination of this study
- The National Heart Lung, and Blood Institute (NHLBI), the National Cancer Institute (NCI) and other government agencies, like the Food and Drug Administration (FDA), involved in keeping research safe for people
- The Children's Oncology Group (COG), a clinical trials cooperative group

Expiration date for retention of records:

The study results will stay in your child's research record at (*insert Institution*) for at least six years or until after the study is completed, whichever is longer. At that time either the research information not already in your child's medical record will be destroyed or your child's name and other identifying information will be removed from such study results. Research information in your child's medical record will be kept indefinitely.

How will the researcher(s) benefit from your child being in this study?

In general, presenting research results helps the career of a scientist. Therefore, the Principal Investigator may benefit if the results of this study are presented at scientific meetings or in the scientific press. In addition, the sponsor (the NIH) is paying the Principal Investigator to conduct this study. The investigators have no financial interest in the drugs used in the study.

HIPAA¹ authorization to use and disclose individual health information for research purposes:

- a. Purpose: As a research participant, I authorize the Principal Investigator and the researcher’s staff to use and disclose my child’s individual health information for the purpose of conducting the research study entitled *Multi-Center, Open-Label, Randomized Trial Comparing Single Versus Double Umbilical Cord Blood (UCB) Transplantation in Pediatric Patients with High Risk Leukemia and Myelodysplasia*.
- b. Individual Health Information to be Used or Disclosed: My child’s individual health information that may be used or disclosed to conduct this research includes: demographic information (e.g., age, date of birth, sex, weight), medical history (e.g., diagnosis, complications with prior treatment), physical examination findings, and laboratory test results obtained at the time of work up and after transplantation (e.g., blood tests, biopsy results). The identities of individuals such as names and addresses will not be shared.
- c. Parties Who May Disclose My Child’s Individual Health Information: The researcher and the researcher’s staff may obtain my child’s (my) individual health information from:
(list: hospitals, clinics or providers from which health care information can be requested)

- d. Parties Who May Receive or Use My Child’s Individual Health Information: The individual health information disclosed by parties listed in item c and information disclosed by my child during the course of the research may be received and used by the following parties:
 - Members of the BMT CTN Data and Coordinating Center and BMT CTN #0501 Protocol Team
 - National Heart, Lung, and Blood Institute (NHLBI) and the National Cancer Institute (NCI), both of the National Institutes of Health (NIH), study sponsors
 - The National Marrow Donor Program and the Center for International Blood and Marrow Transplant Research
 - U.S. government agencies that are responsible for overseeing research such as the Food and Drug Administration (FDA) and the Office of Human Research Protections (OHRP)

1 HIPAA is the Health Insurance Portability and Accountability Act of 1996, a federal law related to privacy of health information

- U.S. government agencies that are responsible for overseeing public health concerns such as the Centers for Disease Control (CDC) and federal, state and local health departments
- Cord Blood Banks providing units
- Children’s Oncology Group, a clinical trials cooperative group
- Other:

- e. Right to Refuse to Sign this Authorization: I do not have to sign this Authorization. If I decide not to sign the Authorization, my child will not be allowed to participate in this study or receive any research-related treatment that is provided through the study. However, my decision not to sign this authorization will not affect any other treatment, payment, or enrollment in health plans or eligibility for benefits.
- f. Right to Revoke: I can change my mind and withdraw this authorization at any time by sending a written notice to the Principal Investigator to inform the researcher of the decision. If I withdraw this authorization, the researcher may only use and disclose the protected health information already collected for this research study. No further health information about my child (me) will be collected by or disclosed to the researcher for this study.
- g. Potential for Re-disclosure: My child’s individual health information disclosed under this authorization may be subject to re-disclosure outside the research study and no longer protected. Examples include potential disclosures for law enforcement purposes, mandated reporting or abuse or neglect, judicial proceedings, health oversight activities and public health measures.
- h. This authorization does not have an expiration date. However, you can elect at any time to withdraw your authorization to participate in the study.

You will receive a copy of this form. If you (or your child) need more information about this study, ask the study doctor.

Storage of blood for future research:

Please note: This section of the informed consent form is about future research studies that will be done using blood samples from children who are taking part in the main study described above. Your child may give samples for these future research studies if you want to. You can say "yes" or "no" to allowing blood samples for future research studies. You can still be a part of the main study even if you say 'no' to allowing these samples to be used for future research studies. Please mark your choice at the end of this section.

Your child will be asked to provide samples of blood to be used for research to determine how the immune system is recovering after transplant. These samples will not require additional procedures. The samples will be taken from blood drawn normally for other tests and will not require additional needle sticks. Your child’s name will not be on these samples. You do not have to agree to provide these research samples from your child in order for your child to participate in the study.

Samples will be labeled with unique codes that do not contain information that could identify your child. A link to this code does exist. The link is stored at the Data Coordinating Center for the Blood and Marrow Transplant Clinical Trials Network (BMT CTN). The staff at the laboratories where your samples are being tested do not have a link to this code. Your samples will be stored at these laboratories until the entire sample has been used for the research tests or until the end of the study.

If you agree, we will collect your child’s (your) blood at Day 100, 6 months, 12 months and 24 months after transplant (approximately 15 mL, 1 tablespoon, each time) for immune recovery studies.

Things to think about regarding use of blood for immune recovery studies:

The choice to let us have a blood samples for immune recovery studies is up to you (and your child). No matter what you decide to do, it will not affect your child’s care. Even if you decide now that your child’s blood sample can be kept for studies, you (or your child) can change your mind later. If this is the case, tell us that you are no longer interested in allowing us to use your child’s blood sample for research. We will then destroy the blood samples.

Benefits: The benefits of research using blood include learning more about what causes cancer and other diseases, what causes complications after transplantation and how to prevent and how to treat them.

Risks: The main risk to you is the release of information from your health records. We will do our best to make sure that your personal information will be kept private. The chance that this information will be given to someone else is very small.

Making Your Choice: Please read each sentence below and think about your choice. After reading each sentence, please indicate your choice below. If you have any questions, please talk to your doctor or nurse, or call our research review board at _____.

No matter what you decide to do, it will not affect your care.

- Yes, I agree to allow my child’s blood used for future research.
- No, I do not agree to have my child’s blood used for future research.

Signature

Date

SIGNATURE

I have read the information in this consent form and have had the study explained to me. My questions have been answered to my satisfaction. I agree to allow my child to participate in the study.

Signature of Subject's Mother/Guardian

Date

Printed Name of Subject's Mother/Guardian

Signature of Subject's Father/Guardian

Date

Printed Name of Subject's Father/Guardian

Signature of Patient/Study Subject (if greater than or equal to 18 years of age)

Printed Name of Patient/Study Subject (if greater than or equal to 18 years of age)

Signature of Physician Obtaining Consent

Date

Printed Name of Physician Obtaining Consent

ASSENT FORM

Multi-Center, Open-Label, Randomized Trial Comparing Single versus Double Umbilical Cord Blood Transplantation in Pediatric Patients with High-Risk Leukemia and Myelodysplasia

You have leukemia. Leukemia is cancer of the blood cells made in your body's "blood factory", which is called the bone marrow. Leukemia is treated with special medicines. These medicines are called chemotherapy. They kill cancer cells. If chemotherapy doesn't kill all of the cancer cells, a special and stronger treatment called a transplant may be needed.

During a transplant, you get a very large amount of chemotherapy medicines and radiation therapy. These kill the leukemia in your body. The chemotherapy drugs you will receive are so strong that they also kill the normal cells in your blood and bone marrow. To make your bone marrow grow new, healthy cells, you are given a transplant from a donor. The cells in the transplant travel to your bone marrow and grow new cells. Your doctors think that a transplant is the best treatment for you. They believe that it will increase your chance of cure.

Cells from the donor's bone marrow or cord blood can be used in a transplant. Bone marrow cells are donated by volunteers. Bone marrow donors have to have the same kind of bone marrow as the patient. If your doctor cannot find a bone marrow donor for you, you can be transplanted with blood cells from a baby's umbilical cord. Umbilical cord blood is the extra blood left over after a baby is born. It used to be thrown away. We know now that it contains blood-forming cells like the ones found in bone marrow. Cord blood can be collected after a baby is born and stored for future use. Collecting cord blood does not hurt the baby or Mom. When a patient, like you, needs a transplant, cord blood can be removed from storage and sent to your hospital for your transplant. There have been many transplants using umbilical cord blood. Sometimes the transplants grow back too slowly or not at all. In this study, your doctors are trying to figure out if the transplant will grow back faster if blood cells from two umbilical cords are used instead of one umbilical cord. They don't know the answer. They need to do this study to find out whether two cord blood units are better than one.

If you agree to participate the following will occur:

1. Your doctor will check to make sure that there are two umbilical cord blood donors available for you.
2. Then a computer program will decide whether you will get one or two cord blood units. This is called randomization. Randomization is like flipping a coin. You have the same chance of receiving one or two cord blood units. By assigning treatment this way, your doctors will be able to learn which of these treatments is better.

Transplant Procedure

Before the transplant, you will be given the drugs cyclophosphamide and fludarabine. These drugs will be given through a central line – an IV that will be placed in your chest. If you do not already have a central line, we will put one in as a surgical procedure (you will be asleep for

this). A central line makes it easier for you to receive drugs and for drawing blood for tests (you will not be poked for blood or receive shots). You will also get radiation to your whole body twice a day for four days. After you have received these drugs and radiation, new blood cells from cord blood will be given through your central line. When the blood gets into your body, you may feel sick to your stomach but that will go away quickly. You will be in the hospital for about four weeks after the cord blood cells are given to you while we are waiting for the cord blood cells to grow up inside your body and for you to recover from the chemotherapy and radiation. You will need to be on a number of medications during your transplant, which will either be given through your line or will be taken by mouth.

It will be necessary to check your blood and bone marrow after the transplant to make sure the cord cells are growing in your body. Your doctors will do blood tests and bone marrow tests. Blood tests will also be done by taking blood through your line.

Risks/Discomforts

The drugs and radiation may cause hair loss, nausea and vomiting, and diarrhea. Your blood counts will fall and you may get fevers, infections or start bleeding. You may also get mouth sores. These are temporary and you will feel better as your new bone marrow grows.

During the period your new bone marrow is growing back after the cord blood transplant, you may need to get antibiotics since you will not be able to fight infections. You may also need to get blood transfusions since your new bone marrow will not be making new blood cells right away. It is possible that your new bone marrow will not grow back. This is unlikely but if it did happen, it may even be necessary to do a second transplant. You may get graft-versus-host disease (GVHD), which happens when transplanted cells attack your body causing skin rash, vomiting, diarrhea and liver problems. These problems could be mild, or they could be very serious. Your doctors will do their best to make you feel better and keep you safe.

The above information has been explained to me. My questions have been answered.

I agree to participate in this study.

Patient

Parent

Physician

Witness

Date

APPENDIX C
LABORATORY PROCEDURES

APPENDIX C

LABORATORY PROCEDURES

1. HLA TYPING

Before Transplantation: HLA typing will be performed for all patients and donors in American Society of Histocompatibility and Immunogenetics (ASHI)-approved laboratories designated by the transplant centers. HLA typing must be performed by DNA methods for HLA-A, and -B, at intermediate resolution, and DRB1 at high resolution, consistent with NMDP standard procedures.

After Transplantation: High resolution HLA typing of cryopreserved patient and UCB samples (from the wash or unused attached segments) is conducted as an ongoing research study by the NMDP. Data will be shared with the BMT CTN.

2. CHIMERISM

Samples of peripheral blood or marrow are collected from the patient and samples from the UCB pre-transplant for chimerism studies according to institutional standards. Patient samples are also collected on Day 28, 42, 60, 100, 180 and 365 post-transplant. Chimerism will be measured by RFLP or microsatellite. On Day 28, chimerism tests will be performed on total, lymphoid and myeloid fractions. At all other time points, only total chimerism is required.

3. PATHOLOGY/CYTOGENETICS STUDIES

A Day 21 bone marrow biopsy/aspirate is required if WBC < 500. Flow cytometry and cytogenetics are required for aspirate. Pathology and cytogenetics studies conducted as per institutional guidelines.

4. IMMUNE RECONSTITUTION – STANDARD OF CARE

While the kinetics of immune reconstitution following peripheral blood and marrow transplantation have been extensively studied, there are limited data on immunologic recovery after UCB transplantation in children. The immune reconstitution studies listed in the table below allow assessment of humoral immunity (total immunoglobulin levels and post-immunization antibody titers) and cellular immune recovery (lymphocyte subset analysis to quantify numbers and proportions of different lymphocyte subpopulations and assessment of lymphocyte function by measuring proliferative responses to tetanus after UCB transplantation. Antigen specific responses have been noted as early as 6-8 weeks post cord blood

transplantation. Patients will thus be immunized at 100 days, and reimmunized at 6 and 12 months following transplantation.

The following standard of care (SOC) tests will be performed by the local institution at the time points shown in the table below.

STANDARD OF CARE TESTS

	100 Days	6 Months	12 Months	24 Months	SOC or Research
Tetanus Immunizations	X	X	X		SOC
Immunophenotyping Lymphocyte subsets: CD3, CD4, CD8, CD19, CD16/56	X	X	X	X ¹	SOC
Immunoglobulin levels ² : IgG, IgA, IgM, IgE		X	X	X ¹	SOC

¹ These tests are only required if the 12 months tests were abnormal or the patient has developed chronic GVHD in the last year.

² Data forms will capture the use of IVIG or other immune globulin products (e.g., Cytogam, Respigam) in the previous two months and the last day of administration of IVIG/Ig.

5. IMMUNE RECONSTITUTION – INVESTIGATIONAL RESEARCH ASSAYS

One of the questions to be addressed by this trial is whether transplantation of two UCB units will result in faster immune reconstitution than a single UCB unit. We plan to compare the tempo of immune recovery in recipients of single vs. double UCB units using the results of standard (described above) and investigational immunologic assays.

Analysis of the COBLT immune reconstitution data suggests a strong association between immune reconstitution and decreased leukemia relapse and improved survival in children with acute leukemia. In order to dissect the mechanisms underlying this, the following assays of immune reconstitution will be performed. Cellular immune reconstitution will be studied by the evaluation of thymopoiesis (by assessing numbers and proportions of subpopulations of naïve/memory CD4 cells, quantifying T-cell receptor excision circles or TRECs and T cell receptor repertoire analysis) and the assessment of recovery of T cell responsiveness to specific antigens such as herpes viruses.

The following investigational assays will be performed by central laboratories at the time points shown in the table below.

INVESTIGATIONAL RESEARCH ASSAYS

	100 Days	6 Months	12 Months	24 Months	SOC or Research
Tetanus and PRP titers	X	X	X	X	Research
T cell Receptor Excision circles (TREC)	X	X	X	X	Research
T cell responses to HSV, CMV, VZV antigens and Tetanus	X	X	X ¹	X	Research

¹ If results for tetanus response are abnormal, repeat at 15 months for T cell blastogenesis (i.e., not antibody titers).

TABLE C-1: SCHEDULE OF LABORATORY EVALUATIONS

Type of Sample	Method	Type of Storage	Dates Samples Obtained	Shipping Specifications	Test Location
HLA Typing	According to institutional practice	According to institutional practice	Any time during preparation for transplant.	N/A	Transplant Center
Chimerism	According to institutional practice	According to institutional practice	Less than four weeks before initiation of conditioning therapy and on Day 28, 42, 60, 100, 180 and 365 post-transplant.	N/A	Transplant Center
Pathology/ Cytogenetic Studies	According to institutional practice	According to institutional practice	Less than four weeks before initiation of conditioning therapy. A Day 21 sample is collected if WBC < 500.	N/A	Transplant Center

Immune Reconstitution – Standard of Care Tests

Immunophenotyping Lymphocyte subsets: CD3, CD4, CD8, CD19, CD16/56	According to institutional practice	According to institutional practice	Samples are collected at Day 100, 6 months, and 12 months after UCB transplantation. Will assess at 24 months if 12-month assessment is abnormal or if patient develops chronic GVHD in the last year.	N/A	Transplant Center
Immunoglobulin levels: IgG, IgA, IgM, IgE	According to institutional practice	According to institutional practice	Samples are collected at 6 months, and 12 months after UCB transplantation. Will assess at 24 months if 12-month assessment is abnormal or if patient develops chronic GVHD in the last year.	N/A	Transplant Center
Tetanus Immunizations	According to institutional practice	N/A	Tetanus immunizations will be done at Day 100, 6 months, and 12 months after UCB transplantation as per Section 2.6.5.	N/A	Transplant Center

**TABLE C-2: SCHEDULE OF LABORATORY EVALUATIONS —
IMMUNE RECONSTITUTION INVESTIGATIONAL RESEARCH ASSAYS**
(Samples shipped to Duke Laboratory on Day 100, 6 months, 1 year, and 2 years)

Assay	Collection	Collection Container ²	Processing	Specimen to be Shipped	Storage and Shipping Container ³	Storage Temperature	Shipping Instructions
Tetanus and PRP titers¹	1 mL	2 - 0.5 mL Red Top BD Microtainer Blood Collection Tubes Cat# 365957	No additional processing; do not centrifuge	Peripheral Blood	Same as collection container	2-8°C	Ship priority overnight FedEx on cold pack to Duke University Laboratory; <u>samples should be stored at 4°C until ready to ship</u>
T cell Receptor Excision circles (TREC)	6 mL	1 - 6 mL Green Top BD Vacutainer Blood Collection Tubes Cat# 367879	No additional processing; do not centrifuge	Peripheral Blood	Same as collection container	2-8°C	Ship priority overnight FedEx on cold pack to Duke University Laboratory; <u>samples should be stored at 4°C until ready to ship</u>
T cell responses to HSV, CMV, VZV antigens and tetanus	6 mL	1 - 6 mL Green Top BD Vacutainer Blood Collection Tubes Cat# 367879	No additional processing; do not centrifuge	Peripheral Blood	Same as collection container	2-8°C	Ship priority overnight FedEx on cold pack to Duke University Laboratory; <u>samples should be stored at 4°C until ready to ship</u>

Notes:

¹ If results for tetanus response are abnormal, repeat at 15 months for T cell blastogenesis (I.e., not antibody titers)

² The transplant center will provide the collection containers specified

³ The shipping labels will be provided to the center by the DCC/EMMES

APPENDIX D

KARNOFSKY AND LANSKY PERFORMANCE STATUS SCALES

APPENDIX D**KARNOFSKY AND LANSKY PERFORMANCE STATUS SCALES****KARNOFSKY SCALE ≥ 16 YEARS**

Percentage	
100	Normal, no complaints, no evidence of disease
90	Able to carry on normal activity; minor signs or symptoms of disease
80	Normal activity with effort; some signs or symptoms of disease
70	Cares for self; unable to carry on normal activity or do active work
60	Requires occasional assistance, but is able to care for most of his/her needs
50	Requires considerable assistance and frequent medical care
40	Disabled; requires special care and assistance
30	Severely disabled, hospitalization indicated. Death not imminent
20	Very sick, hospitalization necessary, active supportive treatment necessary
10	Moribund, fatal processes, progressing rapidly
0	Dead

LANSKY SCALE < 16 YEARS

Percentage	
100	Fully Active
90	Minor restriction in physically strenuous play
80	Restricted in strenuous play, tires more easily, otherwise active
70	Both greater restrictions of, and less time spent in, active play
60	Ambulatory up to 50% of time, limited active play with assistance/supervision
50	Considerable assistance required for any active play; fully able to engage in quiet play
40	Able to initiate quiet activities
30	Needs considerable assistance for quiet activity
20	Limited to very passive activity initiated by others (e.g., TV)
10	Completely disabled, not even passive play
0	Dead

REFERENCE

Karnofsky DA: Meaningful clinical classification of therapeutic responses to anti-cancer drugs.
Editorial: Clin Pharmacol Ther 2:709-712, 1961.

APPENDIX E
SUPPORTIVE CARE GUIDELINES

APPENDIX E**SUPPORTIVE CARE GUIDELINES****1. Anemia**

Transfusions of packed red blood cells are indicated for symptomatic management of anemia. An attempt will be made to maintain the hematocrit >24% and hemoglobin >8 g/dl. Irradiated (1500-3000 cCy) blood products will be used. If CMV negative patient, CMV negative blood products or leukocyte poor blood products will be administered.

2. Thrombocytopenia

Prophylactic platelet transfusions should be given to maintain the platelet count >10,000/ml or above the level at which signs of bleeding are known to occur, whichever is greater. When available, autologous stored platelets may be used. All aspirin-containing drugs are to be avoided. The patients should receive no IM nor SQ injections. Irradiated (1500-3000 cCy) blood products will be used. If CMV negative patient, CMV negative blood products or leukocyte poor blood products will be administered.

3. Fever

Aggressive diagnostic and therapeutic management of fever in the neutropenic patient is mandatory. At the onset of fever, the patient will be thoroughly examined and cultured, after which empiric broad-spectrum antibiotics will be initiated. Anti-mold antibiotics should be considered for patients failing to respond to antibiotics. Granulocyte transfusions may be given at the investigator's discretion. Irradiation (1500-3000 cCy) of blood products is required.

4. Nutrition

All patients will be candidates for total parenteral nutrition; length of use is at the attending physician's discretion.

5. CSA Dose Modifications

To minimize the risk of CSA toxicity, the serum creatinine/BUN will be determined daily for the first 30 days after transplantation. Subsequently, the frequency will be determined by the patient's clinical status. Trough CSA levels will be determined twice weekly for all inpatients with levels obtained on the basis of the patient's clinical status thereafter. CSA dose adjustment on the basis of toxicity or low trough level. The goal is to achieve a CSA trough level of at least 200-400 mg/L without significant renal toxicity.

Adjustment of CSA should be based on CSA levels in conjunction with clinical observations of the biological effects of the drug, i.e., renal and neurologic toxicity, and the physician's assessment of the patient's need for immunosuppression. As a guideline, if the serum creatinine exceeds 2x baseline or is >2.0 mg/dL, then the CSA dose should be decreased by 50%; if the serum creatinine exceeds 3x baseline or the patient requires

hemodialysis, then the CSA dose should be held. As a guideline, if the patient becomes disoriented or develops visual disturbance, paresis, aphasia or seizure, the CSA dose should be held during the evaluation of the neurological disturbance. CSA should be reinstated as soon as possible.

6. G-CSF

All patients will receive G-CSF 5 µg/kg/day IV based on the actual body weight IV beginning on Day +1 after PBSC infusion. G-CSF will be administered daily until the ANC exceeds $2.5 \times 10^9/L$ for three consecutive days and then discontinued. If the ANC decreases to $<1.0 \times 10^9/L$, G-CSF will be reinstated.

APPENDIX F

PROCEDURE FOR THAWING UMBILICAL CORD BLOOD UNITS FROZEN IN TWO COMPARTMENT BAGS USING DEXTRAN-ALBUMIN SOLUTION

APPENDIX F

**PROCEDURE FOR THAWING UMBILICAL CORD BLOOD UNITS FROZEN IN TWO
COMPARTMENT BAGS USING DEXTRAN-ALBUMIN SOLUTION**

6D.720.02

CCBB
5E.410.02

**PROCEDURE FOR THAWING UMBILICAL CORD BLOOD UNITS
FROZEN IN TWO COMPARTMENT BAGS USING DEXTRAN-
ALBUMIN SOLUTION**

Carolinas Cord Blood Bank
Duke University Medical Center
Durham, NC 27710

Implementation Date: 12/31/04

Document History:

Original Version	1.0	
	2.0	12/04

Approvals

Laboratory Manager

Date

CCBB Medical Director

Date

Quality Manager

Date

Document Control Page

Implementation Date: 12/31/04

Annual Review

Signature	Date	Signature	Date

Minor Revision Record

Date	Revision	Comment	Initials
06/15/05	Number Change. No change to procedural steps.	Implementation date remains the same. New sign off date.	

Procedure Superseded By: 5E.410.01 **Date:** Implemented 11/17/03

Procedure Discontinued By: _____ **Date:** _____

Procedure Archived By: _____ **Date:** _____

A. PURPOSE

To maximize viable cell recovery, cryopreserved cord blood units are rapidly thawed in a 37° C water bath. The slushy content is then slowly and gently diluted with a hypertonic solution containing 10% Dextran and 5% human albumin.

Albumin absorbs out the intracellular Dimethyl Sulfoxide (DMSO) improving significantly the post thaw viability. Centrifugation helps in the removal of the solubilized DMSO, free hemoglobin and the majority of cell debris.

B. INTRODUCTION

Umbilical cord blood units are cryopreserved in a solution containing 10% Dimethyl Sulfoxide (DMSO) and 1% Dextran. Stem cells cryopreserved in DMSO have limited viability upon thawing, resulting in the potential for significant loss of cells available for transplantation. DMSO, the cryoprotectant of choice, has cytotoxic effects when warmed to 37°C. Immediately upon thawing, intracellular DMSO creates a hypertonic intracellular environment which leads to sudden fluid shifts that compromise cell viability. In addition, DMSO causes adverse side effects in vivo after reinfusion, including blood pressure instability, fever, chills, and nausea. Lyses of red blood cells leads to accumulation of free hemoglobin that can be nephrotoxic when infused intravenously.

Mixing the thawed cells with a hypertonic solution, immediately upon thawing can ameliorate many of these problems. Typically, the hypertonic thawing solution contains 5% human albumin and 10% Dextran 40 in 0.9% sodium chloride solution. Dextran-Albumin thawing solution helps to restore the osmolarity of the blood cell suspension, promoting colloidal-osmotic intracellular equilibrium. Cell suspensions can then be washed to remove DMSO, free hemoglobin and other cellular products, thus allowing other procedures to be performed prior to reinfusion.

C. SCOPE

This procedure describes in detail the use of a hypertonic solution of Dextran and human albumin for thawing cryopreserved cord blood units. The procedure covers all required steps for the application of the methodology. It starts with the initial preparations and continues from the time the selected unit is removed from the storage Dewar until the product is ready for patient infusion. Cord blood units are stored in double compartment cryopreservation bags, which enables working with the compartments independently if required. (Figure 1)



Figure 1

D. MATERIALS**Reagents**

Albumin (human) USP 25% solution (0.25g/ml)	Baxter	prod. #060-033
Dextran 40(10% Gentrin 40 and 0.9% NaCl), USP	Baxter	prod. #2B-5043
Trypan Blue vital stain at 0.4% solution in DH2O	Sibma Co.	cat # T6126
Aerobic & anaerobic culture bottles Bac-T alert	Organon-Teknika	
0.9% Sodium Chloride Solution (Saline)	Baxter	prod. #BB-5329

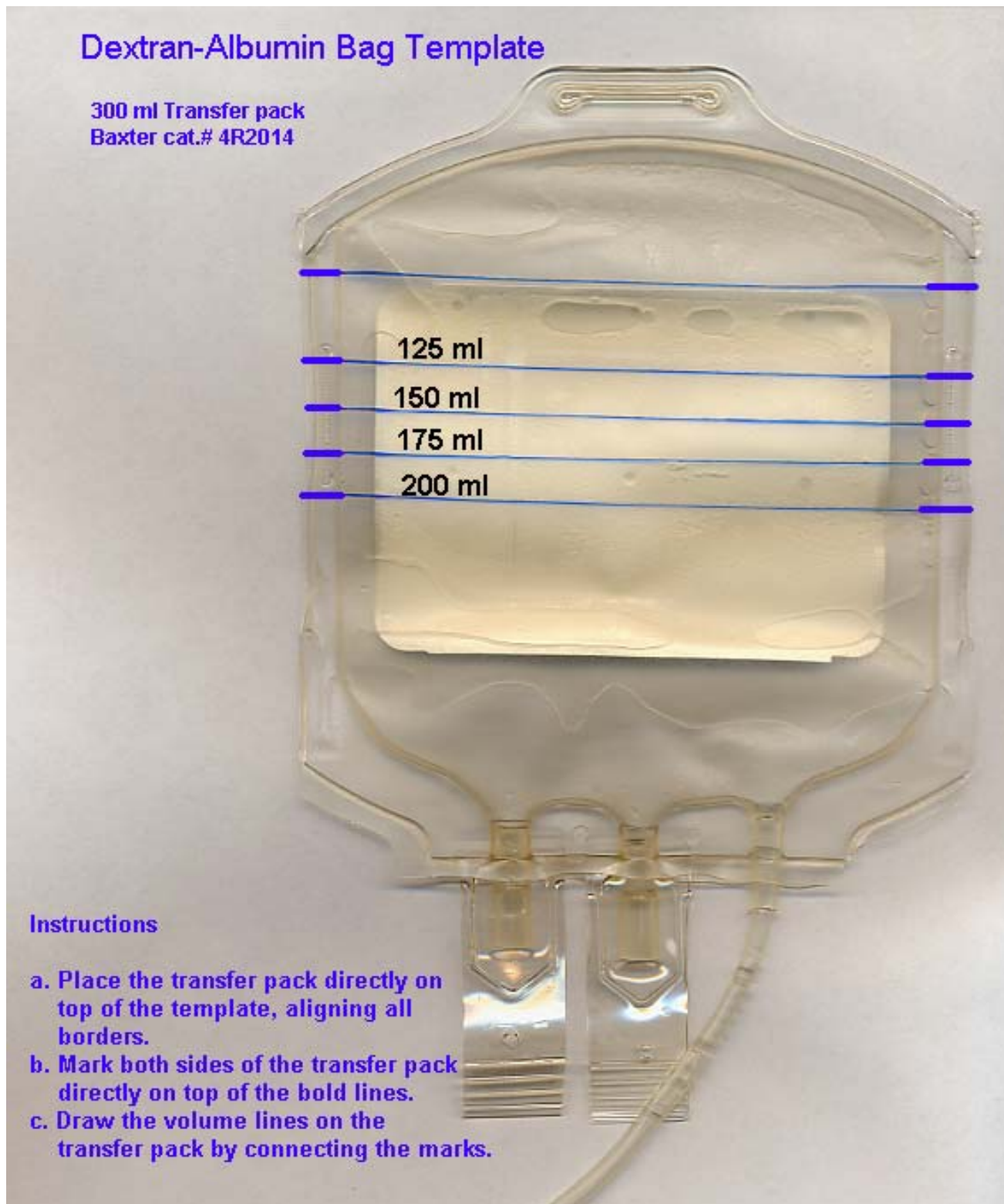
Supplies

Cell infusion bag set	Pall Medical	cat. #791-03
150 ml transfer pack	Baxter	prod. # 4R2001
300 ml transfer pack	Baxter	prod. # 4R2014
Sterile disposable syringes: 3, 20 & 60ml	Becton-Dickinson	
16 gauge injection needles	Becton-Dickinson	prod. #30519
Alcohol cleaned scissors		
5 ml sterile culture tubes (snap cap)	Falcon	cat. #52766
5ml polystyrene tubes	Falcon	cat. #352052
Cryogenic vials	Corning	cat. #430489
Alcohol prep pads	Allegiance	cat. #40000-110
Iodine swab sticks	Allegiance	cat. #40000-040
Sterile (7x8 in.) zip-lock bags	Ziploc – Johnson	
Hemostats (optional)		
Gloves		
Protective freezer gloves.		
Insul-ice mats	Polyfoam Packers Corp.	
100 ml burette hemostat filter	Abbot Laboratories	cat. # 8948
150ml Sorvall centrifuge insert		

E. EQUIPMENT

Class II Laminar flow hood	The Baker Co Inc.
Refrigerated blood bank centrifuge	Sorvall RC/3C
Plasma extractor	Baxter 4R4414
Analytical balance	Mettler PG300
Sterile docker device	Terumo SCD312
Tube heat sealer for PVC plastic	Sebra model 1105
Automated cell counter	Sysmex K1000
Optical microscope	Olympus BH-2
Vortex mixer	Baxter – vortexer MV-1
Waterbath (4 liters or more at 37°C)	Isotemp Waterbaths
TG canister opener	Thermogenesis Co.
Non frost-free refrigerator	

F. PROCEDURE



Procedure notes

- Use aseptic technique in a biological safety cabinet for all processing steps, including all open-container processing and all spiking of blood bags.
- Allow only sterile materials to come in contact with the cellular product.
- Record the manufacturer, lot number and expiration date (if applicable) of all reagents and disposables.
- Assemble all materials before thawing the cryopreserved product.
- Treat the thawed cell suspension very gently. The cell membranes are fragile and the cells are lysed easily.
- Dextran- albumin solution is to be added slowly so that the DMSO is gradually diluted, then removed.
- The infusion time should be set up in advance with the transplant coordinator and the start time for this thawing procedure should be adjusted accordingly.
- Verify if the waterbath is full and the temperature is 37° C.

Preparation of Dextran-Albumin thawing solution

(Human albumin at concentration of 4.2% in Dextran/NaCl solution)

1. Draw volume reference lines on a 300 ml transfer bag using the template shown previously.
2. Sterilely spike the 300ml marked bag to a 500 ml bag of Dextran.
3. Place the empty transfer bag on the scale and tare the scale.
4. Transfer 250 grams of Dextran solution to the 300 ml transfer bag.
5. Heat seal tubing and detach Dextran bag by cutting tubing at the sealed point.
6. Working in the laminar flow hood, insert a sampling site coupler into one of the ports of the 300 ml transfer bag containing Dextran.
7. Clean the rubber stopper of albumin bottle with alcohol wipes.
8. Draw albumin from the flask using a 60 CC syringe.
9. Clean coupler port with alcohol and inject albumin into the Dextran bag.

NOTE: Dextran-Albumin solution will be referred to as thawing solution in future steps.

Assembly of the closed System

1. Clamp all tubing and place labels on the “cell wash/infusion set”.
2. Sterile dock wash/infusion set to thawing solution bag.
3. Place the wash/infusion set inside the Plexiglas tray on the scale. (Figure 2).
4. Tare scale and transfer 125ml of thawing solution into infusion bag as shown in figure 2.
5. Clamp off tubing and wrap the infusion bag with an ice mat.
6. Place thawing set (wash/infusion set joined to the thawing solution bag) inside the hood.
7. Re-tare the scale if necessary.

8. Use the same tared scale to weigh the infusion bag before centrifugation to confirm the volume.

NOTES:

- The cell wash/infusion bag will be referred to as infusion bag in future steps.
- The wash/infusion set docked to the thawing solution bag will be referred to as thawing set in future steps.

Assembly of reagents and supplies in the hood

(Assemble all materials before thawing the cord blood unit, as shown in figure 3).

1. Assemble and bar code the paperwork, completing as much as possible.
2. Prepare and label all tubes and bacterial culture bottles.
3. Place inside the hood the following supplies necessary for the procedure: tube rack, sterile snap-cap tubes, test tubes, syringes, iodine and alcohol swabs, sterile gauze pads, disinfected scissors, sampling site coupler and ice mats.
4. Hang the volume marked thawing solution bag to facilitate the flow.
5. Leave one ice mat ready for the cryobag.

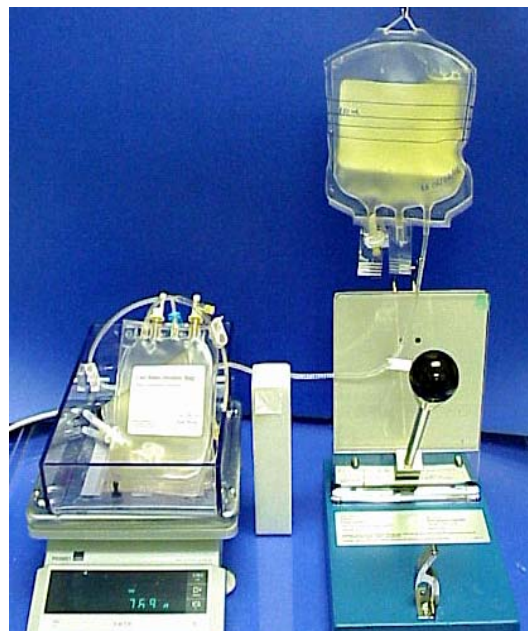


Figure 2



Figure 3

Cord blood thawing

1. Working in the vapor phase of the LN2 tank, remove unit from metal cassette.
2. Perform rechecking and identification per institutional SOP.
3. Remove overwrap plastic sealant, if present. (Figure 4)
4. Cut the cryobag segments as shown in figure 5.
5. Place the segments in a nunc vial labeled with patient information, unit number, date and product type.



Figure 4

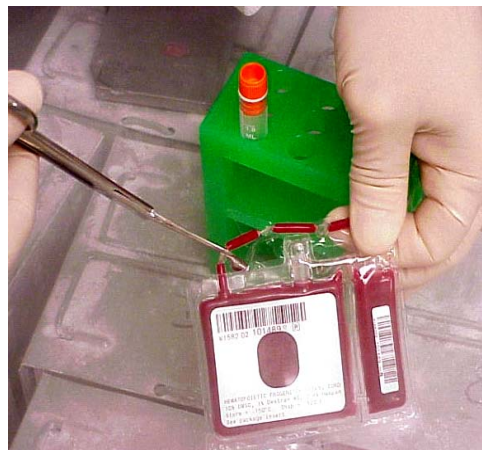


Figure 5

6. Keep nunc vial in vapor phase until finding the definitive storage spot in liquid nitrogen.
7. Place the cryobag inside a sterilized zip-lock bag, let the air out and then seal the bag.
8. Thaw the unit in a 37°C water bath until product reaches a slushy/liquid consistency.

NOTES: To accelerate thawing, carefully agitate unit in water.

From this step on, all manipulations will be performed inside the laminar flow hood.

Cord blood dilution and wash

1. Remove the cryobag from zip-lock bag.
2. Clean outside of the port covers with iodine solution.
3. Cut both port covers with disinfected scissors.
4. Clean cut surfaces, first with iodine, and then with alcohol as shown in figure 6.
5. Dry the cut surfaces with sterile gauze.
6. Insert the spikes of the infusion set in the dry and disinfected ports (one at the time).
7. Wrap the cryobag with an ice mat.



Figure 6

8. Unclamp tubing between infusion and cryo bags.
9. Transfer cold thawing solution from the infusion bag into the cryobag over approximately 1 to 2 minutes until both compartments of the cryobag bulge as shown in figure 7.
10. Gently mix the incoming fluid and slushy product during transfer.
11. Gently rock the cryobag for 4- 5 minutes for complete homogenization of its contents.
12. Elevating the cryobag to gradually transfer the diluted cell suspension from the cryobag into the infusion bag.
13. Mix fluids during transfer by moving bags up and down as shown in figure 8.
14. Leave the remaining residual fluid and cells in the cryobag at this time.
15. Gently rock the infusion bag for 1- 2 minutes to allow complete mixing.
16. Clamp the lines off in preparation for the rinsing process.



Figure 7

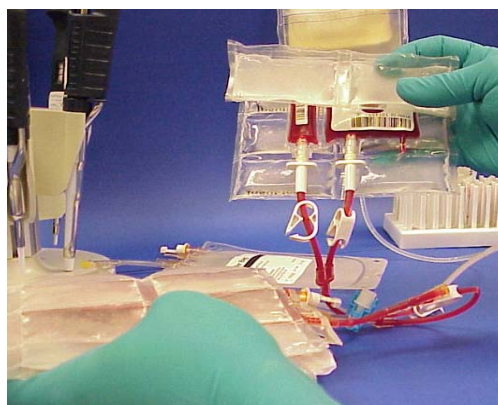


Figure 8

NOTE: Use the volume reference lines in thawing solution bag to deliver the right volume of thawing solution during rinsing steps.

1. Unclamp tubing between thawing solution bag and cryobag.
2. Allow approximately 25 ml of thawing solution, lines 2 →3 in the thawing solution bag to flow into both compartments of the cryobag.
4. Close pinch clamp between thawing solution and cryobags after transfer.
5. Apply pressure and massage the cryobag to dislodge all remaining cells.
6. Swirl thawing solution around the cryobag to resuspend and harvest all remaining cells.
7. Open tubing between cryobag and infusion bag.
8. Elevate cryobag to allow thawing solution and cells to flow into the infusion bag.
9. Mix fluids during transfer by rocking the infusion bag.
10. Compress and roll the cryobag to remove all the remaining cell suspension.
11. Clamp off the line in preparation for the second rinsing.
12. Again, unclamp tubing between thawing solution bag and cryobag.
13. Add approximately 25 ml of thawing solution, lines 3 →4 in the thawing solution bag into the cryobag.
14. Repeat rinsing process steps 4 to 11.
15. After rinsing, heat seal tubing between the infusion bag and cryobag and cut at the sealed point.
16. Remove the unit identifying label, place it on the paperwork and discard empty cryobag.

- Heat seal tubing between infusion bag and thawing solution bag and cut at the sealed point. Retain the thawing solution for potential further use.

NOTE: The volume of the diluted product should be close to 200 ml.

Confirmatory values can be obtained by checking the weight in the previously tarred scale. A volume higher than 225 ml may cause bag breakage.

Centrifugation of thawed/dilute product

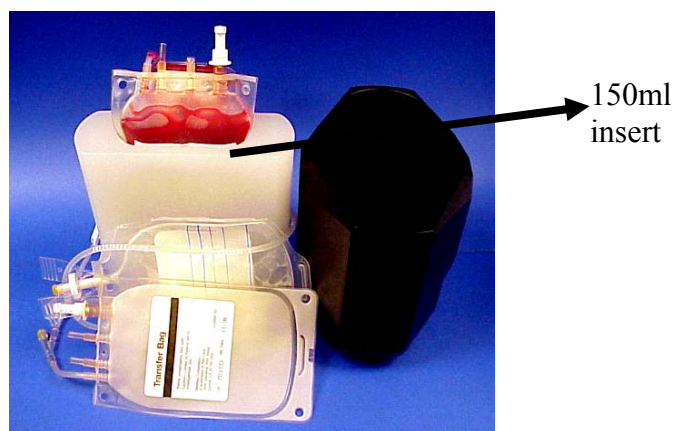


Figure 9

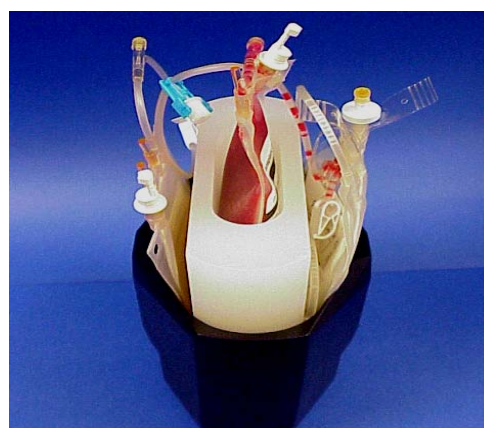


Figure 10

- Place the infusion bag inside a sterile zip-lock bag.
- Place both bags in a specially designed centrifuge insert labeled as "150 ml Sorvall centrifuge insert" as shown in figure 9.
- Arrange the insert and the thawing set inside the centrifuge bucket as shown in figure 10.
- Check if the infusion bag is fully supported inside the insert and all clamps are closed.
- Cross tape tubing inside the bucket as shown in figure 11.
- Pellet the cells at 1800 rpm for 20 minutes at 2-8 °C.



Figure 11

NOTE: In centrifuges with rotor radius equal to 25 cm, the speed of 1800 RPM is equivalent to 880 G.

Express supernatant and add fresh thawing solution:

- Place the centrifuged infusion bag in the plasma extractor as shown in figure 12.
- Place the transfer bag inside the Plexiglas tray on the scale as shown in figure 12.
- Tare the scale.

4. Open the tubing clamp between the two bags.
5. Without disturbing the cell pellet, transfer most of the supernatant to the transfer bag leaving approximately 50 ml (figure 12).
6. Empty tubing between the bags by transferring air from the transfer bag to the infusion bag.
7. Close tubing and remove transfer (waste) bag from scale.
8. Now place the infusion bag on the scale.
9. Tare the scale.
10. Record the weight from the tarred scale on the CBU Thawing form.
11. Heat seal tubing and detach transfer bag.
12. Mix contents of infusion bag (cell suspension #1).
13. Sterilely remove a 0.2 ml aliquot for cell counts and cell viability test.

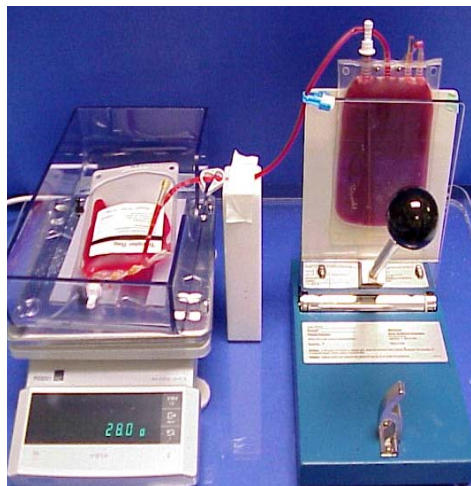


Figure 12

Centrifuge supernatant to pellet remaining cells.

1. Sterile dock the transfer bag containing the supernatant to a processing/freezing set.
2. Place supernatant bag inside a sterile ziplock bag and then inside the insert.
3. Accommodate insert and bags in the centrifuge bucket. Tare the buckets.
4. Pellet the cells at 1800 rpm for 20 minutes at 2-8⁰C.
5. After centrifugation, place the bag in the plasma extractor.
6. Express the leukocyte poor supernatant without disrupting the pellet.
7. Leave 10 to 15 ml approximately.
8. Mix contents of transfer bag (cell suspension #2) and draw contents into a 20 or 30 cc syringe for volume measurement.
9. Sterilely remove a 0.2 ml aliquot for cell counts.
10. Add recovered cells from transfer bag (cell suspension #2) into infusion bag (cell suspension #1) by injecting the volume in the syringe into the infusion bag.
11. Sterilely remove 0.7 ml aliquot for QC tests.
12. Viable cell recovery values are calculated using the following formula:

$$\frac{\text{Total viable nucleated UCB cells on the infusion ready product}}{\text{Total viable nucleated cells of cryopreserved unit provided on the feed beck}}$$

13. Calculate viable cell recovery and record all values on the laboratory thawing form (6D.700, 5E.410 (FRM2)).

Preparation of cells for transplantation:

1. If the final cell suspension contains clumps, filter the product using a burette filter.
2. Label the infusion bag with information per institutional SOP.
3. Sterile dock as shown in figure 13, the infusion bag to a 150 ml transfer bag containing 100 ml of 0.9% NaCl solution. Saline solution will be used to wash the infusion bag after the reinfusion procedure.
4. Fill out forms and paper work (6D.700, 5E.410 (FRM2) and 5E.410 (FRM1)
5. Send product to the transplant unit in a temperature monitored cooler.



Figure 13

Quality Control Tests

- Cell Counts: performed on diluted product, supernatant and infusion ready product.
- Viability test by trypan blue: performed on infusion ready product.
- Colony assay for CFU-GM, GEMM and BFU-E: performed on infusion ready product (9D.212) (FRM1).
- CD34+ determination by flow analysis: performed on infusion ready product. (4D.210.01 (9D.2123.01) (FRM1)
- Sterility Test: performed on 15 ml of supernatant from the final wash.
- RFLP: performed on approximately 1×10^6 cells from cell suspension #2 upon request

Preparation and cryopreservation of the remaining cells as back-up from original CBU reinfusion (if applicable)

1. Place the supernatant bag in the special insert and accommodate bags in the centrifuge bucket.
2. Pellet the cells at 1200 rpm for 20 minutes at 2-8°C.
3. Express out leukocyte poor supernatant using the automated expresser which will leave a volume of 21.5 ml.
4. Heat seal tubing and detach bags.
5. Mix the cell suspension and remove 0.2 ml aliquot for cell count.
6. Place the bag at 4°C for at least 20 minutes.
7. Freeze cells following the procedure for “cryopreserving back-up cells from original UCB reinfusion”

G. RELATED FORMS

- 4D.210.01 (9D.2123.01) (FRM1) Hematopoietic Progenitor Assay Worksheet
- 6D.700, 5E.410 (FRM2) Thawing and Reinfusion Worksheet
- 4D.210.01 (9D.2123.01) (FRM1) Flow Cytometry Worksheet
- 5E.410 (FRM1) Carolina’s Cord Blood Bank CBU Thawing Form

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APPENDIX G
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