FRED HUTCHINSON CANCER RESEARCH CENTER
UNIVERSITY OF WASHINGTON SCHOOL OF MEDICINE
SEATTLE CHILDREN'S

Current version: 06/21/2021
Previous version: 1/8/2021

Transplantation of Umbilical Cord Blood for Patients with Hematological Diseases with Cyclophosphamide/Fludarabine/Total Body Irradiation or Cyclophosphamide/Fludarabine/Thiotepa/Total Body Irradiation Myeloablative Preparative Regimen

Investigators	Professional Title	
Ann Dahlberg, MD	Principal Investigator Assistant Member, FHCRC Assistant Professor of Pediatrics, UW	(206) 667-1959
Colleen Delaney, MD, MSc	Affiliate Member, FHCRC Founder and Chief Scientific Officer Executive Vice President of Research and Development Nohla Therapeutics, Inc.	(206) 519-5304
Fred Appelbaum, MD	Member, FHCRC Professor of Medicine, UW	(206) 667-4412
Effie Petersdorf, MD	Member, FHCRC Professor of Medicine, UW	(206) 667-4453
Filippo Milano, MD, PhD	Assistant Member, FHCRC Assistant Professor of Medicine, UW	(206) 667-5925
Biostatistician:		
Ted Gooley, Ph.D.	Member, FHCRC	(206) 667-6533
Research Staff:		
Jenna Pedersen	Research Coordinator	(206) 667-6013
Denise Ziegler	Research Coordinator	(206) 667-5762
Mary Joy Lopez	Data Coordinator	(206) 667-6139

Emergency Contacts (24 hour):

Local Phone (For Adults)**:** (206) 598-8902; **Fax:** (206) 598-4034

Local Phone (For Children): (206) 987-2032; **Fax** (206) 987-2764

TABLE OF CONTENTS

1.0 OBJECTIVES

1.1 Primary objective

The primary objective of this study is to estimate the one-year survival of patients undergoing umbilical cord blood transplantation (UCBT) after a myeloablative preparative regimen consisting of Regimen A: cyclophosphamide (CY), fludarabine (FLU) and fractionated total body irradiation (TBI) or Regimen B: cyclophosphamide (CY), fludarabine (FLU), thiotepa and fractionated total body irradiation (TBI).

1.2 Secondary objectives are to estimate the:

- Incidence of transplant-related mortality (TRM) at 6 months
- Incidence of neutrophil engraftment at Day 42
- Incidence of platelet engraftment at 6 months
- Incidence of disease free survival at 1 and 2 years
- Incidence of relapse at 1 and 2 years

1.3 Exploratory objectives are to assess the:

- Chimerism at multiple time points
- Incidence of acute graft-versus-host disease (GVHD) grade II-IV and grade III-IV at Day 100
- Incidence of chronic GVHD at Day 100, 1 year and 2 years
- Incidence of clinically significant infections at 6 months, 1 year and 2 years

2.0 BACKGROUND & RATIONALE

Bone marrow transplantation (BMT) is a standard treatment option for an increasing number of malignant and non-malignant disorders. To reconstitute hematopoiesis after an intensive myeloablative therapy, the transplantation of pluripotential hematopoietic stem cells (HSCs) is required. Such HSCs are typically recovered from the bone marrow of related or unrelated donors or the bone marrow or apheresed peripheral blood of the patients themselves [1]. Unfortunately, suitable marrow is frequently not available [2]. Either the patient's own marrow is contaminated with tumor cells or potential allogeneic marrow donors are unsuitable most often on the basis of HLA mismatch. Human umbilical cord blood (UCB) is an alternative source of HSCs that is capable of reconstituting hematopoiesis after intensive myeloablative therapy [3-10].

2.1 Umbilical Cord Blood Transplantation (UBCT)

As a result of the early successes with umbilical cord blood transplantation (UCBT) from sibling donors, pilot programs for the banking of unrelated donor UCB were initiated in many countries around the world. Known benefits of banked UCB include: 1) rapid availability, 2) absence of donor risk, 3) absence of donor attrition, and 4) very low risk of transmissible infectious diseases, such as CMV and EBV, and 5) low risk of acute GVHD despite HLA mismatch. UCB is particularly beneficial for patients of ethnic and racial minority descent for whom adult marrow and blood donors often cannot be identified.

2.2 UCBT Experience at the University of Minnesota

Single Unit UCBT [11].

Between 1994 and 2001, 102 consecutive patients (median age 7.4 years) received a single, unrelated UCB unit after a myeloablative conditioning for malignant (n = 65; 68% high-risk)

and non-malignant diseases (n = 37). Median infused cell dose of UCB was 3.1 x 10^7 NC/kg (range 0.7-57.9), and 2.8 x 10^5 CD34+ cells/kg (range 0.4-39.1). Fourteen percent had an HLA matched unit and 86% had a 1-3 HLA-match. Neutrophil recovery occurred at a median of 23 Days (range 9-54) after UCBT with cumulative incidence of engraftment of 88% (95% CI: 81-95) by Day 42. Speed and likelihood of neutrophil recovery were strongly associated with cell dose, with markedly inferior engraftment (72% at a median of 34 Days) in patients receiving a CD34+ cell dose <1.7 x 10^5 cells/kg.

The incidences of grade II-IV and III-IV acute GVHD were 39% (95% CI: 29-49) and 11% (95% CI: 5-17), respectively, at Day 100, with 10% (95% CI: 4-14) of patients having chronic GVHD at 1 year. One year transplant-related mortality (TRM) was 30% (95% CI: 21-39) which was strongly associated with CD34+ cell dose. The probabilities of 1 and 2-year survival were 58% (95% CI: 49-70) and 47% (95% CI: 36-57), respectively. Importantly, with a graft cell dose ≥1.7 x 10^5 CD34+ cells/kg, survival was 70% (95% CI: 49-90) at 1 year.

The principal conclusions of this study were: 1) an adequate cell dose (≥1.7 x 10^5 CD34+ cells/kg or >2.5 x 10^7 nucleated cells/kg) consistently engraft; 2) GVHD is low despite HLA mismatch; 3) survival and risk of relapse are comparable to that observed after BMT, and; 4) cell dose significantly limits the applicability of UCB, particularly in adult size recipients.

Multi-Unit UCBT [12].

Since cell dose has been clearly identified as a major limitation, often preventing the consideration of UCB for adult recipients, we have explored the possibility of infusing two partially HLA matched units to augment cell dose. Between 2000 and 2005, we have increasingly utilized two UCB units rather than one, particularly in adults and adolescents who cannot find a suitable single UCB unit. The underlying hypothesis of the original study (MT2000-15) was that the addition of the second unit would enhance the engraftment and the speed of hematopoietic recovery.

We have analyzed the results of the first 31 adult and adolescent patients [median age 24 years (range: 13-53); median weight 73 kg (range: 48-120)] with high-risk hematologic malignancy who were transplanted with two partially HLA-matched UCB units after a myeloablative conditioning. Patient breakdown by diagnosis was: acute myelogenous leukemia (AML, n=15), acute lymphocytic leukemia (ALL, n=12), chronic myelogenous leukemia (CML, n=3) and non-Hodgkins Lymphoma (NHL, n=1). The median total infused dose 3.7 x 10^7 nucleated cells per kilogram (range 1.1-6.3) and 4.9 x 10^5 CD34 per kilogram (range, 0.9-14.5).

In this study, 100% of evaluable patients (i.e., survived for 21 Days [n=29]) engrafted at a median of 23 Days (range 14-41). This compares favorably with a 65-75% incidence of engraftment reported for adults [9, 10]. In each case, one unit predominated. Thus far, no factor (nucleated cell dose, CD34+ cell dose, HLA-match, ABO, sex, order infusion) predicts which unit will eventually be responsible for long term marrow recovery. The incidence of platelet recovery (>50,000/uL) was 73% (95% CI, 51-95) at Day 180. Incidence of grades II-IV and III-IV acute graft-versus-host disease (GVHD) was 65% (95% CI, 42-88) and 17% (95% CI, 2-32) at Day 100. Disease-free survival remains 72% at 1 year for patients transplanted in CR with no relapse in this cohort (median follow up: 1.2 years).

These data indicate that: 1) double unit UCBT is safe in terms of engraftment (bi-directional immune rejection between the units was never observed); 2) one unit always predominates within the first 100 Days; 3) >90% of adults will be able to identify two units that are partially HLA matched with the patient and each other; 4) incidence GVHD is similar to that observed after single UCBT; and 5) survival exceeds that of historical data with a single UCB unit.

As a result of these promising early data, we plan to extend this trial in order to 1) confirm the initial observations and 2) establish survival outcomes and relapse risk for individual disease groups. Validation studies at other centers are in progress.

2.3 REGIMEN

This clinical trial will allow variation in preparative regimen but will have identical regimen for GVHD prophylaxis. The protocol-allowed conditioning regimens are:

1. High-dose TBI + Fludarabine + cyclophosphamide
2. Middle-intensity TBI + Fludarabine + cyclophosphamide + thiotepa

The additional regimen (middle-intensity TBI + Fludarabine + cyclophosphamide + thiotepa) was chosen in addition to the more conventional high-dose TBI regimen that was used in our pilot study based on the extensive work published by Dr. Juliet Barker at the Memorial Sloan Kettering Cancer Center [14].This novel regimen was originally developed as an alternative intensive conditioning regimen aimed at reducing the increased risk of lethal regimen-related organ toxicity that has been associated with the more conventional high-dose TBI-based regimen used for myeloablative CBT (using 13.2 Gy TBI). In fact, the known risk of end-organ toxicity with high-dose TBI has led to restriction of this regimen to those 45 years and younger. Although there are nonmyeloablative conditioning regimens that have been used in the CBT setting for those patients older than 45 years, nonmyeloablative conditioning itself is limited by increased risk of graft rejection and relapse in this setting. Thus, there is a significant need to develop a well-tolerated middle-intensity, near-myeloablative regimen to address the limitations of the high-dose TBI and nonmyeloablative regimens. This is particularly true for those patients older than 45 years or younger patients with significant pre-existing co-morbidities precluding them from the conventional high dose TBI regimen.

Dr. Barker published on this regimen for the first time in 2013 which presented results for 30 patients with acute leukemia and MDS, with the majority having acute leukemia (n=26) [14]. The median age was 56 years (range 18 to 69). The overall incidence of engraftment was 97% (1 graft failure), with a median time to engraftment of 26 days (range, 13 to 43).

Expanding our clinical trial to allow the option of this new conditioning regimen as an alternative to the conventional high-dose TBI regimen, with corresponding expanded age range, brings the benefits of cord blood transplant to a wider cohort. The argument for addition of this conditioning regimen is strongly motivated by arguments for the importance of generalizability and for the ability to pool results. The use of the same primary and secondary endpoints for both conditioning regimens is well motivated by the study's primary objective to estimate the one-year survival of patients undergoing cord blood transplantation. Furthermore, the Barker trial results reveal time to engraftment, overall survival and TRM are similar between the cohorts thus supporting the pooling of results from the two conditioning regimens. For these reasons, the inclusion of this new middle-intensity regimen and the inclusion of patients up to age 65 enables a clinically well motivated generalization of the assessment of patients undergoing cord blood transplantation.

The regimen selection for individual patients is flexible to allow for attending physician and site preference. There are some absolute criteria, for example, that patients between the ages of 46 and 65 would have to go on the middle-intensity regimen. However, outside of this, the decision of which conditioning regimen would be based on the underlying disease, presence of MRD, age, co-morbidities and attending preference. Furthermore, there is also a move away from high-dose TBI by some physicians/centers in younger patients to avoid the long-term sequelae of high-dose TBI. In general, it is anticipated that younger patients would continue to accrue to the high dose TBI regimen, especially those with minimal residual disease and the middle-intensity regimen would

accrue those patients between 46 and 65 years of age and patients for whom the attending physician feels this is the more appropriate regimen.

In this study, we will choose the unmanipulated CB donors per a CB selection algorithm (see Section 4.0 and Appendix V) allowing a single or double non-manipulated cell graft.

3.0 STUDY DESIGN

This is a single-arm, phase II study to estimate the one-year survival of patients undergoing umbilical cord blood transplantation (UCBT) after a myeloablative preparative regimen consisting of Regimen A: cyclophosphamide, fludarabine and fractionated total body irradiation (TBI) or Regimen B: cyclophosphamide, fludarabine, thiotepa and fractionated total body irradiation (TBI) for the treatment of study specific hematological malignancies

4.0 PATIENT/DONOR SELECTION

4.1 Graft Criteria

- UCB units will be selected according to current umbilical cord blood graft selection algorithm (Appendix V). One or 2 UCB units may be used to achieve the required cell dose.
- The UCB graft is matched at 4-6 HLA-A, B, DRB1 antigens with the recipient. This may include 0-2 antigen mismatches at the A or B or DRB1 loci. Unit selection based on cryopreserved nucleated cell dose and HLA-A, B, DRB1 using intermediate resolution A, B antigen and DRB1 allele typing.
- If 2 UCB units are required to reach the target cell dose, each unit must be a 4-6 antigen match to the recipient.

4.2 Age and Disease Criteria

1. High-dose TBI regimen: 6 months to ≤ 45 years
2. Middle-intensity TBI regimen: 6 months to ≤ 65 years

Conditioning regimen selection should be based on the underlying disease, presence of MRD, age, co-morbidities, and attending physician.

Patients must have a hematological malignancy as described below:

Acute Myeloid Leukemia, including Biphenotypic Acute Leukemia or Mixed-Lineage Leukemia

- All patients must be in CR as defined by hematologic recovery and <5% blasts by morphology/flow cytometry in a representative bone marrow sample with cellularity ≥ 15% for age. Patients who do not have high-risk features (for example preceding MDS, high-risk cytogenetics, ≥ 2 cycles to obtain CR, erythroblastic or megakaryocytic leukemia or ≥ CR2) must be discussed with the PI prior to enrollment and at the Patient Care Conference or equivalent group such as the pediatric leukemia board as an alternative.
- Patients in whom adequate marrow/biopsy specimens cannot be obtained to determine remission status by morphologic assessment, but have fulfilled criteria of remission by flow cytometry, recovery of peripheral blood counts with no circulating blasts, and/or normal cytogenetics (if applicable) may still be eligible. Reasonable attempts must be made to obtain an adequate specimen for morphologic assessment, including possible repeat procedures. These patients must be discussed

with the PI prior to enrollment. Patients persistently aplastic for greater than one month since completing last chemotherapy are also eligible with PI approval.

Very high risk pediatric/young adult patients with AML. Patients ≤ 25 years, however, are eligible with (M2 marrow) with ≤ 25% blasts in marrow after having failed one or more cycles of chemotherapy. This group of patients will be analyzed separately.

Acute Lymphoblastic Leukemia, including Biphenotypic Acute Leukemia or Mixed-Lineage Leukemia

- All patients must be in CR as defined by <5% blasts by morphology.flow cytometry in a representative bone marrow sample with cellularity ≥ 15% for age. Patients who do not have high-risk disease (High risk CR1, greater than one cycle to obtain CR or ≥CR2) must be discussed with the PI prior to enrollment and at the Patient Care Conference or equivalent group such as the pediatric leukemia board as an alternative.
- Patients in whom adequate marrow/biopsy specimens cannot be obtained to determine remission status by morphologic assessment, but have fulfilled criteria of remission by flow cytometry, recovery of peripheral blood counts with no circulating blasts, and/or normal cytogenetics (if applicable) may still be eligible. Reasonable attempts must be made to obtain an adequate specimen for morphologic assessment, including possible repeat procedures. These patients must be discussed with the Principal Investigator, Ann Dahlberg ((206) 667-1959 or pager, (206) 469-3102) prior to enrollment. Patients persistently aplastic for greater than one month since completing last chemotherapy are also eligible with PI approval.

Chronic myelogenous leukemia excluding refractory blast crisis. To be eligible in first chronic phase (CP1) patient must have failed or be intolerant to imatinib mesylate.

Advanced myelofibrosis

Myelodysplasia (MDS) IPSS Int-2 or High risk (i.e., RAEB, RAEBt) or refractory anemia with severe pancytopenia or high risk cytogenetics. Blasts must be < 10% by a representative bone marrow aspirate morphology.

Lymphoblastic lymphoma, Burkitt's lymphoma, and other high-grade NHL after initial therapy if stage III/IV in PR1 or after progression if stage I/II < 1 year. Stage III/IV patients are eligible after progression in CR/PR.

Chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), marginal zone B-cell lymphoma, lymphoplasmacytic lymphoma or follicular lymphoma that have progressed after at least two different prior therapies. Patients with bulky disease (nodal mass greater than 5 cm) should be considered for debulking chemotherapy before transplant. These patients must be presented at PCC prior to enrollment, given potential competing eligibility on autotransplant protocols.

Mantle-cell lymphoma, prolymphocytic leukemia
Eligible after initial therapy in ≥CR1 or ≥PR1.

Large cell NHL > CR2/> PR2.

- Patients in CR2/PR2 with initial short remission (<6 months) are eligible.
- These patients must be presented at PCC prior to enrollment, given potential competing eligibility on autotransplant protocols.

Multiple myeloma beyond PR2.

- Patients with chromosome 13 abnormalities, first response lasting less than 6 months, or β-2 microglobulin > 3 mg/L, may be considered for this protocol after initial therapy.

4.3 Organ Function and Performance Status Criteria

- **Performance status score**: Karnofsky (for adults) ≥ 70% or ECOG 0-1
  Lansky (for children) ≥ 50%
- **Renal function**: creatinine < 2.0 mg/dL (for adults) or creatinine clearance > 60 ml/min (for children)
- **Hepatic function**: Patients with clinical or laboratory evidence of liver disease will be evaluated for the cause of liver disease, its clinical severity in terms of liver function, histology, and the degree of portal hypertension. Patients with fulminant liver failure, cirrhosis with evidence of portal hypertension or bridging fibrosis, alcoholic hepatitis, esophageal varices, a history of bleeding esophageal varices, hepatic encephalopathy, or correctable hepatic synthetic dysfunction evidenced by prolongation of the prothrombin time, ascites related to portal hypertension, bacterial or fungal abscess, biliary obstruction, chronic viral hepatitis with total serum bilirubin >3mg/dL, and symptomatic biliary disease will be excluded.
- **Pulmonary function**: DLCOcorr > 50% normal or a pediatric patient who is unable to perform PFTs but has adequate pulmonary function
- **Cardiac function**: Left ventricular ejection fraction > 45%, or shortening fraction >26%

4.4 Exclusion Criteria

- Uncontrolled viral or bacterial infection at the time of study enrollment.
- Active or recent (prior 6 month) invasive fungal infection without ID consult and approval.
- History of HIV infection.
- Pregnant or breastfeeding.
- Chemotherapy refractory large cell and high-grade NHL (i.e., progressive disease after > 2 salvage regimens).
- Patients with history of prior myeloablative transplant containing full dose TBI (greater than 8 Gy) will not be eligible for Regimen A; however, they may still enroll on Regimen B if they otherwise meet inclusion and exclusion criteria.
- Any prior myeloablative transplant within the last 6 months.
- Patients ≥ 45 years: comorbidity score of 5 or higher
- Patients who have received Y-90 ibritumomab (Zevalin) or I-131 tostumomab (Bexxar), as part of their salvage therapy are not eligible for Regimen A.

5.0 PATIENT REGISTRATION

Informed consent must be signed prior to the performance of any study related procedures or assessments.

Eligible patients will be identified by the Clinical Coordinators Office. Patients will be registered with the Registration Office (206-667-4728) between 8:30 am and 4:00 PM Pacific Time, Monday through Friday. After hours, the Registration office can be reached by paging (206) 995-7437. Questions regarding eligibility or protocol information should be directed to Ann Dahlberg, MD (206-667-1959).

6.0 TREATMENT PLAN

6.1 Conditioning Regimen

There are two conditioning regimens allowed per protocol. They are:

1. Regimen A: High-dose TBI + Fludarabine + cyclophosphamide
2. Regimen B: Middle-intensity TBI + Fludarabine + cyclophosphamide + thiotepa

REGIMEN A: High-Dose TBI regimen (included ages: 6 months through 45 years old)
Please refer to standard practice with input from radiation oncology as needed.

Fludarabine total 75 mg/m^2 (25 mg/m^2/day IV x 3 days, days -8 to -6), Cyclophosphamide total 120 mg/kg (60 mg/kg IV x 2 days, days -7 to -6), High Dose TBI total 1320 cGy (165 cGy BID, total 8 fractions, days -4 to -1)

Day	Preparative Regimen	Supportive Care/Other
-8	Fludarabine 25 mg/m^2 IV over 30 minutes	
-7	Fludarabine 25 mg/m^2 IV over 30 minutes Cyclophosphamide 60 mg/kg IV	
-6	Fludarabine 25 mg/m^2 IV over 30 minutes Cyclophosphamide 60 mg/kg IV	
-5	Rest	
-4	TBI 165 cGy twice daily	
-3	TBI 165 cGy twice daily	Begin CSA (as per Section 6.2)
-2	TBI 165 cGy twice daily	
-1	TBI 165 cGy twice daily	
0	UCBT	Begin MMF (as per Section 6.2)
+1		Begin G-CSF (as per Section 6.4)

A. Fludarabine: 25 mg/m^2/day IV over 30 minutes x 3 days (days -8 to -6); total dose 75 mg/m^2. For patients > 120% of ideal weight, BSA will be calculated using adjusted weight.
B. Cyclophosphamide
    1. Cyclophosphamide: 60 mg/kg/Day IV x 2 days (Days -7 and -6); total dose 120mg/kg.
    2. Preparation, administration and monitoring will be according to Institutional Guidelines. If patient's actual weight is >100% of IBW, adjusted body weight should be used for calculating initial doses based on per kilogram weight, per Institutional Guidelines. MESNA will be given for bladder prophylaxis according to Institutional Guidelines. Continuous bladder irrigation is an alternative for bladder prophylaxis at the attending physician's discretion.
    3. Cyclophosphamide administration and hydration guidelines for outside centers will be reviewed and approved by FHCRC PI if varying significantly from the above. Monitoring for toxicities will be according to Institutional Guidelines.
C. Total Body Irradiation: TBI 165 cGy will be given twice daily for a total dose of 1320 cGy (days -4 to –1).

REGIMEN B: Middle-Intensity TBI regimen (included ages: 6 months through 65 years old)
Please refer to standard practice with input from radiation oncology as needed.

Fludarabine total 150 mg/m^2 (30mg/m^2 IV x 5 days, days -6 to -2), Cyclophosphamide total 50 mg/kg IV x 1 day, day - 6), Thiotepa total 10 mg/kg (5 mg/kg/day x 2 days, -5 to -40), TBI total 400 cGy (200 cGy/day x 2 days, -2 to -1)

Day	Preparative Regimen	Supportive Care/Other
-6	Fludarabine 30 mg/m^2 IV over 30-60 minutes Cyclophosphamide 50 mg/kg IV	
-5	Fludarabine 30 mg/m^2 IV over 30-60 minutes Thiotepa 5 mg/kg IV over 2-4 hours	
-4	Fludarabine 30 mg/m^2 IV over 30-60 minutes Thiotepa 5 mg/kg IV over 2-4 hours	
-3	Fludarabine 30 mg/m^2 IV over 30-60 minutes	Begin CSA (as per Section 6.2)
-2	Fludarabine 30 mg/m^2 IV over 30 minutes TBI 200 cGy once daily	
-1	TBI 200 cGy once daily	
0	UCBT	Begin MMF (as per Section 6.2)
+1		Begin G-CSF (as per Section 6.4)

A. Fludarabine: 30 mg/m^2/day IV over 30 minutes x 5 days (days -6 to -2); total dose 150 mg/m^2. For patients > 120% of ideal weight, BSA will be calculated using adjusted weight
B. Thiotepa: 5 mg/kg IV x 2 days (days -5 to -4); total dose 10 mg/kg. For subjects > 125% of ideal weight, dose will be calculated based on adjusted weight.
C. Cyclophosphamide:
    a. 50 mg/kg IV x1 day (day -6)
    b. Preparation, administration and monitoring will be according to Institutional Guidelines. If patient's actual weight is >100% of IBW, adjusted body weight should be used for calculating initial doses based on per kilogram weight, per Institutional Guidelines. MESNA will be given for bladder prophylaxis according to Institutional Guidelines. Continuous bladder irrigation is an alternative for bladder prophylaxis at the attending physician's discretion
    c. Cyclophosphamide administration and hydration guidelines for outside centers can be according to Institutional Guidelines. Monitoring for toxicities will be according to Institutional Guidelines
D. Total Body Irradiation: TBI 200 cGy will be given once daily for a total dose of 400 cGy (days -2 to –1)

6.2 Immunosuppressive Therapies

All patients will receive GVHD prophylaxis with 2 drugs as follows:

Cyclosporine (CSA)

- Patients will receive cyclosporine (CSA) therapy beginning Day -3 maintaining a level of > 200 ng/mL. For adults the initial dose will be 2.5 mg/kg IV over 1 hour every 12 hours. For children < 40 kg the initial dose will be 2.5 mg/kg IV over 1 hour every 8 hours.
- Dose adjustments will be made on the basis of toxicity and CSA levels with a targeted trough level of 200-400ng/mL. Once the patient can tolerate oral

medications and has a normal gastro-intestinal transit time, CSA will be be converted to an oral form. Refer to Institutional guidelines for conversion from IV to PO dosing, CSA dosing will be monitored and altered as clinically appropriate.

- Initial cyclosporine dose is calculated using actual body weight except for those patients who are greater than 100% ideal body weight in which case calculation of dose using adjusted body weight is recommended.
- Patients will receive CSA until Day +100. If there is no GVHD, the dose will be tapered 10% per week beginning on Day 101, to discontinue no sooner than 6months post-transplant.

Mycophenolate Mofetil (MMF)

- All patients will begin mycophenolate mofetil (MMF) on Day 0, starting approximately 4 - 6 hours after infusion of the cord blood unit(s) is completed. All patients will receive MMF at the dose of 15 mg/kg (based on adjusted weight) every 8 hours with a maximum of 1 gram/dose. If actual body weight is < ideal body weight, calculation based on actual weight is allowed. Rounding of the dose to the nearest 250 mg capsule size is also allowed.
- Use IV route between days 0 and +7; then, if tolerated, may change to PO beginning on day +8 three times a day.
- All patients will remain on three times a day as permitted after day 7 for a minimum of 30 days. At day +30 **or** 7 days after engraftment (defined as 1st day of 2 consecutive days of absolute neutrophil count [ANC] ≥ 0.5 x 10^9/L), whichever day is later, **if there is no evidence of acute GVHD and donor CD3 engraftment is at least 50% from one donor**, taper MMF to BID. At day +45 (or 15 days after engraftment if engraftment occurred > day +30), if there continues to be no evidence of acute GVHD and donor engraftment has been achieved (defined as <10% host in CD3, CD33 and CD56), taper MMF over the next two to three weeks.
- If there is no donor engraftment, do not stop MMF. If there is no evidence of donor engraftment on the day +28 bone marrow biopsy, notify the PI, Ann Dahlberg (pager: 206-469-3102) or the research coordinator, Jenna Pedersen ((206) 667-6013).

6.3 Umbilical Cord Blood Transplantation (UCBT)

- Procedures for requesting, receiving and characterizing the cord blood unit for infusion will be according to institutional protocol.
- The cord blood unit should be thawed and infused per FHCRC Standard Practice Manual. Cord blood products should be infused without delay as soon as the product arrives on the unit.
  - The thawed product (either one or two units) will be delivered to the patient floor/bedside where the product is double-checked by a nurse with the technologist from the Cellular Therapy Laboratory. Visual inspection of the product is also made at this time. The unit(s) is verified according to 1) the infusion order sheets, 2) the patient's identification number on the cell product, 3) the product (cell) identification number and 4) the patient wrist band.
  - **If the cord blood unit(s) fails to pass inspection or if there is insufficient information to verify the cell product for the patient, notify the Cell Therapy Lab ((206) 606-1200) and the PI, Ann Dahlberg ((206) 667-1959, pager (206) 469-3102) immediately.**
  - The goal infusion time of each cord blood unit is 30 minutes, as clinically possible. Pre-medications (if any) prior to cord blood infusion will be at the

discretion of the attending. Under no circumstances is the cord blood to be irradiated. No medications or fluids should be given piggyback through the catheter lumen that is being used for cord blood infusion.

- The product is infused via IV drip directly into the central line without a needle or pump.
- Vital signs should be monitored before beginning the infusion and periodically during administration. Notify the attending physician, fellow or PA immediately if the patient exhibits signs or symptoms of a reaction.
- Benadryl, epinephrine, and hydrocortisone should be available at the bedside for emergency use if necessary. Oxygen with nasal prongs for standby use should be present in the room.
- If the patient is a double cord blood recipient, the two units may be given sequentially with no wait between infusion of the units. However, infusion of the second unit will **not** begin until any acute toxicities from the first unit have been controlled. The start and stop time of each unit should be recorded on the infusion record.

6.4 Growth Factor

All patients will receive G-CSF 5 mcg/kg/day IV (pediatric patients) or SC (adult patients) (dose rounded to vial size) based on the actual body weight IV beginning on Day +1 after UCB infusion. G-CSF will be administered daily until the ANC exceeds 2.5 x 10^9/L for three consecutive days and then discontinued. If the ANC decreases to <1.0 x 10^9/L, G-CSF will be reinstituted and titrated to maintain an ANC >1.0 x 10^9/L.

6.5 Supportive Care

Patients will receive transfusions, infection prophylaxis (bacterial, fungal, CMV), and other therapy (including GVHD) according to institutional guidelines (at FHCRC this is the FHCRC Standard Practice Manual). FHCRC infection guidelines are provided to outside centers as recommendations.

6.6 Management of Pre-engraftment Immune Reactions

A well-recognized clinical entity consisting of skin rash, fever, and, often, loose stools and respiratory distress has been noted to occur prior to engraftment among cord blood patients, generally between Days +7 and +21. This clinical syndrome likely involves cytokine activation, and though clinically similar to acute or hyperacute graft versus host disease, it appears to be a distinct entity, “pre-engraftment syndrome.” This syndrome is often controlled with brief steroid bursts, thus avoiding a commitment to extended steroid exposure. Patients should be monitored carefully for this syndrome.

If patients have moderate to severe symptoms as described above and alternative etiologies (i.e., infection) have been excluded or are being appropriately evaluated, recommendations for management are:

1. For patients not on steroid therapy when the syndrome occurs: methylprednisolone should be given at 1 mg/kg IV q day for three days. If symptoms have abated, steroids should be stopped. If symptoms persist, 1 mg/kg can be continued through six days then stopped if symptoms have abated. If symptoms persist for more than six days, the patient should be considered to have acute/hyperacute GVHD and should be treated with prolonged steroids as deemed appropriate.

2. For patients already on steroids for other reasons when the syndrome occurs: methylprednisolone should be given at a dose of 3-5 mg/kg IV (max dose 500 mg) q

12 hours x 48 hours, followed by a rapid taper to 1 mg/kg IV q 12 hours. Patients should be weaned after response as tolerated.

6.7 Treatment Related Toxicities

6.7.1 Potential toxicities associated with preparative therapies

Cyclophosphamide

Common Occurs in 21-100 people out of every 100	**Less Frequent** Occurs in 5-20 people out of every 100	**Uncommon** Occurs in <5 people out of every 100
Nausea/vomiting Mucositis Sterility Severe suppression of blood counts Diarrhea Fluid weight gain or edema Alopecia	Hemorrhagic cystitis	Cardiomyopathy Skin rash SIADH (Syndrome of Inappropriate Anti-diuretic Hormone)

Fludarabine

Common Occurs in 21-100 people out of 100	**Less Frequent** Occurs in 5-20 people out of every 100	**Uncommon** Occurs in <5 people out of every 100
Severe suppression of blood counts Diarrhea Anorexia Mucositis Nausea/vomiting Stomatitis Osteoporosis Dysuria	Chills Fever GI bleeding Peripheral edema	Neurotoxicity Agitation and confusion Blurred vision Peripheral neuropathy Hearing loss Headache Cerebellar syndrome Blindness Coma Weakness Depression Insomnia Hemorrhagic cystitis (except in FA) Abnormal renal function test Autoimmune hemolytic anemia Deep venous thrombosis Aneurysms Pruritic skin rash Abnormal liver function/Liver failure Constipation Transient ischemic attack Dysphagia Myalgia Arthralgia Renal failure

Thiotepa

Likely (Over 10%)	Less Likely (1-10%)	Rare (Less than 1%)
• Low white blood cell count with an increased risk of infection (from bacteria, fungi or viruses) • Low platelet count with increased risk of bleeding • Anemia • Nausea/vomiting • Diarrhea • Anorexia (loss of appetite) • Mouth ulcers • Sores in mouth or on lips • Missing or stopping of menstrual periods in women	• Skin rash • Change in skin coloring • Fatigue, weakness • Dizziness • Headache • Permanent sterility (inability to have children)	• Allergic reactions during infusion (fever, chills, itching, hives, flushing, rash, shortness of breath, wheezing, chest tightness, muscle stiffening) • Confusion • Seizures • Liver damage • Secondary cancers

Total Body Irradiation (TBI)

Common Occurs in 21-100 people out of 100	**Less Frequent** Occurs in 5-20 people out of every 100	**Uncommon** Occurs in <5 people out of every 100
Nausea and vomiting Diarrhea Cataracts Sterility Endocrinopathies Growth failure Intestinal cramps Mucositis	Parotitis Interstitial Pneumonitis Generalized mild erythema Veno-occlusive disease	Dysphagia Vertebral deformities Nephropathy

6.7.2 Toxicities potentially associated with the infusion of the UCB graft

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

6.7.3 Potential toxicities associated with Immunosuppressive therapies

Cyclosporine (CSA)

Nephrotoxicity	Thrombotic thrombocytopenic purpura
Seizures	Electrolyte imbalances
Hypertension	Paresthesias/neuropathy
Hirsutism	Gingival hyperplasia
Increased risk of relapse	Increased risk of opportunistic infection

Mycophenolate Mofetil (MMF)

Pancytopenia	Hypertension
Headache	Dizziness
Insomnia	Hyperglycemia
Electrolyte imbalances	Rash
Leg cramps/bone pain	Nausea/diarrhea
Spontaneous abortion	Birth defects
Progressive multifocal leukoencephalopathy	

6.7.4 Toxicities associated with Neupogen (G-CSF)

Bone pain	Insomnia
Headaches	Dyspnea
Body aches	Rash
Fatigue	Edema
Nausea/vomiting	

7.0 STUDY PARAMETERS

See Appendix VIII for schedule of study evaluations.

7.1 Patient Pre-Study Screening per Standard Practice Procedures

(*Results of tests and/or procedures conducted as per standard of care for pre-transplant workups may be used for eligibility determination if conducted within an appropriate window prior to screening. In addition to procedures and evaluations listed below, additional clinical evaluations as directed by the clinical team may be captured for research purposes.)*

- Medical history of allergies, previous chemotherapy, prior radiotherapy and response to treatment
- Karnofsky/ECOG/Lansky performance (Appendix IV) status
- Physical examination
- Complete blood count with leukocyte differential
- Basic metabolic and hepatic function panels
- Urinalysis
- Pregnancy test (blood or urine)
- Viral titers (HSV, CMV, HIV, HBsAg, HBcAb, HCV, HTLV 1, 2). PCR for CMV DNA must be done within 2 weeks prior to the start of conditioning.
- Bone marrow aspirate (+/- biopsy as clinically indicated) collected less than one month prior to the start of conditioning
- Electrocardiogram and MUGA or echocardiography with measurement of the left ventricular ejection fraction (LVEF) and/or shortening fraction.
- A chest CT without contrast to exclude occult infection prior to transplant for patients with a history of the following:
    - MDS/CML blast crisis
    - 2 or more consecutive leukemia inductions
    - prolonged neutropenia, as defined as ≥ 4 weeks of neutropenia within the 2 months prior to study
- Chest radiograph; CXR not required if chest CT performed.
- Pulmonary functions tests
- DNA specimen from cord blood unit(s) for chimerism studies (submitted to CIL)

- Pediatric pre-transplant evaluation qualification: for children that are not able to cooperate to have a MUGA and/or pulmonary function tests, an echocardiogram should be attempted and pulse oximetry with exercise tolerance obtained. If not possible at all, it should clearly be documented in the physician's note.
- Quantitative immunoglobulin levels (IgG, IgA, IgM)
- Immune Reconstitution Research Study (see section 7.8).
- Blood samples for Host and Donor Immunologic Interaction Studies (see Section 7.7)

7.2 Patient Evaluations During Therapy Until Engraftment (Through Day 30)

- Physical examinations daily
- Complete blood count with leukocyte differential daily until the absolute neutrophil count (ANC) $\geq$ 5 X 10^8/L for 3 consecutive measurements. Leukocyte differential is to be performed daily if WBC count > 500.
- Basic metabolic panel daily or as clinically indicated
- Hepatic function panel as clinically indicated or per standard practice
- CMV monitoring per standard practice
- Urinalysis as clinically indicated
- Chest radiographs as clinically indicated
- Day 28 peripheral blood for chimerism studies (as possible sorted for CD3, CD14, CD33, CD56 cells)
- **For patients who received a single cord blood unit** – peripheral blood for chimerism studies (sorted for CD3, CD14, CD33, CD56 cells) on Day 28. **For patients who received double cord blood units** - peripheral blood for chimerism studies (sorted for CD3, CD14, CD33, CD56 cells) on Day 28.
- Bone marrow aspirate (+/- biopsy as clinically indicated) on Day 28 for assessment of UCB engraftment. Bone marrow DNA specimen for chimerism studies.
- Quantitative immunoglobulin levels (IgG, IgA, IgM) at Day 28
- As possible, total T lymphocytes and subset enumeration at Day 28
- GVHD evaluation (Appendix I) weekly or as clinically indicated
- Other tests (e.g., x-rays and tumor markers) as clinically appropriate for assessment of underlying malignancy on Day 28

7.3 Patient Evaluations from Engraftment through Day 100, 6 months, 1 year and 2 years

- Physical examinations weekly and/or as clinically indicated during first 100 days, 6 months, 1 year and 2 years
- Karnofsky/ECOG/Lansky performance status (Appendix IV) once between Day 80-100, at 6 months, 1 year and 2 years
- Complete blood count with leukocyte differential weekly and/or as clinically indicated through Day 100 or until discharge then at 6 months, 1 year and 2 years
- Basic metabolic panel at least weekly or as clinically indicated
- Hepatic function panel as clinically indicated
- CMV monitoring per standard practice
- Chest radiographs, EKG and pulmonary function tests as clinically indicated
- Bone marrow aspirate (+/- biopsy as clinically indicated) on Day 80 and 1 year for assessment of UCB engraftment and evidence of recurrent disease. FHCRC: DNA specimen for chimerism studies (without cell sorting)
- Peripheral blood for chimerism studies as possible (sorted for CD3+, CD33+, CD56+ cells) on Day 56, 80, 6 months, 1 year and 2 years in both single and double cord blood unit recipients

- Quantitative immunoglobulin levels (IgG, IgA, IgM) as possible at Day 56, 80, 6 months, 1 year and 2 years
- As possible, total T lymphocytes and subset enumeration on Day 56, 100, 6 months, 1 year and 2 years
- GVHD evaluation (Appendix I) weekly and/or as clinically indicated through Day 100 (or longer if clinically indicated), then at 6 months, 1 year and 2 years
- Autopsy report, if available, if death occurs before the 2-year follow-up

7.4 UCB Engraftment Evaluation

Chimerism studies will be performed on the bone marrow on Days 28, 80 and 1 year. Subsequently, if the patient's peripheral blood counts drop after an initial recovery, the peripheral blood and bone marrow should again be evaluated unless a cause has been determined (e.g., use of Ganciclovir for treatment of CMV). Patients diagnosed with graft failure as defined in section 10.2 must be reported to the Principal Investigator (Ann Dahlberg, 206-667-1959/pgr 206-469-3102).

7.5 GVHD Evaluation

GVHD assessment, done as part of routine care, will be reviewed once weekly through Day 100. Patients will be assigned an overall GVHD score based on extent of skin rash, volume of diarrhea and maximum bilirubin level. Patients will be rescored at 6 months, and at 1 and 2 years with additional scores as events occur once discharged to home.

7.6 Residual/Recurrent Disease Evaluation

Patients will be evaluated routinely for evidence of recurrent malignancy. If at any time the attending physician suspects recurrent disease, additional analyses will be performed as clinically indicated.

7.7 Immune Reconstitution after UCBT

Clinical Studies:

- Quantitative immunoglobulin levels (IgG, IgA, IgM) will be assessed at Day 28. As possible at Days 56, 80, 6 months, 1 year and 2 years.
- Total T lymphocytes and subset enumeration (Lymphocytes panel) will be performed at Day 28, 56, 100, 6 months, 1 year and 2 years.

8. Guidelines for Serious Adverse Event Reporting

8.1 Monitoring the Progress of Trial and the Safety of Participants

This is a Phase 2 clinical trial that is monitored by the principal investigator (PI), Ann Dahlberg, M.D. For FHCRC patients, the PI reviews the outcome of the data for each individual patient on an ongoing basis. The PI of the study will have primary responsibility for ensuring that the protocol is conducted as approved by the Scientific Review Committee and Institutional Review Board. The PI will ensure that the monitoring plan is followed, that all data required for oversight of monitoring are accurately reported to the Data and Safety Monitoring Committee, that all adverse events are reported according to the protocol guidelines, and that any adverse reactions reflecting patient safety concerns are appropriately reported. The PI will personally review with the Research Nurse the clinical course of all the enrolled patients at least twice monthly.

Until October 2018, this was a multi-institution trial with FHCRC serving as the Coordinating Center. In this capacity, the PI obtained copies of all IRB approvals and had the responsibility for receiving the information required for adverse event reporting and safety monitoring and disseminating that

information to the appropriate Consortium committees. Written agreements were obtained from all participating sites acknowledging their responsibilities for data and adverse event reporting and agreement to provide records, files, case report forms or any other documents needed to verify compliance. The PI reviewed outcome data for each individual patient at a minimum of 3 months after UCBT. Clinical outcome data were summarized and transmitted from collaborating centers as case report forms (CRFs). When possible, primary source documents regarding patient outcomes were collected from the collaborating centers. The CRFs were generated from the collaborating centers at defined time points (day 28, day 100, day 180, 1 year and 2 years post-transplant). The local PI reviewed the official CRF and primary source documents. When the CRFs were verified, the data were entered into a central database managed by the FHCRC Coordinating Center.

8.2. Reporting of Adverse Events

Adverse Event Definitions

Adverse Event

An Adverse Event (AE) is any untoward medical occurrence in a clinical investigation subject administered a medicinal product; the event does not necessarily have a causal relationship with study drug administration or usage. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

Serious Adverse Event

A serious adverse event (SAE) is defined as an untoward medical occurrence that results in any of the following outcomes:

- Death
- Life-threatening situation (i.e., with an immediate risk of death from the event as it occurred but not including an event that, had it occurred in a more serious form, might have caused death).
- In-patient hospitalization or prolongation of existing hospitalization. Inpatient hospitalization comprises formal admission to a hospital for medical reasons, for any length of time, whether or not hospitalization extends overnight. However, hospital admissions for administration of the study drug, procedures required by the study protocol, or tumor-related diagnostic procedures are not considered serious. Hospitalizations commonly associated with transplant including, but not limited, to febrile neutropenia, fevers, infections, and gastrointestinal toxicities will not be recorded as serious adverse events as the majority of patients receiving transplants are hospitalized.
- Persistent or significant disability/incapacity or substantial disruption of the ability to conduct normal life functions.
- Congenital anomaly/birth defect.
- An important medical event that requires intervention to prevent one of the above outcomes.

Unexpected Adverse Event

An unexpected adverse event is defined as an event that has a nature or severity, or frequency that is not consistent with the applicable investigator brochure. “Unexpected,” as used in this definition, refers to an adverse drug experience that has not been previously observed and reported rather than an experience that has not been anticipated based on the pharmacological properties of the study drug.

Recording an Adverse Event and Grading of the Severity of an Adverse Event

Adverse events will be collected and graded according to the modified (for HSCT) NCI Common Toxicity Criteria (Appendix VI). Grade 3 or 4 adverse events (or highly unusual grade 2 adverse events), which occur from the start of study treatment (pre-transplant conditioning) through Day +100 post-transplant will be collected on the Case Report Form (CRF). These adverse events, which are observed by the Investigator or reported by the patient, whether or not attributed to the study, will be reported on the CRF. Attributes will include a description, date of onset, maximum severity, and assessment of relationship to the study agent or other suspect agent(s).

Adverse events will be graded according to CTCAE version 3.0 (Appendix VI). Association or relatedness to the study agent will be graded as follows: 1 = unrelated, 2 = unlikely, 3 = possibly related, 4 = probably related, and 5 = definitely related.

If a patient experiences relapse or graft failure and goes on to further treatment off protocol, adverse events will no longer be collected with the exception of death. The adverse event reporting in this clinical trial will comply with current FHCRC reporting policy (see Appendix VII). For patients being cared for at the FHCRC, health care providers communicate with the PI, trial coordinator or research nurses as events occur triggering subsequent reporting. Toxicities meeting the study stopping rule criteria will be reported to the IRB within 10 days of study staff awareness. All other SAEs and deaths, not meeting the expedited reporting criteria, will be reported to the IRB as part of the annual continuation review report to the IRB. **For the purpose of this study, hospitalizations for protocol-scheduled procedures, blood product transfusions, or for social reasons (i.e., awaiting transport home) will not be considered serious adverse events. Hospitalization will be considered a serious adverse event if it is unexpected or the duration of the hospital stay is unexpected**.

Refer to Appendix III for a list of potential adverse events associated or expected with hematopoietic cell transplantation. PI and the research study team have fulfilled all NIH requirements for training in human subjects protection.

8.3. Plans for assuring data accuracy and protocol compliance

For study enrollment, a signed consent form and eligibility checklist with source documents must demonstrate study eligibility. Fred Hutch Cord Blood Program research staff review CRFs for adherence to the protocol, accuracy, and completeness. The study is monitored under the FHCRC Monitoring Plan. The FHCRC Data and Safety Monitoring Plan details the full scope and extent of monitoring and provides for immediate action in the event of the discovery of major deviations.

8.4. Oversight and Review of Safety Monitoring

An annual review of the progress of the study with respect to the monitoring plan will be performed by a Data and Safety Monitoring Committee (DSMC). As part of the annual renewal process, the PI will submit an accounting of patient enrollment and outcomes defining the monitoring plan in sufficient detail as to permit verification of the report through chart audit. The IRB provides a final level of annual review. Approval by the DSMC is a necessary but not sufficient condition for final approval by the IRB. The IRB will review the same continuation application and materials that are reviewed by the DSMC, and make an independent assessment of the progress of the trial and determine whether the perceived risk-benefit ratio continues to be acceptable.

9.0 RECORDS

Clinical Research Data Systems maintains a patient database at FHCRC to allow storage and

retrieval of patient data collected from a wide variety of sources. The investigator will ensure that the data collected conform to all established guidelines for coding, collection, key entry and verification. Each patient is assigned a unique patient number to assure patient confidentiality. Patients will not be referred to by this number, by name, or by any other individual identifier in any publication or external presentation. The licensed medical records department, affiliated with the institution where the patient receives medical care, maintains all original inpatient and outpatient chart documents. Patient research files are kept in a locked room. Patient research files are scanned and stored in a secure database and maintained by the Fred Hutch data abstraction staff. Access is restricted to personnel authorized by the Division of Clinical Research.

10.0 STATISTICAL CONSIDERATIONS

10.1 Objectives

Primary objective

The principal aim of this study is to estimate one-year overall survival of UCBT after a myeloablative preparative regimen for the combined experience of single unit UCB transplants and double unit UCB transplants.

Secondary objectives

- Transplant related mortality at 6 months
- Chimerism at multiple time points
- Neutrophil engraftment at Day 42
- Platelet recovery at 6 months
- Acute GvHD
- Chronic GvHD
- Clinically significant infections at multiple time points
- Relapse at one and two years
- Progression-free survival at one and two years
- Comparison of single unit UCB transplants with historical controls
- Comparison of single unit UCB transplants with double unit UCB transplants

10.2 Statistical Analysis

The primary objective of this study is to gain experience with both double- and single-unit UCBT's and to estimate the one-year overall survival among patients treated on this study. A non-statistical comparison with historical controls will be made for the endpoints survival and progression-free survival, and the doubles and singles will be assessed separately for these purposes. In addition, we have added a middle-intensity conditioning regimen as described above, this allowing for inclusion of patients > 45 years of age. In addition to assessing results by double vs. single, results will also be examined according to conditioning intensity. Historically, the survival at two years for single-unit UCBT's has ranged from 26-74% [13]. At FHCRC, patients transplanted for CML, acute leukemia, or MDS from an unrelated donor and matched for 10 of 10 alleles have an estimated 2-year survival of 57%; those matched for 9/10 alleles have an estimated 2-year survival of 49%, and those matched for 8/10 alleles have an estimated 2-year survival of 46%. Based on the numbers, we shall use 50% survival at 1 year as the rough benchmark for the current study. We plan to enroll 135 patients, and we expect that approximately 2/3 of these will be double-unit UCBT's. The first 75 patients (or so) received the high-intensity regimen, and we expect the remaining 60 to be distributed equally between the high-dose and middle-intensity regimens. This would result in roughly 105 high-dose and 30 middle-intensity patients, and approximately 90 double-cord recipients and 45 single-cord recipients. Combining each of these, we expect approximately 70 patients to

receive high-dose TBI and a double cord, 35 to receive high-dose TBI and a single cord, 20 to receive middle-dose TBI and a double cord, and 10 to receive middle-dose TBI and a single cord. If the observed 1-year survival for a particular arm is at least 10 percentage points better than the historical benchmark, that arm will be considered to be worthy of further study. The table below shows the probability of observing a 1-year survival that is at least 10 percentage points higher than the benchmark of 50%. The table contains various sample sizes and various assumed-true 1-year survival rates (the varying sample sizes correspond to the expected numbers based on distributions conditioning and number of cords).

N	Group	True 1-year OS	Probability observe > 50% OS
105	High-dose TBI	.65	.88
30	Medium-intensity TBI	.70	.92
90	Double cords	.65	.86
45	Single cords	.65	.81
70	High-dose, doubles	.65	.84
35	High-dose, singles	.70	.93
20	Med-intensity, doubles	.70	.89
10	Med-intensity, singles	.75	.92

Graft failure by Day 42 and transplant-related mortality by day 100 will be carefully monitored throughout the study, and if reasonable evidence exists suggesting that failure for either of these endpoints is excessive, the study will be stopped. Monitoring will take place separately for the single and double UCBT cohorts. Patients will be considered primary graft failure/rejections provided they meet any criteria listed below:

i. Absence of 3 consecutive days with neutrophils ≥500/ul combined with host CD3 peripheral blood chimerism ≥ 50% at day 42
ii. Absence of 3 consecutive days with neutrophils ≥500/ul under any circumstances at day 55
iii. Death after day 28 with neutrophil count <100/ul without any evidence of engraftment (< 5% donor CD3)
iv. Primary autologous count recovery with < 5% donor CD3 peripheral blood chimerism at count recovery and without relapse

A true graft failure rate of 15% will be considered excessive, and a true Day 100 TRM rate of 20% will be considered excessive, and reasonable evidence will be taken to be a ratio of failures to patients treated with a lower limit to the corresponding 80% confidence interval in excess of these rates. These ratios will be assessed after every 5th enrolled patient becomes evaluable and will be assessed separately between the four conditioning/cord-number groups.

Operationally, the stopping limits will be met if any of the following ratios occur:

Graft Failure: 2/5, 3/10, 4/15, 5/20, 6/25, 7/30, 8/35, 9/40, 10/45, 11/50, 11/55, 12/60, 13/65, 14/70

TRM: 3/5, 4/10, 5/15, 6/20, 8/25, 9/30, 10/35, 11/40, 12/45, 13/50, 14/55, 16/60, 17/65, 18/70

If the true probability of graft failure is 5%, then the probability of stopping the study after 20, 40, or 60 patients is approximately 0.03, 0.03, and 0.03, respectively. If the true probability is 25%, these stopping probabilities are approximately 0.70, 0.84, and 0.91, respectively. (It is not expected that 60 patients will be enrolled in either arm, but theoretically it is possible.) If the true probability of TRM is 15%, then the probability of stopping after 20, 40, or 60 patients is 0.11, 0.12, and 0.13, respectively, while a true probability of 30% leads to stopping probabilities of 0.66, 0.81, and 0.89.

Secondary endpoints listed in Section 10.1 will also be evaluated. Cumulative incidence estimates will be used to summarize the time-to-event outcomes.

We anticipate that the data from this study will be useful in the design of future studies, these future studies hopefully culminating in a Phase III trial comparing UCBT to some other alternative source of stem cells. If the data from this trial suggest favorable outcomes as detailed above, then the appropriate groups will be considered for potential inclusion on the clinical trial(s) as appropriate.

10.3 Gender and Ethnicity Statement

This study is open to both males and females and to all racial/ethnic groups. The patient enrollment pattern is expected to be similar to that of other hematological malignancy studies. It is not anticipated that the outcome will be affected by either race or gender. The study will not have separate accrual targets for different subgroups.

11.0 REFERENCES

1. Blume, K., S.J. Forman, and F.R. Appelbaum, *Thoma's Hematopoietic Cell Transplantation*. 3nd ed. 2004, Malden, MA: Blackwell Science, Inc.
2. Confer, D.L., *Unrelated marrow donor registries.* Curr Opin Hematol, 1997. **4**(6): 408-12.
3. Wagner, J.E., N.A. Kernan, M. Steinbuch, et al., *Allogeneic sibling umbilical-cord-blood transplantation in children with malignant and non-malignant disease.* Lancet, 1995. **346**(8969): 214-9.
4. Wagner, J.E., J. Rosenthal, R. Sweetman, et al., *Successful transplantation of HLA-matched and HLA-mismatched umbilical cord blood from unrelated donors: analysis of engraftment and acute graft-versus-host disease.* Blood, 1996. **88**(3): 795-802.
5. Kurtzberg, J., M. Laughlin, M.L. Graham, et al., *Placental blood as a source of hematopoietic stem cells for transplantation into unrelated recipients.* N Engl J Med, 1996. **335**(3): 157-66.
6. Gluckman, E., V. Rocha, A. Boyer-Chammard, et al., *Outcome of cord-blood transplantation from related and unrelated donors. Eurocord Transplant Group and the European Blood and Marrow Transplantation Group.* N Engl J Med, 1997. **337**(6): 373-81.
7. Rubinstein, P., C. Carrier, A. Scaradavou, et al., *Outcomes among 562 recipients of placental-blood transplants from unrelated donors.* N Engl J Med, 1998. **339**(22): 1565-77.
8. Laughlin, M.J., J. Barker, B. Bambach, et al., *Hematopoietic engraftment and survival in adult recipients of umbilical-cord blood from unrelated donors.* N Engl J Med, 2001. **344**(24): 1815-22.
9. Laughlin, M.J., M. Eapen, P. Rubinstein, et al., *Outcomes after transplantation of cord blood or bone marrow from unrelated donors in adults with leukemia.* N Engl J Med, 2004. **351**(22): 2265-75.
10. Rocha, V., M. Labopin, G. Sanz, et al., *Transplants of umbilical-cord blood or bone marrow from unrelated donors in adults with acute leukemia.* N Engl J Med, 2004. **351**(22): 2276-85.
11. Wagner, J.E., J.N. Barker, T.E. DeFor, et al., *Transplantation of unrelated donor umbilical cord blood in 102 patients with malignant and nonmalignant diseases: influence of CD34 cell dose and HLA disparity on treatment-related mortality and survival.* Blood, 2002. **100**(5): 1611-8.
12. Barker, J.N., D.J. Weisdorf, T.E. DeFor, et al., *Transplantation of 2 partially HLA-matched umbilical cord blood units to enhance engraftment in adults with hematologic malignancy.* Blood, 2005. **105**(3): 1343-7.
13. Schoemans, H., et al., *Adult umbilical cord blood transplantation: a comprehensive review*, Bone Marrow Transplantation, 2006. **38**(2): 83-93.
14. Ponce D, Sauter C, Devlin S, Lubin M, Gonzales A, Kernan N, Scaradavou A, Giralt S, Goldberg J, Koehne G, Perales M, Young J, Castro-Malaspina H, Jakubowski A, Papadopoulos E, Barker J. *A Novel Reduced-Intensity Conditioning Regimen Induces a High Incidence of Sustained Donor-Derived Neutrophil and Platelet Engraftment afterDouble-Unit Cord Blood Transplantation*. BBMT, Feb 2013; 19: 799-803.

Appendix I
GVHD Staging and Grading

ACUTE GVHD ASSESSMENT
Staging by Individual Organ Involvement

SKIN: measured by rash first appearing generally between 10 and 70 days after transplant. (excludes rashes of known viral or other origin)

Stage	Description
1	Maculopapular rash <25% BSA
2	Maculopapular rash 25 – 50% BSA
3	Generalized erythroderma
4	Generalized erythroderma with bullous formation and desquamation

LIVER*: measured by total serum bilirubin

Stage	Description
1	2.0 – 2.9 mg/dL
2	3.0 – 5.9 mg/dL
3	6.0 – 14.9 mg/dL
4	≥ 15.0 mg/dL

GUT:** includes only diarrhea occurring after Day +21

Score	Adult	Pediatric***
1	upper GI (anorexia, nausea, vomiting) with diarrhea of <1000 mL/day	upper GI (anorexia, nausea, vomiting) with diarrhea of <555 mL/m^2/day
2	1000 – 1499 mL/day diarrhea	556-833 mL/m^2/day diarrhea
3	≥ 1500 mL/day diarrhea	>833 mL/m^2/day diarrhea
4	severe abdominal cramping, bleeding or ileus caused by GVHD	

* In cases where another cause of hyperbilirubinemia antedated the onset of rash, the liver score should be decreased by one stage.

** In cases where peak GI symptoms are exacerbated by a cause other than GVHD, the gut score should be decreased by one stage.

*** Pediatric patients <17 years of age

APPENDIX I (continued)
GVHD Staging and Grading

ACUTE GVHD ASSESSMENT
Overall Grade

The determination of an overall GVHD grade should be based on the organ stage, response to treatment and whether GVHD was a major cause of death.

Overall Grade	Organ Stage	Qualifying Conditions	Additional Qualifying Conditions
I	Stage 1 -2 skin	No liver or gut	Indicates that the prophylactic immunosuppressive regimen was not sufficient to prevent all manifestations of aGVHD.
II	Stage 3 skin or Stage 1 liver or Stage 1 gut	N/A	Indicates that the prophylactic immunosuppressive regimen was not sufficient to prevent all manifestations of aGVHD, but glucocorticoid treatment after the onset of GVHD was generally sufficient to control the disease.
III	Stage 4 skin or Stage 2-4 liver or Stage 2-4 gut	**without** GVHD as a major contributing cause of death	Indicates that the prophylactic immunosuppressive regimen was not sufficient to prevent all manifestations of aGVHD and that additional treatment after the onset of GVHD did not readily control the disease.
IV	Stage 4 skin or Stage 2-4 liver or Stage 2-4 gut	**with** GVHD as a major contributing cause of death	GVHD was resistant to both the prophylactic immunosuppressive regimen and any additional treatment after the onset of the disease.

References:

1. Leisenring, WM, Martin, PJ, Petersdorf, EW, et al. An acute graft-versus-host-disease activity index to predict survival after hematopoietic cell transplantation with myeloablative conditioning regimens, Blood, 2006:108: 749-755.
2. Przepiorka D, Weisdorf D, Martin PJ, et al. 1994 Consensus Conference on Acute GVHD Grading. Bone Marrow Transplant. 1995:15: 825-828.

APPENDIX I (continued)
GVHD Staging and Grading

CHRONIC GVHD
Patients who develop clinical and laboratory findings of chronic GVHD will be classified according to the schema outlined below:

Clinical Limited cGVHD

1. Oral abnormalities consistent with cGVHD, a positive skin or lip biopsy, and no other manifestations of cGVHD.
2. Mild liver test abnormalities (alkaline phosphatase ≤2 x upper limit of normal, AST or ALT ≤3 x upper limit of normal and total bilirubin ≤1.6) with positive skin or lip biopsy, and no other manifestations of cGVHD.
3. Less than 6 papulosquamous plaques, macular-papular or lichenoid rash involving <20% of body surface area (BSA), dyspigmentation involving <20% BSA, or erythema involving <50% BSA, positive skin biopsy, and no other manifestations of cGVHD.
4. Ocular sicca (Schirmer's test ≤5mm with no more than minimal ocular symptoms), positive skin or lip biopsy, and no other manifestations of cGVHD.
5. Vaginal or vulvar abnormalities with positive biopsy, and no other manifestations of cGVHD.

Clinical Extensive cGVHD

1. Involvement of two or more organs with symptoms or signs of cGVHD, with biopsy documentation of cGVHD in any organ.
2. More than 15% loss of base line body weight not due to other causes, with biopsy documentation of cGVHD in any organ.
3. Skin involvement more extensive than defined for clinical limited cGVHD, confirmed by biopsy.
4. Scleroderma or morphea.
5. Onycholysis or onychodystrophy thought to represent cGVHD, with documentation of cGVHD in any organ
6. Decreased range of motion in wrist or ankle extension due to fasciitis caused by cGVHD.
7. Contractures thought to represent cGVHD.
8. Oral involvement with functional impairment, refractory to topical treatment.
9. Vaginal involvement with functional impairment, refractory to topical treatment.
10. Bronchiolitis obliterans not due to other causes.
11. Positive liver biopsy; or abnormal liver function tests not due to other causes with alkaline phosphatase >2 x upper limit of normal, AST or ALT >3 x upper limit of normal, or total bilirubin >1.6, and documentation of cGVHD in any organ.
12. Positive upper or lower GI biopsy
13. Fasciitis or serositis thought to represent cGVHD and not due to other causes

APPENDIX II: The Hematopoietic Cell Transplant-Comorbidity Index (HCT-CI)

Instructions: Circle applicable scores and provide actual value or cause of' co-morbidity.

Comorbidities	Definitions	HCT-CI weighted scores	Actual Lab Values/Comments
Arrhythmia	Atrial fibrillation or flutter, sick sinus syndrome, and ventriuclar arrhythmias	1	
Cardiac	Coronary artery disease‡, congestive heart failure, myocardial infarction, or EF≤50%	1	
Inflammatory bowel disease	Crohn's disease or ulcerative colitis	1	
Diabetes*	Requiring treatment with insulin or oral hypoglycemics, but not diet alone	1	
Cerebro-vascular disease	Transient ischemic attack or cerebro-vascular a ccident	1	
Psychiatric Disturbance	Depression anxiety requiring psychiatric consult or treatment	1	
Hepatic -mild*	Chronic hepatitis, Bilirubin >ULN- 1.5 X ULN,or AST/ALT >ULN-2.5XULN	1	
Obesity*	Patients with a body mass index > 35kg/ m^2	1	
Infection*	Requiring continuation of anti-microbial Treatment after day 0	1	
Rheumatologic	SLE, RA, polymyositis, mixed CTD Polymyalgia rheumatica	2	
Peptic ulcer*	Requiring treatment	2	
Moderate/severe renal*	serum creatinine>2mg/dl, on dialysis, or prior renal transplantation	2	
Moderate pulmonary*	DLCO and/or FEV1, 66%-80% or Dyspnea on slight activity	2	
Prior solid tumor	*Treated at any time point in the patient's past history, excluding non-melanoma skin cancer*	3	
Heart valve disease*	Except mitral valve prolapse	3	
Severe pulmonary*	DLCO and/or FEV_1 ≤65% or dyspnea at rest requiring oxygen	3	
Moderate/severe Hepatic	Liver cirrhosis, Bilirubin>1.5XULN or AST/ALT>2.5XULN	3	
Please provide Karnofksy performance Score (KPS) =_____%		**Total Score =_____**	

Completed by (Print): _____ Date: _____

Signature: _____

*Comorbidity is currently active or patient requires medical treatment +
‡One or more vessel-coronary artery stenosis, requiring medical treatment, stent, or bypass graft
EF indicates ejection fraction; ULN, upper limit of normal; SLE, systemic lupus erythematosus; RA, rheumatoid arthritis; CTD, connective tissue disease; DLCO, diffusion capacity of carbon monoxide; FEV_1, forced expiratory volume in one second; AST, aspartate aminotransfcrase; ALT, alanine aminotransferas

APPENDIX III

Potential Adverse Events Associated or Expected with Hematopoietic Cell Transplantation

1. Graft versus host disease: GVHD is a major toxicity associated with the infusion of allogeneic donor stem cells. GVHD may be acute or chronic and may affect multiple organ systems, including the skin, liver, and GI tract.

2. Opportunistic infections, including viral and fungal infections, can results in severe pulmonary, neurologic, hepatic and other organ dysfunction, and possible death.

3. Gastrointestinal toxicity. Nausea and vomiting can be anticipated during the entire course of ablative therapy. Mucositis and diarrhea should be expected. Prednisone can cause GI bleeding.

4. Cardiac toxicity. Cardiac toxicity (congestive heart failure, pericardial effusion, EKG changes) is uncommonly associated with the chemotherapy agents and TBI used in the regimen and these sequelae may prove lethal.

5. Pulmonary toxicity. Diffuse interstitial pneumonitis of unknown etiology occurs with some regularity after BMT and interstitial fibrosis occurs much more rarely. Both are well-described complications of intensive chemotherapy and TBI regimens and may prove lethal.

6. Hepatic toxicity. Veno-occlusive disease of the liver is a common toxicity of high-dose chemoradiotherapy and may result in death. Cyclosporine may cause elevation of ALT/AST.

7. Renal dysfunction. Chemoradiotherapy may uncommonly cause renal dysfunction. More commonly, nephrotoxicity results from cyclosporine and generally responds to dose reduction. Rarely, idiopathic or calcineurin inhibitor-associated hemolytic-uremic syndrome may occur and may be progressive and fatal. A syndrome of moderate renal insufficiency and hemolysis has been seen 5-7 months post HSCT after intensive multi-agent conditioning plus TBI.

8. Hemorrhagic cystitis, manifested either as gross or microscopic hematuria, is a common toxicity after high-dose chemoradiotherapy, but usually associated with regimens that include cyclophosphamide. Hemorrhagic cystitis may predispose to a long-term increased risk of bladder cancer.

9. Central nervous system toxicity. Radiation and chemotherapy can cause CNS toxicity, including seizures, depressed mental status, or leukoencephalopathy. Calcineurin inhibitors can cause seizures or other CNS toxicity.

10. Marrow aplasia. Severe neutropenia, thrombocytopenia, and anemia, is expected to occur for a period of 7 to 42 days following infusion of marrow. Transfusion of platelets and red blood cells is expected as supportive care. Transfusion of blood products may be associated with acquisition of HIV or a hepatitis virus. Neutropenia may increase the risk for acquiring serious infection. Thrombocytopenia may increase the risk of life-threatening hemorrhage. Hemorrhagic or infectious complications during the expected period of aplasia may result in death.

11. Miscellaneous. Alopecia and sterility are expected complications of the program as a whole. Cataract development is possible after TBI and/or steroids. Deficiencies of growth hormone, thyroid hormone, and sex hormones are possible after TBI. Calcineurin inhibitors can cause transient gingival hyperplasia, tremor, seizure, hypertension, headache, dysesthesia and hirsutism. Steroid therapy can also contribute to fluid retention, easy bruising, hypertension, aseptic necrosis of bone and increased susceptibility to infection.

MMF can cause spontaneous abortions and birth defects. Hospitalization during conditioning and recovery period is expected to be 5-9 weeks in duration.

APPENDIX IV - Karnofsky and Lansky Performance Status Scales

ECOG Performance Status Scale		Karnofsky Performance Scale	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

LANSKY PLAY PERFORMANCE STATUS SCALE

Percentage	
100	Fully active, normal
90	Minor restrictions in physically strenuous activity
80	Active, but tires more quickly
70	Both greater restriction of, and less time spent in, play activities
60	Up and around, but minimal active play; keeps busy with quieter activities
50	Gets dressed but lies around much of the day, no active play; able to participate in all quiet play and activities
40	Mostly in bed; participates in quiet activities
30	In bed; needs assistance even for quiet play
20	Often sleeping; play entirely limited to very passive activities
10	Unresponsive
0	Dead

APPENDIX V
Umbilical Cord Blood Graft Selection

CB donor selection will be based on institutional guidelines and in general should be selected to optimize both HLA match and cell dose. Additionally, CB grafts shall consist of one or two CB donors based on, but not exclusively determined by, cell dose (TNC/kg and CD34/kg), HLA matching and disease status and indication for transplant. Attending preference will be allowed for single versus double unit as well as the degree of mismatching based on patient specific factors, as long as the following minimum criteria are met:

A. HLA matching:
   i. Minimum requirement: The CB graft(s) must be matched at a minimum at 4/6 HLA-A, B, DRB1 loci with the recipient. Therefore 0-2 mismatches at the A or B or DRB1 loci based on intermediate resolution A, B antigen and DRB1 allele typing for determination of HLA-match is allowed.
   ii. HLA-matching determined by high resolution typing is allowed per institutional guidelines as long as the minimum criteria (#A.i., above) are met.

B. Selection of two CB units is mandatory when a single cord blood unit does not meet the following criteria in the table below.

	Single Unit Allowed for:
Match Grade	**TNC Dose**
6/6	≥ 2.5 x 10^7/kg
5/6, 4/6	≥ 4.0 (± 0.5) x 10^7/kg

If two CB units are used, the total cell dose of the combined units must be at least 3.0 x 10^7 TNC per kilogram recipient weight based on pre-cryopreservation numbers, with each CB unit containing a MINIMUM of 1.5 x 10^7 TNC/kg.

C. The minimum *recommended* CD34/kg cell dose should be 2 x 10^5 CD34/kg, total dose from a single or combined double.

D. The unmanipulated CB unit(s) will be FDA licensed or will be obtained under a separate IND, such as the National Marrow Donor Program (NMDP) Protocol 10-CBA conducted under BB IND-7555 or another IND sponsored by (1) a participating institution or (2) an investigator at FHCRC or one of the participating institutions.

E. **FHCRC only:** Up to 5% of the unmanipulated cord blood product (s), when ready for infusion, may be withheld for research purposes as long as thresholds for infused TNC dose are met. These products will be used to conduct studies involving the kinetics of engraftment and immunobiology of double cord transplantation.

UCB Unit Exclusions

1. Any cord blood units with <1.5 x 10^7 total nucleated cells per kilogram recipient weight.
2. Any cord blood units without full maternal testing and negative results for hepatitis B, C, HIV, and HTLV-1 viruses. Any additional available virology results on the unit itself will be reviewed but are not mandated, complete or always available. Cord blood units are presumed to be CMV negative regardless of serologic testing due to passive transmission of maternal CMV antibodies.

APPENDIX VI: Adapted from COMMON TOXICITY CRITERIA (CTC)

ALLERGY/IMMUNOLOGY		
Adverse Event	**Grade 3**	**Grade 4**
Allergic reaction/ hypersensitivity (including drug fever)	Symptomatic bronchospasm, requiring parenteral medication(s), with or without urticaria; allergy related edema/angioedema	Anaphylaxis
Vasculitis Requiring steroids	Requiring steroids	Ischemic changes or requiring amputation
Allergy/Immunology – Other (specify):_____	Severe	Life-threatening or disabling
BLOOD/BONE MARROW		
Adverse Event	**Grade 3**	**Grade 4**
Hemolysis (e.g., immune hemolytic anemia, drug related hemolysis, other)	Requiring transfusion and/or medical intervention (e.g., steroids)	Catastrophic consequences of hemolysis (e.g., renal failure, hypotension, bronchospasm, emergency splenectomy)
For BMT studies, if specified in the protocol. For pediatric BMT studies, if specified in the protocol.	>4 u pRBC in 24 hours >30mL/kg in 24 hours	Hemorrhage or hemolysis associated with life-threatening anemia; medical intervention required to improve hemoglobin Hemorrhage or hemolysis associated with life-threatening anemia; medical intervention required to improve hemoglobin
CARDIOVASCULAR - ARRHYTHMIA		
Adverse Event	**Grade 3**	**Grade 4**
Cardiovascular/Arrhythmia - Other (specify): _____	Symptomatic, and requiring treatment of underlying cause	Life-threatening (e.g., arrhythmia associated with CHF, hypotension, syncope, shock)
CARDIOVASCULAR – GENERAL		
Adverse Event	**Grade 3**	**Grade 4**
Cardiac left ventricular function	CHF responsive to treatment	Severe or refractory CHF or requiring intubation
Cardiac troponin I (cTnI)	Levels consistent with unstable angina as defined by the manufacturer	Levels consistent with myocardial infarction as defined
Cardiac troponin T (cTnT)	≥ 0.1 - <0.2ng/mL	≥ 0.2ng/mL
Hypotension	Requiring therapy and sustained medical attention, but resolves without persisting physiologic consequences	Shock (associated with acidemia and impairing vital organ function due to tissue hypoperfusion)
Myocarditis	CHF responsive to treatment	Severe or refractory CHF
Pericardial effusion/ pericarditis	With physiologic consequences	Tamponade (drainage or pericardial window required)
Syncope (fainting) is graded in the Neurology category.	-	-
Thrombosis/embolism	Deep vain thrombosis, requiring anticoagulant therapy	Embolic event including pulmonaryembolism
Vein/artery operative injury is graded as Operative injury of vein/artery in the Cardiovascular (general) category. Other (specify):_____		

Cardiovascular/General –	Severe	Life-threatening or disabling
COAGULATION		
Adverse Event	**Grade 3**	**Grade 4**
DIC (disseminated intravascular coagulation) Also consider Platelets. *Note: Must have increased fibrin split products or D-dimer in order to grade as DIC*	Laboratory findings present with no bleeding	Laboratory findings and bleeding
Thrombotic microangiopathy (e.g., thrombotic thrombocytopenic purpura/TTA or hemolytic uremic syndrome/HUS) Also consider Hemoglobin, platelets, creatinine. *Note: Must have microangiopathic changes on blood smear (e.g., schistocytes, helmet cells, red cell fragments).*	Laboratory findings present without clinical consequences Evidence of RBC destruction with creatinine (>3 x ULN) not requiring dialysis	Laboratory findings and clinical consequences, (e.g., CNS hemorrhage/bleeding or thrombosis/embolism or renal failure) requiring therapeutic intervention Evidence of RBC destruction with renal failure requiring dialysis and/or encephalopathy.
Coagulation - Other (specify): _____	Severe	Life-threatening or disabling
CONSTITUTIONAL SYMPTOMS		
Adverse Event	**Grade 3**	**Grade 4**
Weight gain associated with Veno-Occlusive Disease (VOD) for BMT studies, if specified in the protocol. <u>Also consider</u> Ascites Edema, Pleural effusion (non-malignant).	>10% or as ascites	>10% or fluid retention resulting in pulmonary failure
DERMATOLOGY/SKIN		
Adverse Event	**Grade 3**	**Grade 4**
Erythema multiforme (e.g., Stevens-Johnson syndrome, toxic epidermal necrolysis)	Severe or requiring IV fluids (e.g., generalized rash or painful stomatitis)	Life-threatening (e.g., exfoliative or ulcerating dermatitis or requiring enteral or parenteral nutritional support)
Rash/desquamation associated with graft versus host disease (GVHD) for BMT studies, if specified in the protocol.	Symptomatic generalized erythroderma or symptomatic macular, papular or vesicular eruption, with bullous formation, or desquamation covering ≥50% of body surface area.	Generalized exfoliative dermatitis or ulcerative dermatitis or bullous formation
GASTROINTESTINAL		
Adverse Event	**Grade 3**	**Grade 4**
Ascites (none-malignant)	Symptomatic, requiring therapeutic paracentesis	Life-threatening physiologic consequences
Colitis <u>Also consider</u> Hemorrhage/ bleeding with grade 3 or 4 thrombocytopenia,	Abdominal pain, fever, change in bowel habits with ileus or peritoneal signs, and radiographic or biopsy documentation	Perforation or requiring surgery or toxic megacolon

hemorrhage/bleeding without grade 3 or 4 thrombocytopenia, melena/GI bleeding, rectal bleeding/hematochezia, hypotension.		
Diarrhea associated with graft versus host disease (GVHD) for BMT studies, if specified in the protocol. *For pediatric BMT studies, if specified in the protocol.* <u>Also consider</u> Hemorrhage/ bleeding with grade 3 or 4 thrombocytopenia, hemorrhage/bleeding without grade 3 or 4 thrombocytopenia, pain, dehydration, hypotension.	>1500mL of diarrhea/day *>15mL/kg of diarrhea/day*	Severe abdominal pain with or without ileus
Duodenal ulcer (requires radiographic or endoscopic documentation)	Uncontrolled by outpatient medical management; requiring hospitalization	Perforation or bleeding, requiring emergency surgery
Gastric ulcer (requires radiographic or endoscopic documentation) <u>Also consider</u> Hemorrhage/bleeding with grade 3 or 4 thrombocytopenia, hemorrhage/bleeding without grade 3 or 4 thrombocytopenia.	Bleeding without perforation, uncontrolled by outpatient medical management; requiring hospitalization or surgery	Perforation or bleeding, requiring emergency surgeryv
Gastritis <u>Also consider</u> Hemorrhage/bleeding with grade 3 or 4 thrombocytopenia, hemorrhage/bleeding without grade 3 or 4 thrombocytopenia.	Uncontrolled by out-patient medical management; requiring hospitalization or surgery	Life-threatening bleeding, requiring emergency surgery
Pancreatitis <u>Also consider</u> Hypotension. *Note: Amylase is graded in theMETABOLIC/LABORATORY category.*	Abdominal pain with pancreatic enzyme elevation	Complicated by shock (acute circulatory failure)
Mucositis *Note: Radiation-related mucositis is graded as Mucositis due to radiation.*	Painless erythema, edema, or ulcers preventing swallowing or requiring hydration or parenteral (or enteral) nutritional support	Severe ulceration requiring prophylactic intubation or resulting in documented aspiration pneumonia
Typhlitis (inflammation of the cecum) <u>Also consider</u> Hemorrhage/bleeding with grade 3 or 4 thrombocytopenia, hemorrhage/bleeding without	Abdominal pain, diarrhea, fever, and radiographic or biopsy documentation	Perforation, bleeding or necrosis or other life-threatening complication requiring surgical intervention (e.g., colostomy)

grade 3 or 4 thrombocytopenia, hypotension, febrile neutropenia.		
HEMORRHAGE		
Notes: *Transfusion in this section refers to pRBC infusion.* *For any bleeding with grade 3 or 4 platelets (<50,000), always grade Hemorrhage/bleeding with grade 3 or 4 thrombocytopenia. Also consider Platelets, Transfusion: pRBCs, and Transfusion: platelets in addition to grading severity by grading the site or type of bleeding.* *If the site or type of Hemorrhage/bleeding is listed, also use the grading that incorporates the site of bleeding: NS Hemorrhage/bleeding, Hematuria, Hematemesis, Hemoptysis, Hemorrhage/bleeding with surgery, Melena/lower GI bleeding, Petechiae/purpura (Hemorrhage/bleeding into skin), Rectal bleeding/ hematochezia, Vaginal bleeding.*		
Adverse Event	**Grade 3**	**Grade 4**
Hemorrhage/bleeding with grade 3 or 4 theombocytopenia Also consider Platelets, hemoglobin, transfusion: platelets, transfusion: pRBCs, site or type of bleeding. If the site is not listed, grade as Hemorrhage – Other (specify site):_____ *Note: This adverse event must be graded for any bleeding with grade 3 or 4 thrombocytopenia.*	Requiring transfusion	Catastrophic bleeding, requiring major non-elective intervention
CNS hemorrhage/bleeding	Bleeding noted on CT or other scan with no clinical consequences	Hemorrhagic stroke or hemorrhagic vascular event (CVA) with neurologic signs and symptoms
Hemoptysis	Requiring transfusion	Catastrophic bleeding, requiring major non-elective intervention
Melena/GI bleeding	Requiring transfusion	Catastrophic bleeding, requiring major non-elective intervention
Rectal bleeding/hematochezia	Requiring transfusion	Catastrophic bleeding, requiring major non-elective intervention
Vaginal bleeding	Requiring transfusion	Catastrophic bleeding, requiring major non-elective intervention
Hemorrhage – Other (specify site): _____	Requiring transfusion	Catastrophic bleeding, requiring major non-elective intervention
HEPATIC		
Adverse Event	**Grade 3**	**Grade 4**
Bilirubin Bilirubin associated with graft versus host disease (GVHD) for BMT studies, if specified in the protocol.	>3.0 – 10.0 x ULN >6 - <15mg/100mL	>10.0 x ULN >15mg/100mL
INFECTION/FEBRILE NEUTROPENIA		
Adverse Event	**Grade 3**	**Grade 4**
Febrile neutropenia (fever or unknown origin without clinically or microbiologically documented infection).	Present	Life-threatening sepsis (e.g., septic shock)
Infection/Febrile Neutropenia –	Severe	Life-threatening or disabling

Other (specify): _____		
NEUROLOGY		
Aphasia, receptive and/or expressive, is graded under Speech impairment in the NEUROLOGY category.		
Adverse Event	**Grade 3**	**Grade 4**
CNS cerebrovascular ischemia	Transient ischemic event or attack (TIA)	Permanent event (e.g., cerebral vascular accident)
Leukoencephalopathy associated radiological findings	Severe increase in SAS; severe ventriculomegaly; near total white matter T2 hyperintensities or diffuse low attenuation (CT); focal white matter necrosis (cystic)	Severe increase in SAS; severe ventriculomegaly; diffuse low attenuation with calcification (CT); diffuse white matter necrosis (MRI)
Seizure(s)	Seizure(s) in which consciousness is altered	Seizures of any type which are prolonged, repetitive, or difficult to control (e.g., status epilepticus, intractable epilepsy)
PULMONARY		
Adverse Event	**Grade 3**	**Grade 4**
Adult Respiratory Distress Syndrome (ARDS)	-	Present
Apnea	Present	Requiring intubation
Carbon monoxide diffusion capacity (DLCO)	>25 - <50% of pretreatment or normal value	<25% of pretreatment or normal value
FEV1	>25 - <50% of pretreatment or normal value	<25% of pretreatment or normal value
Hypoxia	Decreased O2 saturation at rest, requiring supplemental oxygen	Decreased O2 saturation, requiring pressure support (CPAP) or assisted ventilation
RENAL/GENITOURINARY		
Adverse Event	**Grade 3**	**Grade 4**
Creatinine *Note: Adjust to age-appropriate levels for pediatric patients*	>3.0- 6.0 x ULN	>6.0 x ULN
Renal failure	Requiring dialysis, but reversible	Requiring dialysis and irreversible
SECONDARY MALIGNANCY		
Adverse Event	**Grade 3**	**Grade 4**
Secondary Malignancy – Other (specify type): _____ *Excludes metastasis from initial primary.*	-	Present

APPENDIX VII: FHCRC IRB Policies

http://extranet.fhcrc.org/EN/sections/iro/irb/policy/index.html

APPENDIX VIII Schedule of Study Evaluations

	Screen	Day 1 to engraftment			Days 31-100				Long-Term Follow-up		
		daily	weekly	Day 28 (± 3 days)	weekly	Day 56 (± 7 days)	Day 80 (± 7 days)	Day 100 (± 7 days)	6 months (± 30 days)	1 year	2 years
Informed consent	X										
Medical history	X										
Physical exam	X	X			X				X	X	X
Performance status	X						X Day 80-100		X	X	X
Height/Weight	X						X				
GVHD evaluation			X		X			X	X	X	X
Adverse events		X			X						
CBC with diff	X	X			X				X	X	X
Basic Metabolic Panel	X	X			X				X	X	X
Hepatic Function Panel	X	As clinically indicated or per standard practice									
Viral screening including CMV PCR	X										
CMV Surveillance by PCR		per standard practice									
Pregnancy test	X										
Urinalysis	X	As clinically indicated or per standard practice									
EKG	X	As clinically indicated or per standard practice									
MUGA or echo	X	As clinically indicated or per standard practice									
Chest x-ray or CT	X	As clinically indicated or per standard practice									
PFT	X	As clinically indicated or per standard practice									
Bone marrow asp/bx**	X			X			X			X	
Chimerism – BM				X			X			X	
Chimerism – PB				X		X	X		X	X	X
Disease evaluation	X			X			X			X	X
IgG, IgA, IgM*	X			X		X	X		X	X	X
Lymphocyte panel as possible				X		X		X	X	X	X
HCT-CI (for patients ≥45)	X										

Check Section 7.0: Study Parameters for details. **NOTE:** In certain circumstances (e.g., relapse or terminal illness), study evaluations may be omitted at the physician's or PI's discretion. Every effort will be made to complete the 1- and 2-year evaluations as close to these dates as possible, taking into consideration patient's circumstances at these timepoints.* Per Section 7.3, Quantitative immunoglobulin levels (IgG, IgA, IgM) as possible at Days 56, 80, 6 months, 1 year and 2 years. **Bone marrow biopsy as clinically indicated.