UNIVERSITY OF MINNESOTA BLOOD & MARROW TRANSPLANTATION PROGRAM

Hematopoietic Stem Cell Transplantation in the Treatment of Infant Leukemia and Myelodysplastic Syndrome

MT2005-25
CPRC #2005LS075

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Revision History

Version Date	Amendment #	Details of changes	Consent change?
10/11/05	original		
2/1/2006		Minor clarifications required for CPRC approval	
4/27/2006	1	Eligibility clarification: deleted section 4.1.1. Patients with a matched sibling donor will no longer be excluded from this study	
6/4/2007	2	Modified eligibility to allow for subjects with minimal residual disease (section4.2.5)	
09/14/2011	3	Clinical changes: section 4 change LFT eligibility from 2 x the upper limit of normal to 5 x ULN as on checklist; section 6 - change seizure prophylaxis from Dilantin to Keppra; correct exclusion criteria on page 4 to reflect previous protocol amendment (4/27/2006) Generalized protocol update to current template and language; Make study title consistent throughout – add CPRC #; section 1 – separate out secondary objectives to clinical, transplant and research related; section 4 – minor edits to match eligibility checklist; section 5 – update registration procedures; section 6 –; remove references to and appendices for outdated clinical policies and replace with “per current institutional guidelines” or similar language for UCB infusion, supportive care and slow engraftment; section 7 – split table of tests and procedures into two tables – SOC and research; section 8 – replace previous with current adverse event reporting language; section 8 – replace previous with current data and safety monitoring plan, expand section with other data related language; section 11 – add conduct of study section; renumber and update appendices, move expected toxicities from protocol body to an appendix. Remove Drs. Kumar and Baker from study document (previously done with the IRB).	Reformatted and updated
03/16/2015	4	Restrict graft source to one partially HLA matched unit Update background Revise upper age limit from 2 years of age to 3 Expand enrollment to other high risk leukemic subtypes where transplant may be indicated Update patient registration procedures Update preparative regimen dosing to current practices Remove fludarabine and MMF PK’s from protocol deleting appendices IV and V Add ursodiol for VOD prophylaxis Change toxicity assessment to weekly through day 42, then at day 60 and 100, make LP as clinically indicated Remove research related sample collection, delete correlative endpoints	yes

Version Date	Amendment #	Details of changes	Consent change?
		Update AE documentation and reporting requirements to current requirements Increase enrollment goal for single cord units and update the statistical section to reflect increase Other edits throughout	
05/18/2015	5	Schema, sections 1,1, 3, 4.1.1, and 10.2, and eligibility checklist: Permit matched sibling donors – exclusion was originally removed from the protocol with amendment 1, but was lost in subsequent revisions	Yes
11/30/16	5A	Update PI to Christen Ebens Updated Cancer Center DSMP location Updated eligibility checklist to current template	No
04/12/2017	6	Added donor-specific antibody testing guidelines under section 4.1 Graft criteria, section 7, and eligibility checklist; removed post transplant lansky assessment under section 7; removed Melisa Stricherz from trial	No
07/26/2017	6A	Updated enrollment goals	
03/02/2020	7	Updates to align with institutional practices for: Umbilical cord blood selection criteria Busulfan dosing Graft-versus-host disease prophylaxis -remove eligibility checklist from protocol (checklists are being kept in Oncore)	Yes
11/16/2020	7A	Updated Melphalan administration timing per current pharmacy standard of care; removed year 5 neuropsychological exam; updated G-CSF discontinuation parameters; incorporated updated busulfan units.	No
08/25/2021	8	Updated study committee Removed section 2.3.3 (Background on double UCBT) as study is no longer using double UCBT Section 2.4 Updated reference; removed double cord blood Per pharmacy review: Updated Section 6.2 busulfan AUC goal units, per new local standard clinical care Removed text of Appendix #II – as no longer standard of care References Added reference 27 Minor edits throughout for wording and error correction	Yes

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PROTOCOL SYNOPSIS

MT 2005-25
HEMATOPOIETIC STEM CELL TRANSPLANTATION IN THE TREATMENT OF INFANT LEUKEMIA AND MYELODYSPLASTIC SYNDROME

Study design:	Open label, single institution, non-randomized phase II trial
Primary objective:	Determine incidence of donor derived neutrophil recovery at day 42
Secondary objectives:	•Determine the incidence of transplant-related mortality (TRM) at 6 months after UCBT •Determine the incidence of platelet engraftment at 1 year after UCBT •Determine the incidence of acute graft-versus-host disease (GVHD) grade II-IV and grade III-IV at day 100 after UCBT •Evaluate the developmental outcome after UCBT at 1, 2, and 5 years after UCBT
Eligibility criteria:	Subjects must be ≤ 3 years old at diagnosis of infant leukemia or myelodysplastic syndrome; adequate organ function; Lansky play score ≥50. Matched sibling donor, if available, or an unrelated partially HLA matched UCB graft
Eligible diseases:	Acute leukemia in complete remission (high risk CR1 or subsequent CR); myelodysplastic syndrome or other leukemic sub-types where transplant is indicated and 2 members of the protocol team concur with this decision.
Exclusion criteria:	History of HIV infection or known HIV positive serology; current active serious infection; patients with acute lymphocytic leukemia in relapse or >15% acute myelocytic leukemia blasts.
Accrual objective:	3 to 4 patients per year

TREATMENT PLAN

Day	Drug	Dose
-8	Busulfan	Once daily over 3 hrs with initial dose determined by model-based dosing*
-7, -6, -5	Busulfan	Once daily over 3 hrs with dose adjusted based on PK results
-4, -3, -2	Fludarabine Melphalan	25 mg/ m2/day (0.83 mg/kg/day if < 10 kg) IV over 60 min[&] 50 mg/m2/day (1.7 mg/kg/day if < 10 kg) IV over 15 min
-1	Rest	
0	BM or UBC infusion	

*Initial dose will be determined by model-based dosing utilizing Bayesian pharmacokinetic software
[&]Initial dose may be based on Bayesian pharmacokinetic software if available
Refer to section 6.2 for preparative regimen dosing specifics
Begin GVHD prophylaxis (Tacrolimus and MMF) on day -3 per section 6.3

1 OBJECTIVES

1.1 Primary Objective

The primary objective of this study is to determine the incidence of engraftment (defined as achieving donor derived neutrophil count >500/uL by day 42) in young children with leukemia or myelodysplastic syndrome undergoing a matched sibling or a partially matched unrelated single unit umbilical cord blood transplant (UCBT) after a myeloablative preparative regimen consisting of busulfan, melphalan and fludarabine.

1.2 Secondary Objectives

- Determine the incidence of transplant-related mortality (TRM) at 6 months after UCBT
- Determine the incidence of platelet engraftment at 1 year after UCBT
- Determine the incidence of leukemia relapse at 2 years after UCBT
- Determine the incidence of acute graft-versus-host disease (GVHD) grade II- IV and grade III-IV at day 100 after UCBT
- Evaluate the developmental outcome after UCBT at 1, 2 and 5 years

1.3 Transplant Related Objectives

- Determine the incidence of chronic GVHD at 1 year after UCBT
- Determine the survival and disease free survival at 1 and 2 years after UCBT
- Determine the incidence relapse at 1 and 2 years after UCBT

2 BACKGROUND

2.1 Infant Acute Leukemia

Infantile leukemia is a unique entity in pediatric hematology commonly presenting with hyperleukocytosis, organomegaly, extramedullary disease and CNS leukemia. Infantile leukemia is a relatively rarely occurring entity with ~200 cases/year in the U.S. (reviewed in [1]). Flow cytometry demonstrates a unique immunophenotype (i.e., CD10-) with either lymphocytic (T or B cell) or myeloid features. Cytogenetic analysis frequently shows abnormalities in chromosomal band 11q23, indicative of rearrangements in the mixed lineage leukemia (MLL) gene. Microarray data demonstrate that leukemia with MLL gene rearrangements have a distinct gene expression profile when compared to conventional acute lymphocytic or myeloid leukemia, supporting the concept that infant leukemia is a separate clinical entity [2]. Despite aggressive chemotherapy, the majority of infants with leukemia will suffer early relapse and death. Overall survival on modern chemotherapy protocols range from 20-40% [1,3,4]. Children with high WBC counts (>100,000/ml), or the 4;11 translocation appear to be ultrahigh risk with an event free survival (EFS) of ~5% (reviewed in [1]). Given the unsatisfactory results using standard chemotherapy, some groups have resorted to allogeneic hematopoietic cell transplantation (allo-HCT) in first complete remission (CR1) for such high-risk leukemia patients. The role for this approach has not been fully established. For instance, Pui and coworkers retrospectively examined the outcome of infants with 11q23 chromosomal rearrangements and found that those receiving chemotherapy (n=173) had better overall and disease free survival (OS and DFS) compared to those that undergoing a variety of different types of hematopoietic cell transplantation (autologous, matched and mismatched related and unrelated donors) (n=55) [3]. The difference between the chemotherapy and transplant groups was

attributable to both relapse and toxicity.

While the above study casts doubt on the role of transplantation in infant ALL, other studies show that allo-HCT, in fact, improves outcomes. Balduzzi and coworkers recently published the results from a European cooperative group study (both BFM and EBMT) on infant leukemia [4]. This study used aggressive induction chemotherapy followed by a "genetic" randomization to transplantation. Thus, if infants had an HLA-matched sibling donor, transplantation was to be performed within 2-5 months of achieving CR1. Of the 357 children enrolled, 77 underwent matched sibling donor transplantation with a conditioning regimen of total body irradiation (TBI), etopiside, busulfan and cyclophosphamide. Children that received transplantation had a better 5 yr DFS rate compared to those receiving chemotherapy (56.7% vs. 40.6%, p=0.02). Likewise, a recent publication from the Japan Infant Leukemia Study Group showed favorable results with transplantation using either sibling or unrelated donors [5]. The 3-year OS and EFS for patients undergoing transplantation in this study was 58.2% and 43.6%, comparing favorably to nearly all chemotherapy protocols. Interestingly, infants transplanted in CR1 had significantly better outcome than those transplanted in CR2 (64.4% vs. 22.2%, p=0.0044). A recent single center study (Seattle) showed a similar improvement in DFS for patients transplanted early in the disease course [6]. Comparing the DFS for patients transplanted in CR1 vs. CR2/3 vs. relapse showed an advantage for early transplantation (76% vs. 45% vs. 8%, p<0.001). Like the above protocols, the Seattle study used a TBI-based regimen. Of the 15 patients who had developmental testing, ~2/3rds of patients showed some degree of either motor or language delay—most delays were assessed as mild.

Collectively the above studies demonstrate that infant leukemia is aggressive and outcome with standard chemotherapy is poor. Some recent large studies show a benefit for early transplantation (i.e., CR1) using TBI containing regimens. In addition, the Seattle study showed that such patients have some degree of developmental delay, but whether this is secondary to TBI or other factors (i.e., their chronic illness) is difficult to determine.

2.2 Allogeneic Hematopoietic Cell Transplantation And Umbilical Cord Blood Transplantation (UBCT)

Allogeneic hematopoietic cell transplantation (allo-HCT) is a standard treatment option for an increasing number of malignant disorders. To reconstitute hematopoiesis after an intensive myeloablative therapy, the transplantation of pluripotent 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 [7]. Unfortunately, suitable marrow is frequently not available [8]. 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 [9-16].

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.

2.3 UCBT Experience at the University Of Minnesota

2.3.1 Single Unit UCBT [17].

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 107 NC/kg (range 0.7-57.9), and 2.8 x 105 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 105 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 105
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 105 CD34+ cells/kg or >2.5 x 107 nucleated cells/kg) consistently leads to engraftment; 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.

2.3.2 UCBT in Young Children with Leukemia (COBLT Study) [18]

Between January 1999 and May 2002, the University of Minnesota participated in a multi-institutional trial evaluating the efficacy of a conditioning regimen containing busulfan and melphalan in the treatment of 32 young children with infant leukemia or myelodysplastic syndrome. Infant leukemia was defined as all children diagnosed with leukemia prior to 6 months of age, or diagnosed prior to 12 months of age with cytogenetic rearrangements carrying the MLL gene.

Median age of the patients at transplant was 1.6 years (range 0.5, 3.9 years); 56% were Caucasian and 59% were female. The primary diagnosis was ALL (44%), AML (41%), JMML (6%), MDS (6%) and undifferentiated leukemia (3%). The median cryopreserved cell doses were 10.7 × 107 total nucleated cells (TNC)/kg (range, 4.6-29.2), 2.6 × 105 CD34+ cells/kg (range, 0.7-8.3) and 1.6 × 107 CD3+
cells/kg (range 0.6, 3.3).

The incidence of neutrophil recovery at day 42 was 0.59 (95% CI, 0.44, 0.78). No factor, including age, weight, gender, ethnicity, primary disease, HLA disparity, recipient CMV status, graft nucleated cell dose, CD34+ dose and CD3+ dose, was associated with incidence of neutrophil engraftment. Also, there was no difference in the occurrence of primary graft failure between patients receiving IV versus oral busulfan. The median time to engraftment

was 31 days (range, 11-55).
Maximum Bearman toxicity across all organ systems was reported for the period up to day 42 after UCBT. Five patients exhibited severe toxicity with 59% having a maximum toxicity of grade 1-2. Notably, no patient had severe grade 4 mucositis. However, three patients were reported to have grade 4 cardiac toxicity.

The incidence of grade II-IV and III-IV acute GVHD at day 100 was 0.41 (95% CI, 0.25, 0.56) and 0.25 (95% CI: 0.09, 0.41), respectively. Six patients out of 23 surviving at least 100 days developed chronic GVHD for an incidence of 0.26 (95% CI, 0.09, 0.44).

Ten patients relapsed. The incidence of leukemia relapse at two years was 0.31 (95% CI, 0.16, 0.47) at a median of 200 days (range, 41-464). No difference in the incidence of relapse was observed by disease (ALL versus AML) or disease status at the time of transplant (CR1 versus >CR2). The probability of relapse-free survival at 2 years was 0.28 (95% CI, 0.13, 0.44). Relapse was hematologic (n=9), or extramedullary (n=1); no patient had CNS relapse after CBT. The overall probability of survival was 0.56 (95% CI, 0.39, 0.73) and 0.47 (95% CI, 0.30, 0.64) at day 180 and 1 year, respectively. Age, weight, gender, ethnicity, primary disease, recipient CMV status, TNC dose, CD34+ dose, CD3+ dose and transplant center did not significantly influence survival. Donor/recipient gender match ($p<0.01$) and high resolution HLA match ($p<0.01$) were the only two factors that were associated with improved survival. Twenty-two of the 32 patients died. The most common causes of death were relapse (n=8), graft failure (n=4), acute GVHD (n=5) and chronic GVHD (n=3). One death was attributed to each of viral infection and interstitial pneumonia.

2.3.3 Outcomes of Mismatched Cord Blood Transplantation-UMN Experience

Recently, we have reviewed the outcomes of 342 adult patients undergoing UCB transplantation at the University of Minnesota (Brunstein, manuscript submitted). Approximately one third of patients underwent myeloablative conditioning and the remainder received reduced intensity conditioning. All patients were transplanted with two UCB units. Following double UCB transplantation, the majority of patients have engraftment from a single unit within the first 100 days. To examine transplant outcomes patients were assigned to 3 different groups based on the HLA match of the engrafting unit. The groups consisted of HLA 2- 5/10 (n=108), 6-8/10 (n=202) and 9-10/10 (n=32) based on matching at HLA-A, - B, -C and –DRB1. These groups did not differ with respect to the incidence of neutrophil or platelet recovery (p=0.33 and p0.22). Similarly, the incidence of non-relapse mortality or grade II-IV aGVHD was not different between the groups (p=0.87 and p=0.71). Somewhat paradoxically, after adjusting for the conditioning regimen, there was a 1.8-fold higher (p=.08) risk of relapse for patients receiving a 9-10/10 HLA-matched graft, as compared to a 2-5/10 HLA- matched graft. Similarly, when patients engrafted with a poorly matched unit (2- 5/10) vs. a well matched unit (9-10/10) they were 2.4-fold less likely to relapse (p=0.02). Collectively, these results in adult patients suggest that for patients at high risk for relapse, it may be beneficial to receive purposely mismatched UCB units. Similar results (high relapse rates) in well matched units [20] or lower rates of for poorly mismatched units [19,21] have been reported by some, but not others [22].
Given that relapse is a major barrier to success for infants with leukemia (section 2.1 and 2.2), we will test the hypothesis that poorly matched units are safe and result in lower rates of leukemia relapse in this patient population.

2.3.4 Consequences of TBI in Young Children

In probably the largest study to date, Willard and coworkers studied the outcomes of 183 children undergoing allogeneic transplantation [23]. Patients were assessed using age appropriate neuropsychological testing, sequentially before and after transplantation (pre-transplant and at 1, 3, and 5 years after transplantation).

Approximately half of the patients received TBI, while the others did not. Using a variety of age-appropriate functional assessments (intelligence quotient (IQ), etc.) they demonstrate that all patients, regardless of conditioning, have a decrement in IQ score in the first year after transplantation. However, non-TBI treated patients recover to their pre-transplant baseline by 3 years (and maintain their performance at 5 years). In striking contrast, TBI treated patients were less likely to recover to baseline between 1 to 3 years post-transplant. More importantly, from years 3 to 5 after transplant these TBI-treated patients had further decrement in IQ function, suggesting that the effects of TBI are not completely appreciated for up to 5 years after transplant. This is a time when most transplant physicians are no longer involved in the care of the patient, perhaps leading to the bias, by some transplant physicians, that TBI does not significantly impact infants. These findings we age dependent since patients who were <3 years at the time of TBI had the most significant impairment ($p=0.05$). Thus, these findings strongly support the exploration of a non-TBI containing regimens in young children.

2.4 Summary

Poor engraftment and relapse are the two principal reasons for treatment failure in young patients with leukemia/myelodysplastic syndrome treated with busulfan and melphalan followed by UCBT. While effective, TBI results in long-term, irreversible cognitive dysfunction. Methods to prevent non-engraftment, relapse and long term neurocognitive complications are needed. There have been two recent changes in the field of allogeneic transplantation that have positively impacted outcomes. First, the introduction of fludarabine into the conditioning regimen has enhanced engraftment across histocompatibility barriers and has spawned an era of "non-myeloablative" transplantation due to its potent immune suppressive effects. While the transplantation regimen proposed here is not a non-myeloablative regimen, this drug has also had a positive impact on myeloablative transplants. For instance, Fanconi anemia patients who have received a regimen of cyclophosphamide/TBI had an engraftment rate of 60%, but the addition of fludarabine to this regimen (i.e., flu/ cyclophosphamide/TBI) resulted in 100% engraftment[27]. Thus, we hypothesize that incorporation of fludarabine into a regimen of busulfan/melphalan will, likewise, enhance engraftment in a cohort of infants. The second recent change in UCB transplant is the observation that in UCBT, engraftment with a more mismatched unit results in lower relapse and equivalent leukemia free survival (our unpublished data and [19,21]). Thus, our overall hypothesis is that the addition of fludarabine and the use of a partially matched UCB unit will improve the rate of hematopoietic recovery (engraftment) and overall survival in infants with leukemia.

3 STUDY DESIGN

This is a single institution, phase II study to determine the incidence of engraftment in patients with hematological malignancy undergoing a match sibling donor or an unrelated, partially matched

single unit umbilical cord blood transplant (UCBT) after a myeloablative preparative regimen consisting of busulfan, melphalan and fludarabine.

4 PATIENT SELECTION

4.1 Graft Criteria

4.1.1 Matched sibling donor (HLA 8/8) cord blood or marrow, if available, or an unrelated partially HLA matched single umbilical cord blood (UCB) unit

4.1.2 UCB unit selection: Considering only units containing ≥2 x 10^7 total nucleated cells/kg and ≥1 x 10^5 CD34+ cells/kg:

- 1st priority: HLA-mismatch (HLA-A, -B, -DRB1): 4/6 preferred over 5/6, 5/6 preferred over 6/6
- 2nd priority: Anti-HLA antibody testing will be completed per institutional guidelines with preference to UCB units for which the patient does not have donor specific antibodies. However, the presence of donor specific antibodies does not preclude the use of the UCB unit as long as the risks and potential benefits are discussed with the parent or legal guardian and documented. Antibody debulking should be considered.
- 3rd priority: If above priorities are fulfilled and equal, select the unit with the largest CD34+/kg cell dose
- Additional considerations: If all above priorities fulfilled and equal, consider matching on ABO and race (latter when data available)

4.2 Age and Disease Criteria

4.2.1 Age ≤ 3 years at diagnosis (not age of transplant)

4.2.2 Meeting of one of the following disease criteria:

Acute myeloid leukemia: high risk CR1 as evidenced by:
High risk cytogenetics
t(4;11) or other MLL rearrangements chromosome 5, 7, or 19 abnormalities complex karyotype (>5 distinct changes)
≥ 2 cycles to obtain CR; CR2 or higher Preceding MDS
All patients must be in CR or early relapse (i.e., <15% blasts in BM).

Acute lymphocytic leukemia: high risk CR1 as evidenced by: High-risk cytogenetics:
t(4;11) or other MLL rearrangements hypodiploid
t(9;22)
>1 cycle to obtain CR CR2 or higher
All patients must be in CR as defined by hematological recovery, AND
<5% blasts by light microscopy within the bone marrow with a cellularity of ≥15%.

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.

Persistent or rising minimal residual disease (MRD) after standard chemotherapy regimens: Patients with evidence of minimal residual disease at the completion of therapy or evidence of rising MRD while on therapy. MRD will be defined by either flow cytometry (>0.1% residual cells in the blast gate with immune phenotype of original leukemic clone), by molecular techniques (PCR or FISH) or conventional cytogenetics (g- banding).

New Leukemia Subtypes: A major effort in the field of pediatric hematology is to identify patients who are of high risk for treatment failure so that patients can be appropriately stratified to either more (or less) intensive therapy. This effort is continually ongoing and retrospective studies identify new disease features or characteristics that are associated with treatment outcomes. Therefore, if new high risk features are identified after the writing of this protocol, patients can be enrolled with the approval of two members of the study committee.

4.3 Organ Function and Performance Status Criteria

4.3.1 Lansky play score ≥ 50 (appendix I)

4.3.2 Acceptable organ function defined as:

- Renal: glomerular filtration rate > 60ml/min/1.73m^2
- Hepatic: bilirubin, AST/ALT, and ALP < 5 x upper limit of normal
- Pulmonary function: oxygen saturation >92%

4.3.3 Cardiac: left ventricular ejection fraction ≥ 45%

4.3.4 Voluntary written informed consent before performance of any study- related procedures not part of normal medical care.

4.4 Exclusion Criteria

4.4.1 Active uncontrolled infection at time of transplantation (including active infection with Aspergillus or other mold within 30 days)

4.4.3 History of HIV infection or known positive serology

4.4.4 Myeloablative transplant within the last 6 months

4.4.5 Evidence of active extramedullary disease (including CNS leukemia)

5 PATIENT REGISTRATION

To be eligible for registration to this study, the patient must meet each criteria listed on the eligibility checklist (kept in Oncore) based on the eligibility assessment documented in the patient's medical record.

Participants will be registered in the Masonic Cancer Center's clinical database, OnCore by a BMT CDA.

Any patient receiving unlicensed unrelated umbilical cord blood (UCB) will be co- enrolled in the University of Minnesota protocol MT2011-13R "Infusion of Cell Populations from Unlicensed Umbilical Cord Blood Units" (IND 14797 C. Brunstein S/I).

6 TREATMENT PLAN

In order to provide optimal patient care and to account for individual medical conditions, investigator discretion may be used in the prescribing of all supportive care drug therapy (i.e. acetaminophen, diphenhydramine, methylprednisolone, G-CSF, etc).

6.1 Treatment Plan

Begin Keppra on day -9 and continuing until day – 3 or as medically indicated.

Day	Drug	Dose
-8	Busulfan	Once daily over 3 hrs with initial dose determined by model-based dosing*
-7	Busulfan	Once daily over 3 hrs with dose adjusted based on therapeutic drug monitoring
-6	Busulfan	Once daily over 3 hrs with dose adjusted based on therapeutic drug monitoring
-5	Busulfan	Once daily over 3 hrs with dose adjusted based on therapeutic drug monitoring
-4	Fludarabine Melphalan	25 mg/ m2 (0.83 mg/kg if <10 kg) IV over 60 min[&] 50 mg/m2 (1.7 mg/kg if <10 kg) IV over 15 min
-3	Fludarabine Melphalan	25 mg/ m2 (0.83 mg/kg if <10 kg) IV over 60 min[&] 50 mg/m2 (1.7 mg/kg if <10 kg) IV over 15 min
-2	Fludarabine Melphalan	25 mg/ m2 (0.83 mg/kg if <10 kg) IV over 60 min[&] 50 mg/m2 (1.7 mg/kg if <10 kg) IV over 15 min
-1	Rest	
0	UCB or BM infusion	

*Initial dose will be determined by model-based dosing utilizing Bayesian pharmacokinetic software if available
[&]Initial dose may be based on Bayesian pharmacokinetic software if available

6.2 Conditioning Regimen

6.2.1 Busulfan

BUSULFAN DOSING AND ADMINISTRATION:
Busulfan compounding, administration and monitoring should be performed per institutional guidelines.

The first busulfan dose will be determined by model-based dosing utilizing Bayesian pharmacokinetic software with a cumulative area under the curve (cAUC) target of 62 mg*hr/L.
An IP pediatric BMT pharmacist will be consulted for assistance in first (and subsequent) busulfan dose determinations.

Busulfan will be given every 24 hours (i.e., on a daily basis). Busulfan will be administered over 3 hours per the University of Minnesota Blood and Marrow Transplant Program busulfan SOC guidelines on days -8, -7, -6, and -5.

- Levetiracetam (Keppra) will be administered in accordance with Busulfan SOC guidelines as seizure prophylaxis during busulfan therapy.

- Concomitant administration of interacting medications such as azole anti-fungal agents (except fluconazole) should be avoided.
- It is recommended that acetaminophen administration, particularly on 72 hours prior to or after busulfan administration, be limited to necessary indications (such as pre-medication for the prevention of ATG reactions). When acetaminophen use is deemed necessary on or near the days of busulfan administration, conservative dosing of 10 mg/kg/dose (max 500 mg/dose) is recommended.

BUSULFAN THERAPEUTIC DRUG MONITORING (TDM):
Area under the curve (AUC) analyses will be calculated in-house per University of Minnesota BMT SOC guidelines for all BMT patients. The AUC will be calculated for each dose using serum busulfan concentrations analyzed by the Special Chemistry/Drug Analysis Lab.
Busulfan therapeutic drug monitoring (TDM) will be used to target a cumulative (or overall) exposure for the entire course **cAUC = 58-67 mg*h/L (Target cAUC = 62 mg*h/L).** Results of TDM performed with the first dose will inform subsequent busulfan dosing (doses #2, #3 and #4).
An IP pediatric BMT pharmacist will be consulted for TDM. Subsequent busulfan doses will be determined by busulfan AUC calculations. Refer to Busulfan SOC Guideline for blood sample collection process, busulfan results reporting, and therapeutic drug monitoring procedures.

6.2.2 Fludarabine

Fludarabine will be administered at a dose of 25 mg/m^2/day x 3 days, total dose 75 mg/m^2 (days –4 to –2). For children < 10 kg, fludarabine dosing will be 0.83 mg/kg/day x 3 days, total dose 2.49 mg/kg (days -4 to -2). If available, dosing may be calculated using Bayesian pharmacokinetic software. Dosing is based on actual body weight. The fludarabine dose will be administered IV over one hour at a constant rate. If the normalized GFR is <70 ml/min, a 20% dose-reduction of fludarabine may be considered after discussion between the protocol PI, patient's primary BMT physician and pediatric BMT pharmacist.

6.2.3 Melphalan

Melphalan will be administered at a dose of 50 mg/m^2/day x 3 days, total dose150 mg/m^2 (days -4 to - 2). For children <10 kg, melphalan dosing will be 1.7 mg/kg/day IV x 3 days, total dose 5.1mg/kg (days -4 to -2). Melphalan is to be administered 12 hours after fludarabine, infused over 15 minutes, with maintenance hydration (>1500ml/m^2/day) during administration and for 24 hours following infusion.

6.3 Immunosuppressive Therapies

All patients will receive GVHD prophylaxis with Tacrolimus and Mycophenolate mofetil (MMF) as per institutional guidelines:

6.3.1 Tacrolimus

Tacrolimus will start day -3 and will be administered as a continuous IV infusion at a starting dose of 0.03 mg/kg/day. Goal trough levels will be 10-15 ng/mL for the first 14 days post-transplant and then decreased to a goal of 5-10 ng/ml thereafter. Dose adjustments will be

made on the basis of toxicity and/or tacrolimus levels outside of goal range. Conversion from IV to oral tacrolimus will be done as the patient tolerates and prior to discharge. Potential toxicities are detailed in Appendix III.

Patients will receive tacrolimus until day +100. If no GVHD, the dose will be tapered 10% per week beginning on day 101, to discontinue at approximately day +180.

In the presence of severe tacrolimus toxicities, other alternative agents may be used after review and approval by the co-PIs.

6.3.2 Mycophenolate mofetil (MMF)

MMF will start on day -3 at a dose of 15 mg/kg/dose IV every 8 hours (max dose 1 gm/dose).

Stop MMF at day +30 or 7 days after engraftment, whichever day is later, if no acute GVHD. Definition of engraftment is 1st day of 3 consecutive days of absolute neutrophil count [ANC] > 0.5 x 10^9/L. If the patient has acute GVHD requiring systemic therapy, MMF should continue for at least 7 days after initiation of systemic therapy for acute GVHD. Afterward, use of MMF is at the discretion of the treating physician.

6.4 Umbilical Cord Blood Transplantation (UCBT)

The unit will be infused on day 0 according to the current institutional guidelines for a single cord UCB transplant.

6.4.1 Pre Infusion Treatment Plan

All patients will receive intravenous hydration plus pre-medication with acetaminophen and diphenhydramine hydrochloride (or similar alternatives) with doses adjusted for the patient's age and weight approximately 30 minutes prior to the infusion of the UCB product.

Emergency drugs such as epinephrine, hydrocortisone, diphenhydramine and atropine in appropriate dosages and dilutions should be available and administered according to institutional guidelines. Oxygen should be available in the patient's room.

6.4.2 Infusion of Minimally Manipulated UCB Unit

After positive identification and crosschecking according to institutional SOPs, the UCB unit will be infused through a central line according to institutional guidelines. The UCB infusion rate is not to exceed 10 mL/min regardless or recipient age or size. However for patients <20 kg, the final volume is adjusted upon washing such that the unit is re-suspended in a maximum volume 100 mL (range 60-100 mL).

6.4.3 Monitoring of Vital Signs

Based on existing Transplant Unit SOPs, vital signs are obtained prior to and every 15 minutes during the infusion and at the completion of infusion. This monitoring may be intensified or prolonged if clinically indicated

6.4.4 Management of Infusion Reactions

In case of a life threatening infusion related reaction, such as severe allergic reaction or cardiac arrhythmia, the infusion will be stopped and the patient will be evaluated and treated

by the medical team. Once the patient has improved and is medically stable, the team will determine whether or not the UCB infusion should be completed.

In case of a non-life-threatening/serious reaction (e.g. hypertension), the infusion may be slowed or stopped and the patient treated, as deemed appropriate by the medical team. Once the patient has improved and is medically stable, the infusion may be restarted at a slower rate.

6.5 Growth Factor

All patients will receive G-CSF 5 mcg/kg/day IV based on the actual body weight IV beginning on day +1 after UCB infusion. G-CSF will be administered daily until the ANC exceeds 1.5 x 10^9/L for three consecutive days, or if ANC exceeds 3 x 10^9/L on a single day, then discontinue. If the ANC decreases to <1.0 x 10^9/L, G-CSF will be provided as needed.

6.6 Treatment Related Toxicities

Refer to appendix III for expected treatment related toxicities.

6.7 Supportive Care Guidelines

Patients will receive transfusions, infection prophylaxis, and nutritional support according to the current University of Minnesota supportive care guidelines.

For veno-occlusive disease (VOD) prophylaxis, patients should receive ursodiol 10 mg/kg enterally TID upon admission until day +30 or hospital discharge, whichever occurs earlier.

7 STUDY PARAMETERS

Scheduled evaluations prior to engraftment (day 30) may be performed +/-3 days from the targeted date; assessments to be performed between engraftment and day 100 may be done +/-7 days of the targeted date; assessments after day 100 may be performed +/-30 days of the targeted date. In addition, targeted days may be altered as clinically appropriate.

	Screen	Day 1 to engraftment2				Days 31-100			Follow-up
		daily	weekly	Day +21	Day +28	weekly	Day +60	Day +100	6 months, 1 & 2 yrs
Informed consent	X								
Medical history	X	X				X			
Physical exam	X	X				X			X
Lansky play score	X								
Height/Weight	X								X
GVHD evaluation			X			Per institutional guidelines			
CMV Surveillance						Per institutional guidelines			
Toxicity assessment			X			X thru day +42	X	X	
Laboratory									
CBC, diff	X	X2				X			X
Platelet	X								
PT/PTT	X								
AST ALT, alk phos, bili (T/D)	X								
Creatinine, Na, K, HCO3	X	prn							
Viral serology	X								
Urinalysis	X		prn						
GFR	X								
Bone marrow bx/asp w/chimerism	X			X	X3			X	X
Lumbar puncture[4]	X						X	X	X
Chimerism – PB	X			X	X		X	X	X
PRA: Initial[5]	X								
PRA: Confirmatory w/in 21 days of treatment[5]	X								
Procedures									
EKG	X		prn			prn			prn
MUGA or echocardiogram	X		prn			prn			1 yr then prn
Chest x-ray or CT	X1		prn			prn			prn
Oxygen saturation (or PFTs if old enough)	X		prn			prn			prn
Additional disease evaluations[4]	X		X					X	X
Neuropsych Eval	X								X (1 & 2 years)

1 – CT without contrast to exclude occult infection for patients with a history of the following:

- MDS
- 2 or more consecutive leukemia inductions
- prolonged neutropenia, as defined as ≥ 4 weeks of neutropenia within the 2 months prior to BMT

2 – Complete blood count with leukocyte differential daily until the absolute neutrophil count (ANC) > 0.5 x 10^9/L for 3 consecutive measurements

3 – Day 28 Bone marrow biopsy if slow engraftment and proceed per U of MN guidelines

4 – as clinically indicated

5 – UCBT recipients only

8 ADVERSE EVENT MONITORING AND REPORTING

8.1 Definitions

An adverse event (AE) is any symptom, sign, illness or experience, regardless of causality, that develops or worsens in severity during the course of the study. The occurrence of an adverse event will be based on changes in the patient's physical examination, laboratory results, and/or signs and symptoms. Intercurrent illnesses or injuries should be regarded as adverse events if at least possibly related (i.e. temporal relationship to the study treatment, known toxicity of the treatment, etc.).

Attribution:

- **Unrelated** - The AE *is clearly NOT related* to the intervention
- **Unlikely** - The AE *is doubtfully related* to the intervention
- **Possible** - The AE *may be related* to the intervention
- **Probable** - The AE *is likely related* to the intervention
- **Definite** - The AE *is clearly related* to the intervention

Unanticipated (unexpected) problems/events are those that are *not* already described as potential risks in the consent form, *not* listed in the Investigator's Brochure or *not* part of an underlying disease.

Anticipated (expected) problems are those that are already described as potential risks in the consent form, listed in the Investigator's Brochure or part of an underlying disease.

8.2 Monitoring and Documentation of Toxicity

Toxicity assessments will be done once weekly for the start of any study therapy through day 42 and at each milestone visit through day 100.

Refer to appendix III for a list of expected toxicities.

Adverse event documentation requirements will be determined based on grade, expectedness and relationship to study therapy as follows:

	Grade 1	**Grade 2**		**Grade 3**		**Grade 4 and 5**
	Expected or Unexpected	**Expected**	**Unexpected**	**Expected**	**Unexpected**	**Expected or Unexpected**
Unrelated Unlikely	Not required	Not required	Not required	Not required	Not required	Required
Possible Probable Definite	Not required	Not required	Not required	Not required	Required (Non-hematologic only)	Required

Stopping rule events: The following events count toward a study stopping rule per section 10.4 and must be reported to the MCC SAE Coordinator using the Study Stopping Rule Report Form:

- Graft failure by day 42 (defined as failing to achieve an ANC > 500/uL of donor origin by day 42)

- Treatment related mortality by day 100

Events that count toward an early stopping rule do not necessarily constitute a an adverse event requiring expedited reporting and should be reported as such only if they meet the criteria for expedited reporting to the IRB as defined in section 8.3.

8.3 Required Reporting to University Of Minnesota IRB and Masonic Cancer Center

The reporting period for this study is from initiation of any study treatment through day +100; however after day +100, the investigator must report upon knowledge any study treatment related event meeting the expedited reporting criteria below.

Agency	Criteria for reporting	Timeframe	Form to Use	Submission address/ fax numbers	Copy AE to:
U of MN IRB	Events requiring prompt reporting including, but not limited to unanticipated death of a locally enrolled subject(s); new or increased risk; any adverse event that require a change to the protocol or consent form or any protocol deviation that resulting in harm. For a complete list and the Report Form refer to http://www.research.umn.edu/irb/guidance/ae.html# definition	Within 5 business days of event discovery	IRB's Report Form	irb@umn.edu	SAE Coordinator mcc-saes@umn.edu
	All problems/events that do not meet the IRB's requirements for prompt reporting	Report to the IRB in summary form at the time of continuing review.			Not applicable
Masonic Cancer Center SAE Coordinator	Events that meet the criteria for early study stopping rule	At time of reporting	OnCore Form	SAE Coordinator mcc-saes@umn.edu	Not applicable

The SAE Coordinator, if applicable, will provide the Masonic Cancer Center's Data and Safety Monitoring Council (DSMC) with the SAE in an appropriate format depending on the individual SAE (as reported or in a summary format).

9 DATA COLLECTION AND MONITORING PLAN

9.1 Data Management

Patients will be registered in The Online Enterprise Research Management Environment (OnCoreTM), a web based Oracle® database. Specific transplant related endpoints will be recorded in the University Of Minnesota Blood and Bone Marrow Database as part of the historical database maintained by the department.

9.2 Data and Safety Monitoring Plan

The study's Data and Safety Monitoring Plan will be in compliance with the University of Minnesota Masonic Cancer Center's Data & Safety Monitoring Plan (DSMP), which can be accessed at http://z.umn.edu/dmsp .

For the purposes of data and safety monitoring, this study is classified as moderate risk.

Therefore the following requirements will be fulfilled:

- The PI will complete and submit a twice yearly Trial Progress Report to the Masonic Cancer Center Data and Safety Monitoring Council (DSMC) with the understanding the Cancer Protocol Review Committee (CPRC) may require more frequent reporting.
- The PI will comply with at least yearly monitoring of the project by the Masonic Cancer Center monitoring services.
- The PI will oversee the submission of all reportable adverse events per the definition of reportable in section 8.3 to the Masonic Cancer Center's SAE Coordinator and the University of Minnesota IRB.

In addition, at the time of the continuing review with the University of Minnesota IRB, a copy of the report with any attachments will be submitted to the Cancer Protocol Review Committee (CPRC).

9.3 Record Retention

The investigator will retain study records, including source data, copies of CRF's and all study correspondence in a secure facility for at least 6 years after the study file is closed with the IRB. In addition, the Clinical Trials Office will keep a master log of all patients participating in the study with sufficient information to allow retrieval of the medical records.

9.4 Monitoring

The investigator will permit study-related monitoring, audits, and inspections by the University of Minnesota compliance groups and government regulatory bodies. The investigator will make available all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data, etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.) will be available for trial related monitoring, audits, or regulatory inspections.

10 STATISTICAL CONSIDERATIONS

10.1 Objectives

10.1.1 Primary objective

The principal aim of this study is to estimate the incidence of neutrophil recovery by day +42 after UCBT after a myeloablative preparative regimen.

10.1.2 Secondary Endpoints

- Determine the incidence of transplant-related mortality (TRM) at 6 months after UCBT
- Evaluate pattern of chimerism after double UCBT days 21, 100, 6 months, 1 year and 2 years
- Determine the incidence of platelet recovery at 1 year after UCBT
- Determine the incidence of acute graft-versus-host disease (GVHD) grade II-IV and grade III-IV at day 100 after UCBT
- Evaluate the developmental outcome after UCBT

10.1.3 Transplant Related Endpoints

- Determine the incidence of chronic GVHD at 1 year after UCBT
- Determine the survival and disease free survival at 1 and 2 years after UCBT
- Determine the incidence relapse at 1 and 2 years after UCBT

10.2 Statistical Analysis

Survival and disease-free survival will be estimated by the Kaplan-Meier method. Non-relapse mortality, relapse, neutrophil and platelet engraftment, acute and chronic GVHD will be estimated by cumulative incidence treating non-event deaths as competing risks. Chimerism will be evaluated both by simple proportions and median (range) values among assessable patients. Ninety-five percent confidence intervals will be used to make inferences. Comparisons of time-to-event endpoints by various factors will be completed by the Log-rank or Gray's test. Comparison of simple proportions will be evaluated by the Chi-square test and the general Wilcoxon test will be used to evaluate differences between continuous factors. Simple descriptive statistics and plots will be given for the neuropsychiatric parameters.

Addendum (03/02/2020): Since we are changing the UCB selection, Busulfan dosing and GvHD prophylaxis, endpoints will be estimated separately for patients enrolled before and after this addendum. Methods will stay the same.

10.3 Rationale for Sample Size

We expect to enroll 3 to 4 patients per year. Based on prior experience with fludarabine and double UCB, we hope to get better than 75% engraftment and estimate that we will achieve 90% engraftment by day 42 post-transplant. If 95% of patients have access to two UCB donors, this will give a lower 95% confidence bandwidth of at least 0.15. This is assuming that there are no patients with early death prior to an evaluable graft assessment. Enrollment on the double unit and single unit arms of the study is expected to be a minimum of 14 and 1 patients respectively. No more than 20 patients will be enrolled on this protocol before evaluation of the primary endpoint. Transplantation at the University of Minnesota of patients that fit the eligibility criteria in this study averaged 3 patients per year in recent years.

addendum (03/16/2015 and 5/19/15): We have reached the maximum sample size of 20 patients and therefore the protocol will be redesigned. To permit continued access to transplant until that time, 7 additional patients will be enrolled using a single UCBT or a matched sibling donor. Among the currently enrolled 14 patients in the double UCBT cohort, engraftment is 100%. However, the observation that in both single and double UCBT, engraftment with a more mismatched unit results in lower relapse and equivalent leukemia free survival (our unpublished data and [19,21]), single mismatched UCB transplants will now be performed instead of double UCBT in this protocol. There is currently 1 graft failure among 6 SUCBT patients. The cumulative incidence of engraftment is 83% (95% CL, 48-99%). To improve precision, 7 patients will be added for a total of 13 single UCB transplants. With 13 patients and an expected engraftment of 93%, the lower 95% confidence band will be 65% and the upper limit will be 100%. Due to a very limited number of matched sibling donor transplants expected, results from these patients will be listed descriptively.

Addendum (07/26/2017): After reaching the revised accrual goal, we will enroll an additional 10-15 subjects in order to give patients access to transplant until a replacement study is

approved. The replacement protocol is currently being written and should be submitted to the IRB/CPRC within 3 months. The current regimen continues to be safe with no additional graft failures. Although allowing time for a newly developed protocol is the primary reason for revising the accrual goal, the additional patients will also help to improve precision of our primary endpoint of neutrophil engraftment. With potentially 28 patients and an expected engraftment of 93%, the lower 95% confidence band will be 77% and the upper limit will be 99%.

Addendum (03/02/2020): We are updating the protocol to be more in line with institutional practices for UCB selection, Busulfan dosing and GvHD prophylaxis. We will enroll an additional 10-15 subjects in order to give patients access to transplant until a replacement study is approved.

10.4 Toxicity Monitoring and Stopping Rules

Monitoring guidelines will be set up for graft failure by day +42 and treatment related mortality by day +100 post-transplant. Monitoring guidelines are for the single UCBT cohort only using a continuous monitoring strategy based on an adaptation of Pocock stopping boundaries[26]. In the event that a stopping rule is triggered, enrollment will be halted and reviewed by the full study committee and if appropriate by the IRB, prior to initiation of re-enrollment.

Addendum (07/26/2017): Since the regimen has not changed, the stopping rules will include prior events from the single UCBT cohort

10.4.1 Graft Failure by day +42

The goal is to construct a boundary based on graft failure (defined as failing to achieve an ANC >500/uL of donor origin by day +42) such that the probability of early stopping is at most 10% if the true graft failure rate is equal to 7% and our sample size is 28. Given these parameters, the upper stopping boundary for graft failure is 2/5 or 3/11, 4/19 or 5 patients fail to engraft.

10.4.2 Treatment Related Mortality by 100 days

Stopping rules were developed for excessive non-event mortality (TRM) by day +100 post-transplant. The goal is to construct a boundary based on TRM such that the probability of early stopping is at most 10% if the true rate is equal to 30% and our sample size is 28. Given these parameters, the upper stopping boundary for TRM is 3 deaths out of 3 patients, 4/5, 5/7, 6/9, or 7 /11, 8/13, 9/16, 10/18, 11/21, 12/24, 13/26 or 14 patients with TRM by 100 days.

Addendum (03/02/2020): Since the regimen is changing, the stopping rules will start over after this addendum among the single UCB cohort. We do not expect more than 15 additional patients.

10.4.1 Graft Failure by day +42

The goal is to construct a boundary based on graft failure (defined as failing to achieve an ANC >500/uL of donor origin by day 42) such that the probability of early stopping is at most 10% if the true graft failure rate is equal to 7% and our sample size is 28. Given these parameters, the upper stopping boundary for graft failure is 2/5 or 3/11, 4/19 or 5 patients fail to engraft.

10.4.2 Treatment Related Mortality by 100 days

Stopping rules were developed for excessive non-event mortality (TRM) by day +100 post-transplant. The goal is to construct a boundary based on TRM such that the probability of early stopping is at most 10% if the true rate is equal to 30% and our sample size is 28. Given these parameters, the upper stopping boundary for TRM is 3 deaths out of 3 patients, 4/5, 5/7, 6/10, or 7 /12, 8/14 or 9 patients with TRM by 100 days.

Due to the addition of stopping rules, it is understood that the overall power may be slightly decreased in this study.

10.5 Gender and Ethnicities 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.

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Appendix I - Lansky Play Score

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 II – Busulfan Dose Selection, AUC Monitoring and Algorithm for Dose Modification Using Once Daily IV Dosing

Refer to University of Minnesota BMT Busulfan Standard of Care Guidelines for blood sample collection process, busulfan results reporting, and therapeutic drug monitoring procedures.

Appendix III – Expected Toxicities of Standard Therapies

Conditioning Regimen:

Busulfan		
Common	**Less Common**	**Rare**
• low white blood cell count with increased risk of infection • low platelet count with increased risk of bleeding • low red blood cell count (anemia) which may cause tiredness, headache, dizziness • hair loss or thinning, including face and body hair (usually grows back after treatment) • long-term or short-term infertility (inability to have children) in men and women	• tiredness (fatigue) • sores in mouth or on lips • fever • nausea • vomiting • rash • loss of appetite • diarrhea	• abnormal blood tests results which suggest that the drug is affecting the liver • allergic reaction with hives, itching, headache, coughing, shortness of breath, or swelling of the face, tongue, or throat • scarring of lung tissue, with cough, difficulty breathing, and shortness of breath that may occur after prolonged use, or even months or years after stopping the drug • seizure • leukemia (several years after treatment) • darkened skin • heart problems with high-dose treatment, most often in people with thalassemia • problems with the hormone system that cause weakness, tiredness, poor appetite, weight loss, and darker skin • death due lung damage, bone marrow shutdown, or other causes

Fludarabine		
Common	**Less Common**	**Rare**
• low white blood cell count with increased risk of infection • low platelet count with increased risk of bleeding • low red blood cell count (anemia) with tiredness and weakness • tiredness (fatigue) • nausea • vomiting • fever and chills • infection	• pneumonia • diarrhea • loss of appetite • weakness • pain	• numbness and tingling in hands and/or feet related to irritation of nerves • changes in vision • agitation • confusion • clumsiness • seizures • coma • cough • trouble breathing • intestinal bleeding • weakness • death due to effects on the brain, infection, bleeding, severe anemia, skin blistering, or other causes

Melphalan		
Common	**Less Common**	**Rare**
• nausea • vomiting • low white blood cell count with increased risk of infection • low platelet count with increased risk of bleeding • low red blood cell count (anemia) with tiredness and weakness	• short-term or long-term infertility (inability to have children) • weakness	• severe allergic reaction • loss of appetite • scarring (fibrosis) or inflammation of lungs • hair loss, including face and body hair • rash • itching • second type of cancer (may happen years after treatment) • death from lung damage or other causes

Immunosuppressive Therapies for GvHD prophylaxis:

Tacrolimus	Mycophenolate mofetil (MMF)
• high blood pressure • abnormalities in blood chemicals • seizures • headaches • renal dysfunction to renal failure requiring dialysis	• nausea and vomiting • diarrhea • constipation • lowering of blood counts • leg cramps • skin rash • difficulty sleeping • chemical imbalances including high blood sugar • headaches • dizziness • high blood pressure

Hematopoietic Cell Transplantation – regardless of stem cell source

With the cell infusion

- nausea and vomiting
- possible allergic reaction (including itching, hives, flushing [red face], shortness of breath, wheezing, chest tightness, skin rash, fever, chills, stiff muscles, or trouble breathing)

As result of cord blood or marrow transplant

- Slow recovery of blood counts
- Graft failure
- Graft-Versus-Host Disease (GVHD)
- Other complications including:
  - Damage to the vital organs
    - Serious infections
    - Relapse of disease or a new blood cancer
    - Risk to the unborn

Additional Risks associated with the use of Umbilical Cord Blood

- **DMSO Toxicity** is a possible complication of UCB infusion and occurs due to the presence of DMSO in the thawed product. The risk of toxicity increases with the number of units (total volume per kg body weight) infused. Symptoms are due to histamine release and include:
  - cough
  - flushing
  - rash
  - chest tightness
  - nausea and vomiting
  - bradycardia and tachycardia
  - hypertension

- **Bacterial/Endotoxin Contamination** of cellular therapy products may occur, but rarely cause acute, severe or life threatening effects. However, the onset of high fever (>2ºC or >3.5ºF rise in temperature), severe chills, hypotension, or circulatory collapse during or immediately after infusion should suggest the possibility of bacterial contamination and/or the presence of endotoxin in the product.

- **Transmission of Infectious Disease** may occur because cellular therapy products are collected from human body and/or tissues. The donor selection criteria do not totally eliminate the risk of transmitting the agents currently tested such as HIV, HTLV, HBV, HCV, CMV, T. pallidum (Syphilis), West Nile Virus, and Trypanosome (Chagas). For some other infectious disease there are no routine tests to prevent disease transmission including Parvovirus spp., Plasmodium spp. (Malaria), the coronavirus associated with severe acute respiratory syndrome (SARS), and the agents of human transmissible spongiform encephalopathies (TSEs).

G-CSF		
Common	**Less Common**	**Rare**
• none	• bone and muscle pain • abnormal blood tests which suggest that the drug is affecting the liver	• fast heartbeat • low blood pressure • allergic reaction (may include shortness of breath, wheezing, swelling in the mouth or throat, hives, itching, flushing, or fever)