St. Jude Children's Research Hospital

HAPCORD – NCT02199041

Initial Version, dated: 04/02/13 Resubmitted to IRB 02/10/2014 (IRB Approved: 02/26/2014)
Activated:
Revision 0.1, dated: 08/22/14 (IRB Approved: 10/17/14)
Activated: 12/09/2014
Amendment 1.0, dated: 12/12/14 (IRB Approved: 05/04/16) Activated:
Amendment 2.0, dated 7/16/2016 (IRB Approved: 8/3/2016) Activated: 8/11/2016

COMBINED T CELL DEPLETED HAPLOIDENTICAL PERIPHERAL BLOOD STEM CELL AND UNRELATED UMBILICAL CORD BLOOD TRANSPLANTATION IN PATIENTS WITH HEMATOLOGIC MALIGNANCIES USING A TOTAL LYMPHOID IRRADIATION BASED PREPARATIVE REGIMEN

IND: 16036

Principal Investigator

Brandon Triplett, MD[1]

Sub-investigators

Guolian Kang, PhD[3]
Shane Cross, PharmD[5]
Ewelina Mamcarz, MD[1]
Terrence Geiger, MD, PhD[4]
Matthew J. Krasin, MD[6]
John Choi, MD, PhD[4]
Renee Madden, MD[1]
Lea Cunningham, MD[1]
Ashok Srinivasan, MD[1]
Amr Qudeimat, MD[1]
Paul Thomas, PhD[7]
William Janssen, PhD[1]
Benjamin Youngblood, PhD[7]

Department of Bone Marrow Transplantation and Cellular Therapy[1]
Department of Oncology[2]
Department of Biostatistics[3]
Department of Pathology[4]
Department of Pharmaceutical Sciences[5]
Department of Radiological Sciences[6]
Department of Immunology[7]

St. Jude Children's Research Hospital
262 Danny Thomas Place
Memphis, TN 38105
(901) 595-3300

COMMON ABREVIATIONS

Absolute neutrophil count (ANC)
Acute lymphocytic leukemia (ALL)
Acute myeloid leukemia (AML)
Adverse event (AE)
Alanine aminotransferase (ALT)
American Association of Blood Banks (AABB)
Aspartate aminotransferase (AST)
Body surface area (BSA)
Bone marrow (BM)
Bone marrow transplantation (BMT),
Bone Marrow Transplantation Follow Up (BMTFU)
Center for International Blood and Marrow Transplant Research (CIBMTR)
Central Nervous System (CNS)
Central Protocol and Data Monitoring Office (CPDMO)
Chronic Myeloid Leukemia (CML)
Clinical Data Update System (CDUS)
Common toxicity criteria (CTC)
Complete Remission (CR)
Cord Blood Transplantation (COBLT)
Creatinine Clearance (CrCL)
Cyclosporine (CSA)
Cytomegalovirus (CMV)
Dimethyl sulfoxide (DMSO)
Disease-free survival (DFS)
Epstein-Barr virus transformed lymphoblastoid cell lines (EBV-LCL)
Event-free survival (EFS)
Forced vital capacity (FVC)
Foundation for the Accreditation of Cellular Therapy (FACT)
Graft versus host disease (GVHD)
Granulocyte colony-stimulating factor (G-CSF)
Haploidentical HCT with NK infusion (HAPNK)
Health Insurance Portability Act (HIPPA)
Hematopoietic cell transplantation (HCT)
Hematopoietic stem cell (HSC)
Human Application Lab (HAL)
Human Immunodeficiency Virus (HIV)
Human leukocyte antigen (HLA)
Human T-lymphotropic virus (HTLV)
Institutional Biosafety Committee (IBC)
Institutional Review Board (IRB)
International Cord Blood Transplant Registry (ICBTR)
Juvenile Myelomonocytic Leukemia (JMML)
Killer-immunoglobulin receptors (KIR)
Matched related/sibling donor (MSD)
Matched unrelated donor (MURD)
Minimal Residual Disease (MRD)
Mycophenolate mofetil (MMF)
Myeloid Dysplastic Syndrome (MDS)
National Cancer Institute (NCI)
National Marrow Donor Program (NMDP)
Natural killer (NK)
New York Blood Center (NYBC)
Overall survival (OS)
Pharmacokinetics (PK)
Polymerase chain reaction (PCR)
Protected health information (PHI)
Pulmonary Function Testing (PFT)
Reduced Intensity Conditioning (RIC)
Shortening Fraction (SF)
Standard operating procedures (SOP)
T cell receptor excision circle (TREC)
Total body irradiation (TBI)
Total lymphoid irradiation (TLI)
Total nucleated cell (TNC)
Total Research and Knowledge System (TRACKS)
Transplant related mortality (TRM)
Umbilical cord blood (UCB)
Umbilical cord blood transplantation (UCBT)

TABLE OF CONTENTS

STUDY SUMMARY

HAPCORD:

Principal Investigator: Brandon Triplett, MD

IND/IDE: 16036

Brief Overview: In this study, participants with high-risk hematologic malignancies undergoing hematopoietic cell transplantation (HCT), who do not have a suitable human leukocyte antigen (HLA)-matched related/sibling donor (MSD), matched unrelated donor (MURD) or killer-immunoglobulin receptors (KIR) ligand mismatched haploidentical donor identified, will receive a combined T cell depleted (TCD) haploidentical peripheral blood stem cell (PBSC) and unrelated umbilical cord blood transplantation (UCBT) using a total lymphoid irradiation (TLI) based preparative regimen. Patients who have a suitable KIR ligand mismatched haploidentical donor will be preferentially enrolled in available haploidentical protocol if clinically appropriate. Those with a suitable HLA matched sibling, unrelated donor or KIR mismatched haploidentical donor identified may be eligible for participation if the donor is not available in the necessary time. Participants must be ≤ 21 years old and with sufficient multi-organ function as specified in protocol. The assessments and follow-up evaluations noted in the protocol follow the St. Jude standard operating procedures (SOP) for all recipients of allogeneic HCT.

Intervention: Combined TCD haploidentical PBSC graft with an unrelated UCBT graft using a TLI-based preparative regimen. The haploidentical graft will undergo processing for T cell depletion and $CD34^+$ stem cell selection.

Brief Outline of Treatment Plan: The preparative regimen includes total lymphoid irradiation (TLI) (8 Gy), fludarabine (150 mg/m^2), cyclophosphamide (60mg/kg), melphalan (140 mg/m^2) and thiotepa (10mg/kg). TLI will be administered in 4 fraction at 200 cGy/fraction over 3 days on day -9 to day -7, fludarabine will be given once a day at 30 mg/m^2 for five days on day -8 to day -4, cyclophosphamide will be given once a day at 60mg/kg for one day on day -6, thiotepa will be given twice a day at 5 mg/kg for one day on day -3, and melphalan will be given once a day for 2 days on day -2 and day-1. Post-transplantation immunosuppression with tacrolimus will begin on day -2 and MMF on day 0. Haploidentical donor product will be processed for $CD45RA^+$ depletion and $CD34^+$ selection and will be infused on day 0 and day +1. Cord Blood infusion will occur on day +2. G-CSF may start on day +3, but may be held at the discretion of the attending physician.

Objectives:

Primary objective

- Estimate the incidence of donor derived neutrophil engraftment by day +42 post-transplant for participants with high-risk hematologic malignancies undergoing a total lymphoid irradiation (TLI)-based hematopoietic cell transplantation (HCT) using a T cell depleted (TCD) haploidentical donor peripheral blood stem cell (PBSC) donor combined with a unrelated umbilical cord blood (UCB) donor.

Secondary objectives:

- Estimate the incidence of malignant relapse, event-free survival, and overall survival at one-year post-transplantation.
- Estimate the incidence and severity of acute and chronic graft versus host disease

(GVHD).

- Estimate the incidence of secondary graft failure, transplant related mortality (TRM) and transplant related morbidity in the first 100 days after transplantation.

Exploratory Objectives

- Assess the relationship between pre-transplant minimal residual disease (MRD) with transplant outcomes.
- Record immune reconstitution parameters, including chimerism analysis, quantitative lymphocyte subsets, T cell receptor excision circle (TREC) and spectratyping. Immunophenotyping and functional assays of T, B and NK cells and lymphocytes will also be evaluated.
- Characterize the recovery of Gamma Delta (γδ) T cells after HCT, including T cell receptor analysis, phenotyping and functional analysis.
- Characterize influenza infection during HCT by monitoring viral isolates and key host factors associated with influenza susceptibility.

Hypotheses/Estimates:

Of the patients with hematologic malignancies requiring allogeneic HCT, approximately 30% will have a suitable matched related/sibling donor (MSD) and another 30% will have a matched unrelated donor (MURD) identified.[1-4] The lack of an adequate MURD for 25-60% of eligible pediatric recipients, and the lengthy duration of the donor search process in those with high risk of relapse have prompted investigators to use an alternative source of HSC, namely UCB.[3] Although UCB has been shown to be a suitable source of HSC, initial studies suggested UCBT is associated with increase incidences of TRM when compared to recipients of MSD or MURD.[5,6] A pilot study in adults that combined TCD haploidentical PBSC and an unrelated UCB graft resulted in rapid neutrophil engraftment with minimal GVHD and durable remission.[7] Here, we propose to investigate the outcomes of pediatric patient with high-risk malignancies undergoing a combined TCD haploidentical PBSC and unrelated UCB using a TLI-based preparative regimen at St. Jude Children's Research Hospital. The primary objective of the study is to estimate the rate of donor derived neutrophil engraftment by day +42 post-transplant. Secondary aims will assess graft failure, overall survival, event-free survival, risk of relapse, graft versus host disease (GVHD), transplant related mortality (TRM), transplant related morbidity, and immune reconstitution.

Criteria for Evaluation:

Safety Evaluation

- The primary measures of safety will be the rate of therapy related death and the rate of severe graft versus host disease.
- Ongoing assessment of toxicity will be done using the NCI CTC version 4. Acute GVHD will be evaluated using the standard grading criteria (Appendix B).

Efficacy

- Neutrophil and platelet engraftment will be determined using the parameters put forth by the Center for International Blood and Marrow Registry**.** Assessments will be made upon review of daily complete blood count and serial chimerism studies.
- BM studies for disease status evaluation will be performed at approximately 28 days, 100 days, and yearly post-transplant.

- Acute GVHD will be assessed using the standard grading criteria put forth by Prezpiorkia et al 1995. Chronic GVHD will be assessed using the NIH Consensus Criteria for Chronic GVHD 2005 (Appendix B).

Study Design: Phase II - Non Randomized

Study Population: Patients with high-risk hematologic malignancies who are in complete remission or AML in first relapse with less than 25% blasts at the time of evaluation are eligible for enrollment. Participant must be less than or equal to 21 years old.

Candidates must have a suitable UCB product available (matched ≥ 4 of 6 to the patient sufficient with sufficient cell dose). Those with a suitable HLA matched sibling, matched unrelated donor, or KIR mismatched haploidentical donor identified will be eligible for participation if the donor is not suitable or available in the necessary time. For example, patients who have a KIR mismatched haploidentical donor but have relapsed after a KIR mismatched haploidentical HCT are eligible for this protocol. Additional eligibility criteria are specified to assure sufficient multi-organ system function.

Inclusion criteria for transplant recipient

- Age less than or equal to 21 years old.
- Has a partially HLA-matched single UCB product available.
- Has a partially HLA-matched haploidentical related donor available.
- High-risk hematologic malignancy.
  - Very high risk ALL in CR1, ALL in High risk CR2, ALL in CR3 or subsequent.
  - AML in high risk CR1, AML in CR2 or subsequent
  - Therapy related AML, with prior malignancy in CR > 12mo
  - MDS, primary or secondary
  - NK cell, biphenotypic, or undifferentiated leukemia in CR1 or subsequent.
  - CML in accelerated phase, or in chronic phase with persistent molecular positivity or intolerance to tyrosine kinase inhibitor.
  - Hodgkin lymphoma in CR2 or subsequent after failure of prior autologous HCT, or unable to mobilize stem cells for autologous HCT.
  - Non-Hodgkin lymphoma in CR2 or subsequent.
  - JMML
  - Refractory hematologic malignancies (ALL, AML, CML in blast crisis, Hodgkin or non-Hodgkin lymphoma) due to chemoresistant relapse or primary induction failure.
  - All patients with evidence of CNS leukemia must be treated and be in CNS CR to be eligible for study.
- Patient must fulfill pre-transplant evaluation:
  - Left ventricular ejection fraction ≥ 40% or cardiac shortening fraction ≥ 25%.
  - Creatinine clearance or GFR ≥ 50 ml/min/1.73m^2.
  - Forced vital capacity (FVC) ≥ 50% of predicted value or pulse oximetry ≥ 92% on room air.
  - Karnofsky (≥ 16 years) or Lansky (<16 years) performance score ≥ 50

- Bilirubin ≤ 3 times the upper limit of normal for age.
- Alanine aminotransferase (ALT) ≤ 5 times the upper limit of normal for age.
- Aspartate aminotransferase (AST) ≤ 5 times the upper limit of normal for age.

Exclusion Criteria for Transplant Recipients:

- Patient has a suitable MSD, volunteer MURD, or KIR mismatched haploidentical donor available in the necessary time for stem cell donation.
- Patient has any other active malignancy other than the one for which HCT is indicated.
- Patient is pregnant as confirmed by positive serum or urine pregnancy test within 14 days prior to enrollment.
- Patient is breast feeding
- Patient has Down Syndrome
- Patient has a current uncontrolled bacterial, fungal, or viral infection per the judgment of the PI.

Sample Size: Up to 49 evaluable.

Randomization: N/A

Data Analyses: Statistical considerations and ongoing analysis will be conducted by Dr. Guolian Kang and designated associates within the St. Jude Department of Biostatistics.

Anticipated Primary Completion Date: (5 years)

Anticipated Study Completion Date: (6 years)

Timeframe for Primary Outcome Measure: 42 days

Data Management: Protocol compliance, data collection including safety data, and reporting will be carried out by the Department of Bone Marrow Transplantation and Cellular Therapy Research Office.

Human Subjects: The risks to subject are primarily related to the infusion and the conditioning regimen. The allogeneic stem cells may induce serious and possibly fatal disorders such as GVHD, veno-occlusive disorder and post-transplant lymphoproliferative disease. Because of the required conditioning, recipients are at high-risk for serious and possibly life-threatening infection, bleeding, and anemia. Adverse events will be treated, monitored, and reported appropriately.

Possible benefits of participation include obtaining and/or sustaining disease remission. In addition, there is the possibility of psychological benefit from knowing participation has helped researchers gain more understanding about the efficacy of UCBT.

Alternatives to participation are identified as chemotherapy, research treatment if available, and/or supportive therapy.

The possible benefits, alternatives to participation, and side effects, including that there may be unknown side effects of treatment, are detailed in lay language within the respective informed consent documents.

1.0 OBJECTIVES

1.0 Primary objective

1.1.1 To estimate the incidence of donor derived neutrophil engraftment by day +42 post-transplant for participants with high-risk hematologic malignancies undergoing a total lymphoid irradiation (TLI)-based preparative regimen using a T cell depleted (TCD) haploidentical peripheral blood stem cell (PBSC) combined with an unrelated umbilical cord blood (UCB) graft.

1.1 Secondary objectives

1.2.1 Estimate the incidence of malignant relapse, event-free survival (EFS), and overall survival (OS) at one-year post-transplantation.

1.2.2 Estimate the incidence and severity of acute and chronic graft versus host disease (GVHD) in the first 100 days after transplantation.

1.2.3 Estimate the incidence of secondary graft failure, transplant related mortality (TRM) and transplant related morbidity in the first 100 days after transplantation.

1.3 Exploratory Objectives

1.3.1 Assess the relationship between pre-transplant minimal residual disease (MRD) with transplant outcomes.

1.3.2 Record immune reconstitution parameters, including chimerism analysis, quantitative lymphocyte subsets, T cell receptor excision circle (TREC) and spectratyping. Immunophenotyping and functional assays of T, B and NK cells and lymphocytes will also be evaluated.

1.3.3 Characterize the recovery of Gamma Delta (γδ) T cells after HCT, including T cell receptor analysis, phenotyping and functional analysis.

1.3.4 Characterize influenza infection during HCT by monitoring viral isolates and key host factors associated with influenza susceptibility.

2.0 BACKGROUND AND RATIONALE

2.1 Overview

Allogeneic HCT has become a widely accepted curative therapy for many hematologic malignancies that cannot be cured with chemotherapy alone.[9,10] Studies have demonstrated that HCT is a potentially curative therapy for patients with CML, AML, ALL and MDS.[11-14] Furthermore, patients with non-Hodgkin or Hodgkin's lymphoma who recur after an autologous HCT may be successfully treated with an allogeneic HCT.[15,16]

Of the patients with hematologic malignancies requiring allogeneic HCT, approximately 30% will have a suitable matched related/sibling donor (MSD) and another 30% will have a matched unrelated donor (MURD) identified.[1-4] The lack of an adequate MURD for 25-60% of eligible pediatric recipients, and the lengthy duration of the donor search process in those with high risk of relapse have prompted investigators to use an alternative source of HSC, namely UCB.[3] Recent comparative studies report no significant difference in OS in patients undergoing UCBT compared to MURD HCT.[17-21] Thus, the number of patients undergoing UCBT has rapidly increased over the past 10 years. In the pediatric

population, UCB is now the most common source of HSC in unrelated donor transplant. However, the limited number of progenitor cell in the UCB unit result in delayed and unpredictable count recovery. The delayed neutrophil recovery predisposes patients to infection and malignant relapse. Recipients of UCBT are associated with increase incidences of TRM when compared to recipients of MSD or MURD HCT.[5,6] The goal of the protocol is to decrease the risks associated with delayed neutrophil recovery.

Recently a pilot study demonstrated that by combining a TCD haploidentical PBMC product and an unrelated UCB graft resulted in rapid neutrophil engraftment with minimal GVHD and durable remission.[7] St. Jude has gained considerable expertise with haploidentical HCT in children [22-29] and we propose to investigate the outcomes of pediatric patient with high-risk malignancies receiving a combine TCD haploidentical PBSC and UCB graft using a TLI-based preparative regimen at St. Jude Children's Research Hospital.

2.2 Umbilical Cord Blood Transplantation (UCBT)

2.2.1 Historical Background:

The use of UCB as a clinical source of HSC was first considered in the late 1960's when UCB from eight donors were pooled and infused into a 16 year old male with acute lymphocytic leukemia (ALL).[30] Although full reconstitution was not observed, there was evidence suggesting transient mixed chimerism. Thereafter, additional studies were performed that suggested that if adequate numbers of HSC could be collected; UCB has the potential for long-term reconstitution. In 1989 the first successful matched sibling UCBT was performed for a child with Fanconi anemia. In 1995, the International Cord Blood Transplant Registry (ICBTR) reported the results of the first 44 matched sibling UCBT demonstrating UCB contained sufficient number of hematopoietic progenitors to reliably engraft transplant recipients after a myeloablative conditioning regimen.[31]

The first successful unrelated UCBT in a pediatric patient occurred at Duke University in 1993.[32,33] In 1996, Kurtzberg et al and Wagner et al simultaneously published the unrelated UCBT experiences at Duke and Minnesota, respectively.[33,34] These initial studies showed the median time to neutrophil recovery was 22 and 24 days, respectively. Combining the 43 patients, 88% engrafted, 54% had Grade II-IV GVHD and 9% had Grade III-IV GVHD. The OS at 100 days was 53%. Together, these studies suggested that UCB is a suitable alternative source of allogeneic HSC for pediatric patients.

In nearly every large single center or registry analysis of outcomes after UCBT, cell dose is identified as an important factor influencing the incidence and rate of hematopoietic recovery, risk of TRM, and probability of survival. Furthermore, data suggest that infusion of two partially HLA-matched UCB units, which always augments the graft cell dose, is safe and may improve neutrophil recovery and survival. To determine whether the infusion of two UCB units enhances survival, the Blood and Marrow Transplant Clinical Trials Network (BMTCTN) proposed a multi-center randomized trial in 2006. Preliminary data suggest that patients receiving double unit UCBT have a higher risk for acute GVHD albeit a lower risk for relapse. However, until data clearly demonstrate a survival

advantage, double UCBT is currently only recommended for those patients who do not have an adequate single unit.[35]

Lastly, in the past decade, large comparative studies evaluating the outcomes of transplantation using UCB versus other HSC sources have been performed. Reports demonstrate that the incidence of GVHD was lower in patients undergoing UCBT compared to HLA-matched bone marrow (BM) recipients. Furthermore, recent studies that include long-term analysis demonstrate that outcomes are similar for patients undergoing transplantation using MURD or UCB sources.[14-18, 25] Thus, UCBT should be considered as a viable options for patients with high-risk malignancies.[17-21,36 7-11, 25]

2.2.2 Cell Dose Threshold and Interaction with HLA match

Cell dose remains one of the single most important determinants of a successful UCBT.[37-39] Numerous clinical studies have shown that the total nucleated cell (TNC) dose and the $CD34^+$ cell dose in the UCB graft are highly correlated with neutrophil and platelet engraftment as well as the incidence of graft failure and early TRM.[3,38,40,41] The first Eurocord Registry report by Gluckman et al, 1997, demonstrated that among 527 patients undergoing UCBT, the most important predictor in outcome was the infusion of $\geq 3 \times 10^7$ TNC per kilogram body weight.[37,42] Moreover, adult recipients had increased TRM by day 180 compared to children (56% versus 32%), and deaths were related to the number of cells infused, with patients who received $\leq 1 \times 10^7$ TNC/kg having a 75% probability of death compared to 30% for those who received $\geq 3 \times 10^7$ TNC/kg. Rubinstein et al, 1998, published the New York Blood Center experience with 562 patients undergoing UCBT and similarly demonstrated a strong correlation between the number of leukocytes infused and neutrophil engraftment.[39] Thus, the collective data point to improved outcome of UCBT for patients receiving a larger number of $CD34^+$ cells. Delayed neutrophil engraftment has also been correlated with HLA mismatch at greater than 2 loci, but recent studies have suggested that the impact of HLA disparity on survival can be partially overcome by increasing cell dose.[43] Combined, these studies identified a minimum threshold for cell dose and HLA matching which lead to a significant reduction in TRM after UCBT. Although the exact threshold criteria for each degree of HLA mismatch is not know, the principle for the dose algorithm is clear.[35,44,45]

2.2.3 Role of Double UCBT

For most adults and large children, a single UCB unit that meets the cell dose requirement is not available. To overcome this obstacle, investigators at the University of Minnesota began infusing two partially HLA-matched units to augment cell dose. They found that neutrophil engraftment was achieved more rapidly and in a higher proportion of patients than the previous cohort who received only a single UCB unit.[46] Preliminary evidence suggests that double unit UCBT may be associated with a decreased risk of relapse in patients with good disease control at the time of transplant, but is also associated with a increased incidence of mild to moderate acute GVHD compared to single UCBT.[47,48,49] Currently, it is unclear whether double UCBT offers any benefit other than

extending the application of UCBT by achieving the cell dose threshold.[50] Until data clearly demonstrate a survival advantage, double UCBT is currently only recommended for those patients who do not have an adequate single unit.[51] An adequate single unit is defined as: $\geq 3.0 \times 10^7$ nucleated cells/kg for 6/6 HLA-matched units, $\geq 4.0 \times 10^7$ nucleated cells/kg for 5/6 HLA-matched units and $\geq 6.0 \times 10^7$ nucleated cells/kg for 4/6 HLA-matched units.[49,51,52]Despite adequate cell dose, patients undergoing double UCBT continue to have delayed neutrophil engraftment compared to MSD and MURD HCT.

2.2.4 UCBT Results

Neutrophil Engraftment: Most studies report the time to hematopoietic recovery after UCBT is delayed with the median time to neutrophil recovery ranging between 20 - 30 days and the incidence of neutrophil engraftment after single UCBT ranges from 65-92%.[32,34,38,39,41,42,53-61] Studies have consistently demonstrated the rate and incidence of neutrophil engraftment after UCBT is slower than bone marrow transplantation (BM), dependent on cell dose and the threshold for cell dose depends on HLA match grade.

GVHD: The first report from the New York Blood Center experience by Rubinstein et al indicated that GVHD after UCBT occurred at a lower rate compared to those with MURD transplantation.[39] Thereafter, Rocha et al published the first comparative analysis between UCBT and MURD transplantation in pediatric patients that substantiated the claim that acute and chronic GVHD were less common in recipients of UCB.[19] In larger studies, the incidence of acute GVHD is reported to range from 33-44% and 11-22% for grades II-IV and III-IV acute GVHD respectively. The incidence of chronic GVHD ranges from 0-25%.[38,39,58,60,61]

Survival: Nearly all studies demonstrate a significant relationship between UCB cell dose and survival and the reported probability of survival after single UCBT ranges from 18-78%.[17,21,41,46,48,52,62-66] One of the largest reports from the Center for International Blood and Marrow Transplant Research (CIBMTR) by Eapen et al in 2007 compared the outcomes of 785 pediatric patients with acute leukemia who received a UCBT (n=503) or an unrelated BMT (n =282). The most notable finding was that UCBT compared favorably to an 8/8 allele-matched unrelated BMT. In comparison with allele-matched BMT, the 5 year disease free survival (DFS) was similar to that after either one or two antigen mismatched UCBT and possibly higher after 6/6 allele-matched UCBT.[17] These and other studies support that UCB should be considered an acceptable source of HSC grafts in the absence of an HLA-matched donor. [17,18,21,38,39,41,46,48,52,58,60-67]

2.3 Haploidentical HCT

2.3.1 Haploidentical HCT Background

Haploidentical donors are another viable alternative donor source when a MSD or MURD are not available. Also, family members are highly motivated, easily accessible, and readily available for most patients. Along with other institutions, St. Jude has shown haploidentical HCT to be an effective therapy for patients with hematologic malignancies.[68-72] Due to the high potential for GVHD with the

degree of HLA mismatch seen in haploidentical HCT, most haploidentical grafts are extensively TCD prior to infusion.[73] Significant progress was achieved when high cells dose of $CD34^+$ cells were infused after high-intensity conditioning.[71] It has been well established that prompt neutrophil engraftment with rapid immune reconstitution can be achieved with $CD34^+$ enriched haploidentical cells, purified by positive ($CD34^+$) and negative ($CD3^-$ lectin agglutination) selection.[74,75]

Building on the initial studies above, St. Jude has gained considerable expertise with haploidentical HCT in children.[22-27,29,76] Over the past decade (2001-2010), more than 220 mismatched related donor HCT have been performed at St. Jude. Initially, most haploidentical HCT performed in this institution were for patients with relapsed and refractory hematologic malignancies, however as it became a more successful therapeutic maneuver, it has become a primary transplant option for patients who lack a well matched donor.[28]

Recent studies suggest that alloreactive NK cells play a role in graft versus leukemia (GVL) and influence outcomes of patients with hematologic malignancies after haploidentical HCT.[77-84] The NK cell alloreactivity depends on the balance of signals mediated through activating and inhibitory KIRs on the NK cell. In patients who received haploidentical HCT for high-risk leukemia at SJCRH the presence of KIR mismatch dramatically reduced the risk of relapse.[25] Thus, patients with high risk hematologic malignancies that lack an HLA-matched donor but have a KIR mismatched haploidentical donor available, would be expected to benefit from haploidentical HCT.

2.3.2 Rationale for immunomagnetic TCD of hematopoietic progenitor cell graft

Donor T cells in the haploidentical graft play a major role in mediating GVHD, with as few as 3 x 10^4 T cells/kg can cause GVHD.[85] TCD allows for a HSC graft with very low T cell content, such that the risk of GVHD is decreased. T cells can be removed from the HSC graft by direct removal of T cells (negative selection) or positive selection of $CD34^+$ progenitors. St. Jude has had success using either method of TCD in haploidentical donor transplantation.

In this study, $CD34^+$ selection by CliniMACS will be the used for TCD of the haploidentical graft. Although positive selection allows for extensive TCD, the graft is devoid of other important cell populations such as NK cells and myeloid cells such as dendritic cells and monocytes.[86,87] In addition, this extensive degree of TCD has significant negative effects on the time to donor immune competency. Donor T cells are critical for reconstituting the allogeneic host immune system, and lymphocyte recovery is an important determinate of outcome post-transplant.[88,89] Transplants that employ TCD result in elimination of most memory T cells, leading to protracted immune dysfunction- an effect that becomes more severe with higher intensity conditioning regimens.[22,90] This results in an increased rate of opportunistic infections. Indeed, viral infections are the most common cause of death of children receiving haploidentical transplants.[91] The majority of these infections occur within the first 6 months following transplantation, when T cell immunity is the lowest.[92] Reconstitution of immunity can be partially restored by therapeutic infusions of donor cytotoxic

lymphocytes.[88,93-95] However, in addition to requiring significant resources and expertise, the cells must be engineered or selected for specific infections - making this approach impractical for broad application.[96]

A more ideal approach would be to infuse a cell product with diverse lymphocyte repertoire capable of effectively recognizing a variety of pathogens, as well as malignant cells without additional GVHD risk. One strategy would be to target naïve T cells for lymphocyte depletion. Naïve T cells are fully matured but do not proliferate until they encounter their receptor specific ligand.[97] After ligand recognition, there is activation of proliferative signals that initiates a marked, antigen-specific cell expansion and inflammatory response. While many of these naïve T cells will undergo apoptosis after the initial response, others are rescued from immune retraction and will persist as memory T cells. Once generated, memory cells persist in the circulation as a diverse cell pool that is critical for long-term infection control.[98] Furthermore, memory T cells are capable of more rapidly responding to future infectious challenges.[99]

The isoform of the leukocyte common antigen, CD45RA, selectively identifies naïve T cells. Human studies have shown that sorted donor $CD45RA^+$ naïve T cells are far more alloreactive than all memory subsets tested.[100] In animal models, naïve T cells, are potent inducers of GVHD.[96] Moreover, in similar models, lymphocyte infusions specifically depleted of $CD45RA^+$ naïve T cells do not cause GVHD.[101] Conversely, $CD45RA^-$ memory T cells do not cause physical or histologic evidence of GVHD. Among $CD4^+$ T cells, the $CD45RA^-$ subset has equivalent "helper" functions as $CD45RA^+$ cells in the generation of alloreactive cytotoxic T cells.[102] Further, it is the $CD45RA^-$ memory cells that are responsible for aiding B-cell differentiation and antibody production.

After demonstrating promising pre-clinical results, selective CD45RA depletion in patients undergoing HCT are underway, including two at SJCRH (RADIANT and HAPNK1). Depletion of $CD45RA^+$ cells by magnetic beads has been shown to be a feasible and effective method for depletion of naïve T cells, with up greater than 3-log depletion in number of $CD45RA^+$ cells (Data provided by Miltenyi). Furthermore, experiments were performed in the HAL to confirm and qualify the $CD45RA^+$ depletion procedures. HSC products were obtained from G-CSF mobilized normal donors by apheresis. The HPC, Apheresis products were depleted of $CD45RA^+$ cells following a procedure provided by Miltenyi Biotech. Briefly, the cells were incubated with the CD45RA microbead reagent followed by washing to remove unbound beads. The labeled cells were then applied to the CliniMACS device (Depletion Tubing Set) and the $CD45RA^+$ cells removed using the Depletion 3.1 program. Flow cytometric analysis before and after depletion was performed following procedures provided by Miltenyi Biotech. The results of the experiments are presented in the table below. An example flow cytometric analysis of $CD3^+$ cells is also included. We hypothesize combining a $CD34^+$ enriched product (HPC, Apheresis 1) along with a CD45RA+ depleted product (HPC, Apheresis 2) will provide a HSC graft with good stem cell content (3-5 x 10^6 $CD34^+$ cells/kg) and a useful memory T cell fraction without additional GVHD risk to the recipients on this trial. Although some patients will have a graft

derived from the UCB donor, we hypothesize that the novel TCD haploidentical PBSC graft may provide immune protection during the period of severe lymphopenia and/or help facilitate the engraftment of the new donor.

CELL COUNTS PRE AND POST DEPLETION				
	Experiment 1		Experiment 2	
	Pre	Post	Pre	Post
No. of TNC (10^6)	29748	10270	26166	10981
No. of $CD34^+$ (10^6)	636	311	693	395
No. of $CD3^+$ (10^6)	12238	4555	6353	1985
No. of $CD3^+CD45RA^+$(10^6)	7877	4	3520	2.2
$CD3^+$ $CD45RA^+$ Log Depletion		3.28		3.21

2.4 Rationale for TLI-based Preparative Regimen

TLI-based preparative regimens have the potential to improve the outcomes of allogeneic HCT by decreasing the acute TRM compared to regimens using total body irradiation (TBI).[103-105] TLI was initially developed with curative intent in patients with Hodgkin Lymphoma, and it was its use in these patients that led to the discovery of TLI's alteration of T-cell specific immune responses. Years of pre-clinical research on TLI have shown that the use of TLI promotes neutrophil engraftment and reduces GVHD.[106] This work was then followed up with therapeutic trials in humans with hematologic malignancy, which showed that conditioning with TLI and ATG alone allowed a high rate of durable donor engraftment, a low rate of GVHD, and preservation of evidence of graft versus malignancy effect, in patients who received HLA-matched related or unrelated donor grafts.[107,108] We have significant institutional experience using TLI-based regimens for allogeneic transplantation (n=29 as of June 2012). Importantly, TLI-based conditioning has allowed successful salvage HCT in patients who failed previous allo-HCT. Nine patients have received TLI-based conditioning with haploidentical donor HCT after experiencing previous allograft failure (3 had primary graft failure, 4 had initial neutrophil engraftment with acute rejection, and 2 had late graft failure). The same haploidentical donor was utilized in 5 of the 9 salvage HCT, with 4 patients receiving a new (haploidentical) donor. Eight of 9 patients (89%) experienced durable neutrophil engraftment at a median of 12 days (range 10 – 27 days). The remaining patient had primary graft failure due to progressive disease. This experience indicates that TLI is effective for facilitation of neutrophil engraftment, even in patients receiving a mismatched haploidentical donor graft and with a history that indicates a very high risk of graft failure. There is additional published experience from Germany in which TLI – given as a single 7Gy fraction – was utilized in 14 adult and pediatric patients for reconditioning after graft failure/rejection.[109] Despite most of the patient having haploidentical donors, neutrophil engraftment was obtained in all evaluable patients. In addition, TLI was well tolerated in this pediatric population that had recently received another (typically myeloablative) preparative regimen. Finally, 10 patients in Chile received TLI-containing preparative regimen, similar to our proposed regimen, as a part of their haploidentical donor preparative regimen.[110] TLI was given as a single dose at 7Gy. Nine out of 10 patients experienced rapid donor neutrophil engraftment, with relapse as the cause of the one primary graft failure. Six of 10 patients were alive and disease free at one year, 3 died of progressive disease, and one of infection.

2.5 Rationale for Study

In the past 20 years, over 20,000 UCBT have been performed worldwide and over 400,000 UCB units have been stored in more than 100 UCB banks.[33,40,111] The main practical advantages of using UCB as an alternative source of HSC are (1) rapid availability, (2) absence of donor risk, (3) relative ease of procurement, (4) absence of donor attrition, (5) very low risk of transmissible infectious diseases and (5) low risk of acute GVHD despite HLA mismatch. Furthermore, UCB is particularly beneficial for patients of ethnic and racial minority descent for whom adult marrow and blood donors often cannot be identified.[33,39,40,111] According to data from the National Marrow Donor Program (NMDP) donor registry, the probability of finding an 8 out of 8 HLA-matched donor is 51% for Caucasians, 30% for Hispanics, 20% for Asians and 17% for African Americans. Since patients who do not have a MURD can undergo a mismatched unrelated UCB transplantation and achieve similar results, the number of patients undergoing UCBT has rapidly increased over the past 10 years. The CIBMTR reports that from 2004 to 2008, UCB is now the most common graft source and BM is no longer the most common unrelated donor graft source for patients 20 years or less. Furthermore, in 2007 and 2008, 46% of all unrelated donor transplantations used UCB grafts for patients 20 years or less.

Patients who do not have an appropriate MSD or MURD donor available in a timely manner often undergo transplantation using an alternative HSC source from UCB or haploidentical donors. Often, the type of donor is chosen based on various factors related to urgency of transplantation, patient, disease, and transplant-related factors as well as the center's experience. Although a prospective randomized clinical trial is the accepted standard to compare different treatment regimens, the feasibility and acceptability of such a clinical trial is problematic and has not been performed. However, retrospective studies comparing the two donor sources have demonstrated no difference in survival.

The Eurocord group performed a retrospective study comparing the outcome of pediatric patients with high-risk ALL undergoing either UCBT or haploidentical HCT. Patients had received either haploidentical (n=118) or UCB (n=341) transplantation in Eurocord centers between 1998 and 2004. The median follow-up was 56 and 24 months for haploidentical and UCBT patients, respectively. Failure of neutrophil engraftment was significantly higher following UCBT than after haploidentical HCT (23% vs. 11%, p=0.007). In a multivariate analysis, relapse incidence was higher in haploidentical HCT recipients compared to UCBT (relative risk 1.7, p=0.01), but TRM and DFS were not significantly different. In conclusion, in pediatric patients with ALL, UCBT is associated with inferior rate of neutrophil engraftment, higher incidence of grades II–IV acute GVHD and lower incidence of relapse compared to haploidentical HCT; however, there was no difference in terms of TRM and DFS. Therefore, in the absence of an HLA-identical donor, both strategies were reported to be suitable options to treat a child with high-risk ALL.

At our institution, patients who do not have an appropriate MSD or MURD donor available often undergo a haploidentical HCT. Studies at St. Jude have shown the presence of natural killer (NK) KIR mismatch in a haploidentical donor dramatically reduced the risk of relapse in patients who received HCT for high-risk leukemia.[25,26] KIR mismatch is predictive of NK alloreactive effects in haploidentical HCT.[25,26,79,81] Thus, patients who

have a KIR mismatch haploidentical donor will be enrolled into an available haploidentical HCT. However, one-third of all patients posses all relevant KIR ligands and will not have a KIR mismatched haploidentical donor. These patients will require an alternate donor for best clinical outcome. Patients who require an alternative donor HSCT and do not have a KIR mismatched haploidentical donors are likely to benefit most from UCBT for best clinical outcome. Contradictory results have been reported on the effect of KIR matching in UCBT. The European group reported KIR-ligand incompatible UCBT improved DFS (hazards ratio = 2.05, p = .0016) and OS (hazards ratio = 2.0, p = .004) and decreased relapse incidence (hazards ratio = 0.53, p = .05).[112] However, the Minnesota group found no advantage using KIR matching in UCBT.[113] Currently, the effect of KIR matching in UCBT is controversial and UCB selection based on KIR matching is not recommended. Some patients may have relapsed after receiving a KIR mismatched haploidentical HCT and would be eligible for this protocol despite having a KIR mismatched donor.

To date, UCBT01, the first UCBT protocol at SJCRH has accrued 9 patients, with 5 patients receiving a single UCB unit and 4 patients receiving two UCB units. One patient on the observational arm died due to TRM before day 100. All 8 remaining patients engrafted however, 2 patients would be categorized as significant graft delay/failure with neutrophil engraftment after day +42. One patient engrafted at day 49 and another at day 51. The mean time to neutrophil engraftment is 28±13 days, (range; 12-51). Thus, despite optimizing HLA match and cell dose, neutrophil engraftment continues to be significantly delayed in patients undergoing UCBT.

In order to abrogate the increased TRM and complication from graft failure and delayed neutrophil engraftment, we propose to provide a combined haploidentical donor with a single UCBT. A pilot study reported the results of 45 adults with a mean age of 50 yrs (range; 20-69) who underwent a RIC regimen using fludarabine, melphlan and ATG for high risk malignancies. The graft consisted of a single UCB with minimum cell dose of 1.0 x 10^7 TNC/kg along with a TCD haploidentical PBSC donor graft. GVHD prophylaxis was with MMF and tacrolimus. The mean time to neutrophil engraftment was 11 days and platelet engraftment was 19 days. Chimerism analysis demonstrated that the haploidentical graft dominated early after HCT and is replaced by the UCB graft by day 100 in majority of the patients. However, 6 of the 45 patients had neutrophil engraftment derived from the haploidentical donor with no evidence of UCB donor. On the converse, 6 patients had had neutrophil engraftment derived from the UCB donor with no evidence of the haploidentical donor. . The risk for GVHD was not increased with the reported cumulative incidence of acute GVHD of 25% and chronic GVHD of 5%.

The primary objective of this study is to optimize the time to donor derived neutrophil engraftment and decrease the risk associated with delayed neutrophil engraftment such as infections and relapse. Briefly, we propose to provide a $CD45RA^+$ depleted haploidentical PBSC graft to allow for rapids donor derived neutrophil engraftment. The graft will contain "memory" T cells that may help facilitate engraftment and/or provide immune protections. The UCB graft provides long-term allogeneic graft and contains majority of the CD45RA naïve T cells.

2.6 Minimal residual disease (MRD)

Despite the fact that the vast majority of patients with high-risk leukemia are in clinical remission prior to HCT, approximately 30-40% ultimately relapse after HCT. Detection of MRD and early intervention may improve the clinical outcome. Detection of leukemic cells that are below the limits of detection by standard morphologic examination allow early interventions when the patients are MRD positive but still in remission. By using flow cytometry and polymerase chain reaction (PCR) amplification of antigen receptor genes in tandem, investigators at our institution have been able to conduct MRD studies in 80 consecutive ALL cases.[114] Results of St. Jude institutional studies have shown that detection of MRD by immunologic techniques at any point in the treatment course is a powerful predictor of relapse in children with ALL.[114-116] However, other studies suggest that eradication of all acute leukemia cells may not be a prerequisite for cure.[117,118]

Similar to conventional-dose therapy, controversy also exists on the implication of MRD in the setting of HCT. Unlike CML, there is a paucity of data on the natural history of AML and ALL patients who have MRD after HCT, and how pre-transplant MRD levels influence post-transplant outcomes.[119,120] It is unclear whether they are also at greater risk of relapse; and whether further pharmacological or immunologic therapy indeed prolongs survival and increases cure rates. Thus, for the participants who are enrolled in this protocol who are unable to proceed to post-transplant immunomodulatory protocols, we will gather the MRD information together with hematopoietic chimerism in a descriptive manner to study the relationship between MRD and chimerism in this large cohort of patients. The knowledge gained from this study should allow their future application to guide therapeutic interventions.

2.7 Rationale for the evaluation of immune reconstitution after UCBT

Delayed immune reconstitution is a major complication after HCT and impacts the overall survival in patients. Recent studies demonstrate that the rate and quality of immune recovery after UCBT is an important predictor of overall outcomes. Unfortunately, the rate of immune reconstitution directly correlates with the number of infused HSC and UCB patients have delayed immune reconstitution secondary to the limited numbers of HSC.[71,121-123] Though transplantation with multiple unrelated UCB units has been used as a strategy to increase the total number of cells infused, the effect on immune reconstitution is not clear. In this study, we propose to monitor the rate of immune reconstitution after single and double UCBT. De novo generation of thymic derived T cells is critical in reconstituting a functional immune system, thus newly derived thymic T cell will be monitored by measuring the concentration of TREC DNA by quantitative PCR in the peripheral blood of transplant recipients. Furthermore, the time to recovery (defined as the median time to reach the normal value of age-matched healthy individual) of $CD3^+$, $CD4^+$, $CD8^+$, $CD19^+$ and $CD56^+$ cells in patients undergoing UCBT will be investigated. Various subsets of T cells will be investigated including naïve, memory, central memory, effector memory, regulatory T cells as well as naïve and memory B cells. Functional measures of immune recovery will be examined by T cell proliferative response to nonspecific and specific antigen and NK lytic function. Antigen specific T cell response to various infections will be monitored in patients over time. Using a direct ex vivo, single-cell-based assay for clonotypic analysis of human epitope-specific receptor, we will characterize T cell response to specific infections. Along with T

cell receptor specificity or antigen specific responses, epigenetic regulation of immune recovery will be evaluated.

Studies demonstrate that the rate of neutrophil engraftment of a single unit was significantly enhanced after addition of a second unrelated UCB unit. It is clear that infusion of a second UCB unit facilitates the neutrophil engraftment of the dominant unit, however, the mechanism has yet to be investigated. Preliminary data from the Dallas laboratory suggest that dendritic cells and regulatory T cells play a critical role in facilitating neutrophil engraftment in the BM and thymus.[124,125] Data obtained from this study addresses a key question regarding the immunological mechanism for enhanced neutrophil engraftment by facilitating cells. Furthermore, published studies consistently report that after a double UCBT, cells from only one of the UCB unit engraft. However, recent results from a multi-institutional clinical trial have demonstrated that small number of patients who undergo a double UCBT have evidence of mixed chimerism in the BM from the two UCB units one-year after HCT. Furthermore, studies performed at the Fred Hutchinson Cancer Research Center, demonstrated that single-unit dominance after double-unit UCBT coincides with NK cell dose and a specific $CD8^+$ T-cell response against the non-engrafted unit.[126]

3.0 PROTOCOL ELIGIBILITY CRITERIA

3.1 Inclusion criteria for transplant recipient

3.1.1 Age less than or equal to 21 years old.

3.1.2 Does not have a suitable MSD or volunteer MUD available in the necessary time for stem cell donation.

3.1.3 Has a suitable partially HLA-matched (≥ 3 of 6) family member donor.

3.1.4 Has a partially HLA-matched single UCB unit (≥ 4 of 6) with adequate cell dose. UCB units must fulfill eligibility as outlined in 21 CFR 1271 and agency guidance.

3.1.5 High-risk hematologic malignancy.

3.1.5.1 Vey high risk ALL in CR1.

Examples include, but not limited to hypodiploid, M2 or greater marrow at the end of induction, infants with MLL fusion or t(4;11).

3.1.5.2 ALL in High risk CR2.

Examples include but not limited to BM relapse <36 mo. CR1, T-ALL, very early (< 6mo CR1) isolated CNS relapse.

3.1.5.3 ALL in CR3 or subsequent.

3.1.5.4 AML in high risk CR1.

Examples include but not limited to preceding MDS, 5q-, -5, -7, FAB M6, FAB M7 not t(1;22), MRD ≥ 5% on day 22 (AML08), M3 marrow after induction 1, M2 marrow after two cycles of induction, FLT3-ITD.

3.1.5.5 AML in CR2 or subsequent.

3.1.5.6 Therapy related AML, with prior malignancy in CR > 12mo

3.1.5.7 MDS, primary or secondary

3.1.5.8. NK cell, biphenotypic, or undifferentiated leukemia in CR1 or subsequent.

3.1.5.9 CML in accelerated phase, or in chronic phase with persistent molecular positivity or intolerance to tyrosine kinase inhibitor.

3.1.5.10 Hodgkin lymphoma in CR2 or subsequent after failure of prior autologous HCT, or unable to mobilize stem cells for autologous HCT.

3.1.5.11 Non-Hodgkin lymphoma in CR2 or subsequent.

3.1.5.12 JMML

3.1.5.13 Refractory hematologic malignancies (ALL, AML, CML in blast crisis, Hodgkin or non-Hodgkin lymphoma) due to chemoresistant relapse or primary induction failure.

3.1.5.14 All patients with evidence of CNS leukemia must be treated and be in CNS CR to be eligible for study.

3.1.6 Patient must fulfill pre-transplant evaluation:

3.1.6.1 Cardiac Function: Left ventricular ejection fraction (LVEF) ≥ 40% or shortening fraction (SF) ≥ 25%.

3.1.6.2 Creatinine clearance (CrCL) or glomerular filtration rate (GFR) ≥ 50 ml/min/1.73m^2.

3.1.6.3 Forced vital capacity (FVC) ≥ 50% of predicted value or pulse oximetry (Pox) ≥ 92% on room air.

3.1.6.4 Karnofsky or Lansky performance score ≥ 50 (See APPENDIX A).

3.1.6.5 Bilirubin ≤ 3 times the upper limit of normal for age.

3.1.6.6 Alanine aminotransferase (ALT) ≤ 5x the upper limit of normal for age.

3.1.6.7 Aspartate aminotransferase (AST) ≤ 5x the upper limit of normal for age.

3.2 Exclusion Criteria for Transplant Recipient:

3.2.1 Patient has a suitable MSD, volunteer MURD, or KIR mismatched haploidentical donor available in the necessary time for stem cell donation.

3.2.2 Patient has any other active malignancy other than the one for which HCT is indicated.

3.2.3 Patient is pregnant as confirmed by positive serum or urine pregnancy test within 14 days prior to enrollment.

3.2.6 Patient is breast feeding.

3.2.7 Patient has Down Syndrome.

3.2.8 Patient has a current uncontrolled bacterial, fungal, or viral infection per the judgment of the PI.

3.3 Inclusion criteria for haploidentical donor

3.3.1 At least single haplotype matched (≥ 3 of 6) family member

3.3.2 At least 18 years of age.

3.3.3 HIV negative.

3.3.4 Not pregnant as confirmed by negative serum or urine pregnancy test within 14 days prior to enrollment (if female).

3.3.5 Not breast feeding.

3.3.6 Regarding eligibility, is identified as either:

3.3.6.1 Completed the process of donor eligibility determination as outlined in 21 CFR 1271 and agency guidance; OR

3.3.6.2 Does not meet 21 CFR 1271 eligibility requirements, but has a declaration of urgent medical need completed by the principal investigator or physician sub-investigator per 21 CFR 127

INCLUSION CRITERIA SUMMARY		
Donor	Haploidentical (≥ 3 of 6) and single UCB (≥ 4 of 6) HLA matched donor	
Age	≤ 21 years	
Malignancy	ALL	Very HR ALL CR1 ALL CR2 ALL CR3 or subsequent
	AML	AML CR1 AML CR2 or subsequent 2° AML with prior disease in CR > 12 mo.
	MDS	Primary or Secondary
	NK leukemia	CR1 or subsequent
	Biphenotypic leukemia	CR1 or subsequent
	Undifferentiated leukemia	CR1 or subsequent
	CML	Accelerated Phase Chronic with persistent molecular positivity OR intolerance to tyrosine kinase inhibitor
	Hodgkin's Lymphoma	CR2 or subsequent after failure of auto-HCT OR unable to mobilize HSC for auto-HCT
	Non-Hodgkin Lymphoma	CR2 or subsequent
	JMML	
	Refractory disease	ALL, AML, CML in blast crisis, Hodgkin or non-Hodgkin Lymphoma
Pre-Evaluation	Cardiac Function	LVEF ≥ 40% or SF ≥ 25%
	Renal Function	CrCL or GFR ≥ 50 ml/min/1.73m^2
	Lung Function	FVC ≥ 50% or Pox ≥ 92% on room air
	Performance Score	Karnofsky or Lansky ≥ 50
	Liver Function	Bilirubin ≤ 3x upper limit of normal for age ALT ≤ 5x upper limit of normal for age AST ≤ 5x upper limit of normal for age
EXCLUSION CRITERIA SUMMARY		
Donor	Has a suitable MSD, volunteer MURD, or KIR mismatched haploidentical donor available in the necessary time for HSC donation	
Malignancy	Other active malignancy other than one being treated	
Pre-Evaluation	Pregnant - Serum or Urine pregnancy within 14 days of enrollment	
	Breast feeding	
	Downs Syndrome	
	Uncontrolled infection	

3.4 Enrollment on Study

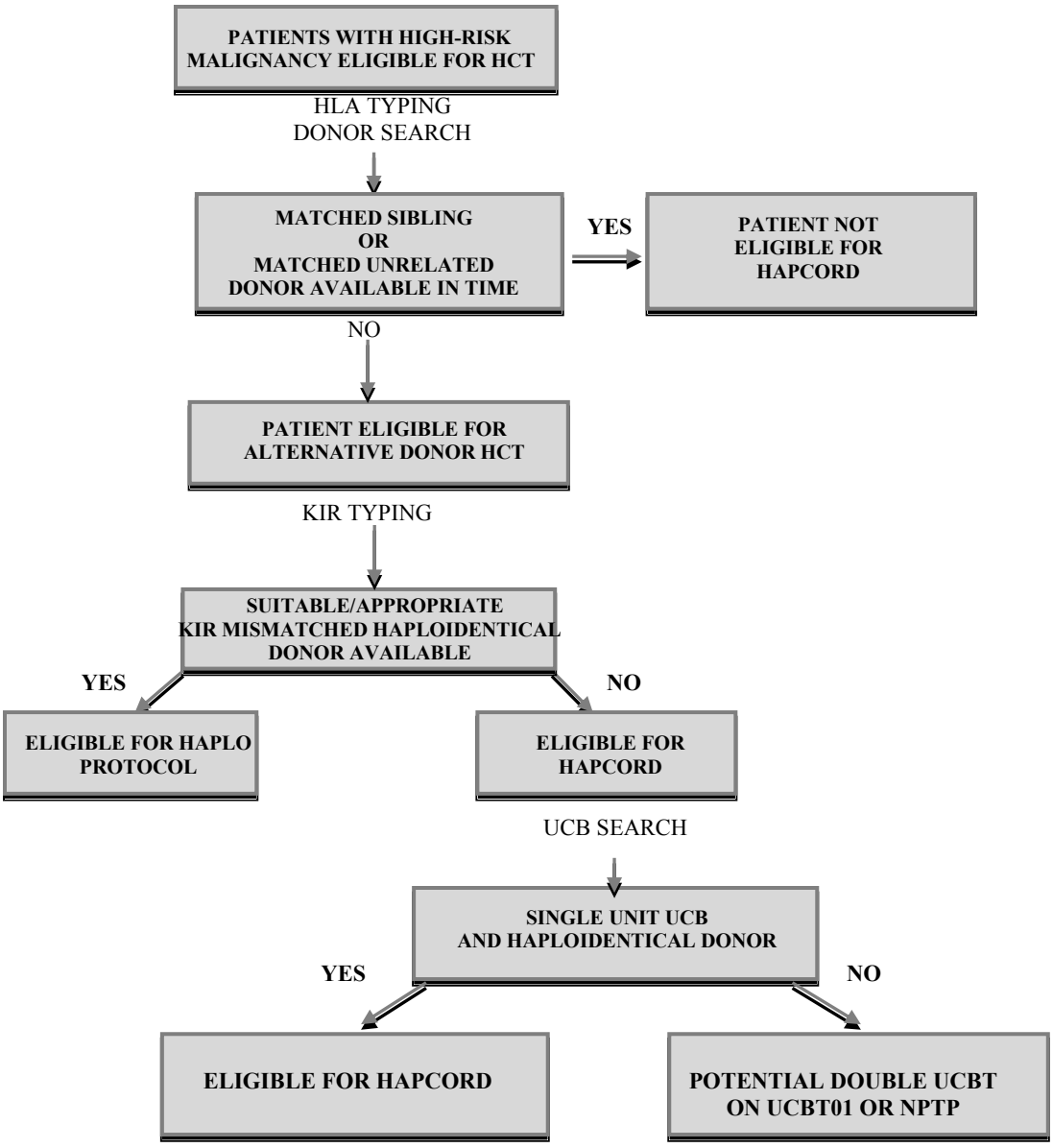
A member of the study team will confirm potential participant eligibility as defined in Section 3.1 – 3.3, complete and sign the 'Participant Eligibility Checklist'. The study team will enter the eligibility checklist information into the Patient Protocol Manager (PPM) system. Eligibility will be reviewed, and a research participant-specific consent form and assent document (where applicable) will be generated. The complete signed consent/assent document form(s) must be faxed or emailed to the CPDMO at [redacted] to complete the enrollment process.

The CPDMO is staffed 7:30 am – 5:00 pm CST, Monday through Friday. A staff member is on call Saturday, Sunday, and holidays from 8:00 am to 5:00 pm. Enrollments may be requested during weekends or holidays by calling the CPDMO "On Call" cell phone [redacted] or referencing the "On Call Schedule" on the intranet.

4.0 TREATMENT PLAN

4.1 Schema for Protocol Prioritization

Participant with high-risk hematologic malignancies undergoing HCT, who do not have a suitable HLA-matched MSD, MURD or KIR ligand mismatched haploidentical donor identified in time, will receive a combined TCD haploidentical PBSC product and an unrelated UCB graft using a TLI-based preparative regimen.



4.2 Reduced Intensity Preparative Regimen

DAY	MEDICATION	DOSE	DOSE #
-9	Total Lymphoid Irradiation (TLI)*	2Gy per fraction x 2 fractions	1-2 of 4
-8	Total Lymphoid Irradiation (TLI)* Fludarabine	2Gy per fraction x 1 fraction 30 mg/m^2 intravenous once daily	3 of 4 1 of 5
-7	Total Lymphoid Irradiation (TLI)* Fludarabine	2Gy per fraction x 1 fraction 30 mg/m^2 intravenous once daily	4 of 4 2 of 5
-6	Cyclophosphamide (with mesna) Fludarabine	60 mg/kg intravenous once daily 30 mg/m^2 intravenous once daily	1 of 1 3 of 5
-5	Fludarabine	30 mg/m^2 intravenous once daily	4 of 5
-4	Fludarabine	30 mg/m^2 intravenous once daily	5 of 5
-3	Thiotepa	5 mg/kg intravenous twice daily	1-2 of 2
-2	Melphalan Tacrolimus	70 mg/m^2 intravenous once daily Maintain until at least 6 months	1 of 2 start
-1	Melphalan	70 mg/m^2 intravenous once daily	2 of 2
0	HPC A Infusion MMF	CD34$^+$ selected Maintain until at least 45 days	HPC A1 start
+1	HPC A Infusion	CD45RA depleted	HPC A2
+2	HPC C Infusion		HPC C
+3	G-CSF	5mcg/kg subcutaneous or intravenous may be started after Day +3	

4.2.1 TLI

A total of 800 cGy of TLI may be administered in one fraction or in divided fractions given at a minimum of 6 hours apart. Administration of 4 fractions at 200 cGy/fraction over three days on day -9 to -7 for a total of 800 cGy is recommended.

*Timing of TLI administration can vary per the Radiation Oncologist. Testicular boosts should be used for all males with ALL with 200 cGy per fraction over two days.

4.2.2 Fludarabine

Fludarabine at 30 mg/m^2/day will be given over 30-60 minutes intravenous infusion on day -8 through day -4 for a total of 5 doses (150 mg/m^2 total). Fludarabine dose may be reduced per PI if concerns of toxicity (i.e. CNS). Infant dosing will apply to fludarabine as follows: patients less than or equal to 10kg will receive 1 mg/kg/day for scheduled doses. Infant dosing will apply to fludarabine as follows: patients less than or equal to 10kg will receive 1 mg/kg/day for scheduled doses.

4.2.3 Cyclophosphamide with Mesna

Cyclophosphamide at 60 mg/kg/day will be administered as a 2 hour intravenous infusion with a high volume fluid flush on day -6 for one dose (60mg/kg total). For

patients weighing more than 125% of their ideal body weight should have cyclophosphamide dose based on the adjusted ideal body weight.

Mesna is administered prior to cyclophosphamide and after the cyclophosphamide infusion per institutional practice. Mesna dose and administration schedule may vary based on physician recommendation.

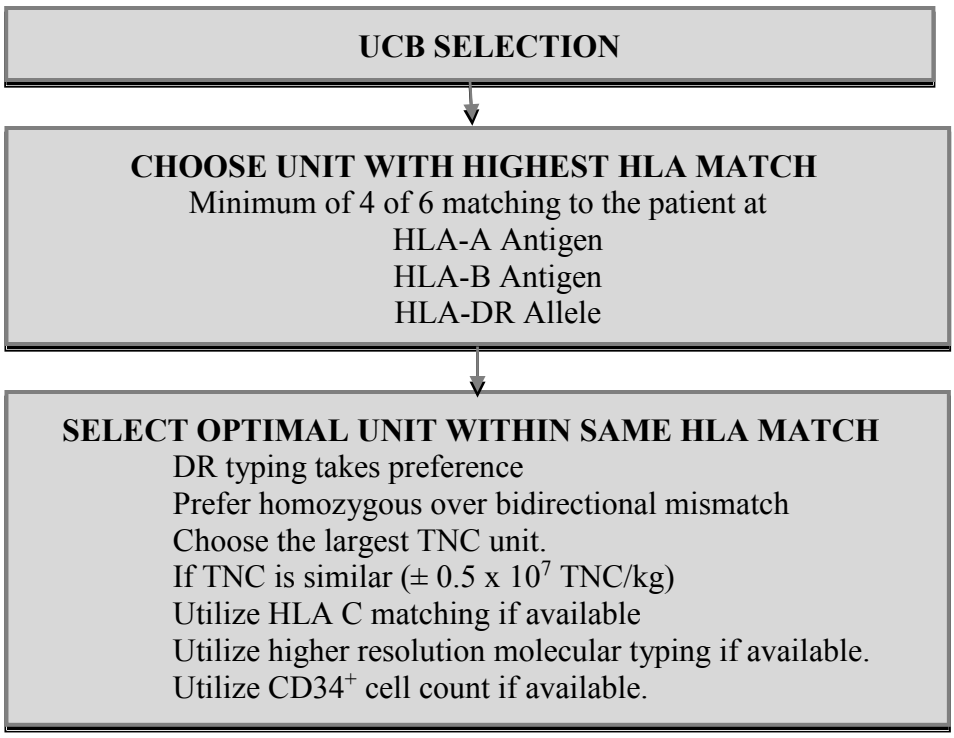
4.2.4 Thiotepa

Thiotepa will be given over 30-60 minutes intravenous infusion at 5 mg/kg twice a day on day -3 for a total of 2 doses (10mg/kg total).

4.2.5 Melphalan

Melphalan will be given over 30-60 minutes intravenous infusion at 70 mg/m^2 once a day on day -2 and -1 for a total of 2 doses (140 mg/m^2 total). Infant dosing of melphalan will apply as follows: patients less than or equal to 10kg will receive 2.3 mg/kg/day for scheduled doses. Infant dosing of melphalan will apply as follows: patients less than or equal to 10kg will receive 2.3 mg/kg/day for scheduled doses.

4.3 UCB Product



4.3.1 UCB Selection:

- Choose the unit with highest HLA match (6/6, 5/6 then 4/6)
  - Matching at HLA typing at HLA-A, B antigen level and DRB1 allele level.
  - The patient and the UCB unit(s) must be matched at least 4 of 6 loci.

- The unit must meet cell dose threshold of 1.0 x 10^7 TNC/kg

- If > one unit is available for a HLA match grade, chose unit based on:
  - *DRB1:* Matching at DR takes preference, followed by
  - *Locus of mismatch*: A unit that is homozygous at the locus of mismatch should be chosen over a unit that is bidirectional at the locus of mismatch, even if the latter unit is larger, followed by
  - *Cell Dose:* Choose the unit containing the greatest TNC.
- If two units are of equivalent HLA match grade, DR□1 matching, locus of mismatch and cell dose (± 0.5 x 10^7 TNC/kg), choose the unit with:
  - HLA match by higher resolution molecular typing, if data available
  - HLA-C antigen/allele level typing, if data available
  - Larger $CD34^+$ cell dose, if data available
  - UCB banks located in the United States are preferred.

4.3.2 UCB Unit Exclusions

4.3.2.1 Unit fails to meet cell dose threshold criteria

Cell dose is < 1.0 x 10^7 TNC per kilogram recipient weight.

4.3.2.2 Fails screening

Any UCB units without full maternal testing and negative results for hepatitis A, B, C, Human Immunodeficiency Virus (HIV), and Human T-lymphotropic virus-1 **(**HTLV-1) viruses. Any additional available virology results on the unit itself will be reviewed but are not mandated, complete or always available. The majority of UCB units will come from banks that operate under the guidelines outlined in 21 CFR 1271 and the *Guidance for Industry: Minimally Manipulated, Unrelated Allogeneic Placental/Umbilical Cord Blood Intended for Hematopoietic Reconstitution for Specified Indications*. All NMDP banks must fulfill this requirement. Foreign banks may have differing screening procedures even if they are American Association of Blood Banks (AABB) or Foundation for the Accreditation of Cellular Therapy (FACT) accredited. It is important to clarify the screening process employed, and additional plasma may be requested if additional testing is needed to fulfill Eligibility Determination requirements. Currently UCB units are available for use under federal IND. The NMDP holds an IND for use, and a few of the older banks hold their own IND. There is no need to adjust for nucleated RBCs when considering TNC. The UCB unit(s) must be present at St. Jude prior to the start of conditioning.

4.3.3 UCB Graft Preparation

Preparation for administration will take place in the Human Applications Lab (HAL) in the Department of Bone Marrow Transplantation and Cellular Therapy (BMTCT) of St. Jude using established Standard Operating Procedure (SOP). UCB units must be prepared for infusion in a manner that assures high cell recovery, maintains cellular viability, and avoids contamination. Infants < 10 kg should not receive reconstitutive thawed product and the UCB unit should be washed. When unit volume and Dimethyl sulfoxide *(*DMSO) concentration are not considered clinically prohibitive, and the wash procedure is deemed by the PI

to have a potential adverse impact on the UCB unit, a reconstitutive thawing procedure may be utilized. In this case, the unit is thawed, and then reconstituted at least 1:1 with an infusion grade solution (Dextran/albumin). In general, a wash is desired, particularly when there are concerns about volume, DMSO dose, red cell replete product, etc. For UCB products to be washed, a modified version of the New York Blood Center's Placental Blood Thaw Procedure will be used. Briefly, the unit will be thawed and diluted; the plasma will then be expressed away after gentle centrifugation. In each case, 1-2 mL of the final product will be removed for testing prior to infusion.

4.4 Haploidentical Donor Graft

4.4.1 Haploidentical Donor Selection

If more than one family member donor is acceptable, then donor selection will be based on the preference of the primary transplant attending. Factors in selection will include donor-recipient matching of CMV serology, donor-recipient red blood cell compatibility, degree of HLA matching, size of the potential donor, previous use as a donor, presence of donor-specific antibody, and overall health of the potential donor.

Donor eligibility for cell collection will be determined through the guidelines outlined in 21 CFR 1271 and the Guidance for Industry: Eligibility Determination for Donors of Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps). Potential donors will undergo an initial screening process that will include at least a complete physical exam, history and testing for relevant communicable diseases. Physical exams to evaluate donor candidacy will be conducted by a non-Department of BMTCT physician (St. Jude or non-St. Jude). For subsequent therapeutic cell collection procedures, if a complete screening procedure has been performed within the previous 6 months, an abbreviated donor screening procedure may be used for these repeat donations. The abbreviated screening procedure must determine and document any changes in the donor's medical history since the previous donation that would make the donor ineligible, including changes in relevant social behavior.

If a donor is determined to be ineligible, the donor is not automatically excluded. Part 21 CFR 1271.65 (b)(1)(i) allows use of ineligible donors who are first or second degree blood relatives. In this situation, the physician will document the necessity of using the ineligible donor by providing a statement of "Urgent Medical Need" as explained in the 21 CFR 1271.3 (u). The cell therapy products will be labeled as required in 21 CFR 1271.65 (b)(2). Recipients or their legal guardians will be informed of the use of an ineligible donor.

Please see Departmental SOP 30.05 "Determination of Eligibility and Suitability for Stem Cell and Therapeutic Cell Allogeneic and Autologous Donors" for additional information.

4.4.2 Haploidentical Donor Mobilization and Donor HSC Graft Collections

A G-CSF mobilized peripheral blood stem cell (PBSC) product (identified as HPC, Apheresis) is the preferred progenitor cell graft source. Our desired target goal will be 3-5 x 10^6 $CD34^+$ cells/kg. This number of cells will be necessary to provide an adequate graft, following the various *ex vivo* manipulations, for prompt reconstitution. Two days of collection are typically needed to achieve this goal. However, on rare occasions, additional days may be necessary. Donors will undergo a standard HSC mobilization regimen consisting of 6 days of G-CSF given subcutaneously at 10 mcg/kg. The graft will be collected by leukapheresis on days -1 and 0. The HPC, Apheresis product will typically be collected and infused fresh, however there may be patients or logistical situations that require the HPC, Apheresis product to be collected early, processed, and stored frozen. The decision to use a fresh versus frozen HPC, Apheresis will be made by the PI and/or primary transplant attending based on patient and donor factors, as well as potential scheduling conflicts.

MOBILIZATION TIME LINE			
DAYS	**MEDICATION**		**APHERESIS**
Day -5	G-CSF 10 mcg/kg/day SC*	1 of 6	
Day -4	G-CSF 10 mcg/kg/day SC*	2 of 6	
Day -3	G-CSF 10 mcg/kg/day SC*	3 of 6	
Day -2	G-CSF 10 mcg/kg/day SC*	4 of 6	
Day -1	G-CSF 10 mcg/kg/day SC*	5 of 6	Apheresis for HSC graft**
Day 0	G-CSF 10 mcg/kg/day SC*	6 of 6	Apheresis for HSC graft**

* G-CSF may may be reduced if the donor's WBC is >75.0 x 10^6/

**Do not start apheresis if platelet count < 50,000/mm^3 or hemoglobin of < 12.5 g/dL pre-phresis

The dose of G-CSF may require modification based on the complete blood counts (CBC). If the donor's white blood count (WBC) is >75.0 x 10^6/ml the dose of cytokine administered will be reduced. The guidelines for dose modification can be found in the St. Jude Children's Research Hospital Department of BMT and CT SOP 30.06.00 "The practice for the evaluation, preparation and care of allogeneic and autologous donors mobilized with growth factor." Ongoing updates of this document can be located at the following St. Jude intranet website: http://home.web.stjude.org/bone_marrow/

The daily leukapheresed volumes for HPC, Apheresis collection is generally 3–4 total blood volumes based on $CD34^+$ cell counts. Two additional days of leukapheresis may be performed at the physician's discretion (no more than 4 total) to reach the cell dose target, however, this is expected to be rare.

Leukapheresis may be terminated early upon request of donor, or when deemed medically necessary per the judgment of the treating sub-investigator physician or PI. All HPC, Apheresis products will be collected as per FACT guidelines. Donors will be monitored during the period of the mobilization and leukapheresis procedure with appropriate laboratory evaluation (Appendix D).

If we are unable to collect the minimum dose of 2 x 10^6 $CD34^+$ cells/kg of recipient weight from the first donor, and the recipient has not yet initiated the preparative regimen, then an alternative family member may be used if he/she fulfills all donor criteria described in section. If the donor is unwilling or unable to complete the mobilization process or leukapheresis procedure, a BM product may be used. The BM product will be processed using the same cell selection methodology on the CliniMACS device.

4.5 Graft Preparation

Graft evaluation and preparation will take place in the Human Applications Laboratory (HAL) in the Department of Bone Marrow Transplantation and Cellular Therapy (BMTCT) using established SOP.

The initial HPC, Apheresis product(s) will be TCD using the investigational CliniMACS device and CD34 Microbead reagent as directed by the manufacturer (Miltenyi Biotech). See section 5.2 for additional CliniMACS device information. Briefly, HPC, Apheresis products from the mobilized donor will be initially assessed in the HAL and stored overnight at 4ºC. The next morning, the product will be washed to remove platelets and adjusted to an appropriate cell concentration for incubation with the CliniMACS CD34 Microbead reagent in the manufacturer provided media. The cells will be washed to remove unbound microbeads. These cells will be applied to the CliniMACS device and the enrichment will be performed using the program "CD34 Enrichment 2.1" as described by the manufacturer.

After enrichment is complete, the cells will be washed and resuspended in an infusion grade solution. The graft product will be enumerated and assessed for viable $CD34^+$ cell and $CD3^+$ T cell content by flow cytometry. The processed HPC, Aphersis CD34 Enriched product will be infused fresh or frozen for future use after completion of release testing and evaluation. Cryopreservation will be performed per SOPs of the HAL. Target cell doses are listed in the following table:

HPC Graft	Target Dose	Minimum Dose	Maximum Dose
$CD34^+$ cells/kg	$\geqslant$ 2 x 10^6	2 x 10^6	50 x 10^6
$CD3^+$ cells/kg	$\leqslant$ 0.5 x 10^5	1 x 10^3	1 x 10^5

Once the target dose is obtained for the $CD34^+$ enriched product, one additional day of apheresis will be performed. This HPC, Apheresis product will be processed for $CD45RA^+$ depletion using the investigational CliniMACS device as directed by the manufacturer (Miltenyi Biotech). See section 5.1 for additional CliniMACS device information. Briefly, HPC, Apheresis products from the mobilized donor will be initially assessed in the HAL and stored overnight at 4ºC. The next morning, the product will be washed to remove platelets and adjusted to an appropriate cell concentration for incubation with the CliniMACS CD45RA Microbead reagent in the manufacturer provided media. The cells will be washed to remove unbound microbeads. These cells will be applied to the CliniMACS device and the depletion will be performed using the "Depletion 3.1" software as described by the manufacturer. When combined with the $CD34^+$ enriched product, the target $CD34^+$ dose will be 3-5 x 10^6/kg. Release criteria of the product will include at least a ≥ 2.00 $\log_{10}$ depletion of $CD45RA^+$ cells and a maximum dose of $CD3^+CD45RA^+$ cells of 0.05x10^6/kg.

HPC Graft	Target Dose	Minimum Dose	Maximum Dose
$CD34^+$ cells/kg	3-5 x 10^6	2 x 10^6	50 x 10^6
$CD3^+CD45RA^+$ cells/kg	$\leq$0.05 x 10^6	N/A	0.05x10^6
$CD45RA^+$	$\geq$ 2.0 $\log_{10}$ depletion ($CD45RA^+$ depleted graft only)		

4.6 Additional Progenitor Cell Graft Administration

Infusion of an additional HSC graft from the original or an alternative haploidentical donor may be performed for participants when clinically indicated for graft failure, poor immune reconstitution, or poor hematopoietic recovery. The use of and content of a conditioning regimen is left to the discretion of the PI and/or primary transplant attending such that the most appropriate therapy is chosen for the clinical situation.

The HSC graft will typically be obtained by apheresis (HPC, Apheresis) and be infused fresh. The target dose for this additional CD 34^+ infusion is $\geq$5 x 10^6 cells/kg. If the participant has quiescent or active BOOP, acute Grade III-IV GVHD, or any other reason that a severely T-cell depleted graft may be indicated, then a graft from the donor processed on the CliniMACS^{TM} device using either $CD34^+$ selection (using established SOPs) or $CD3^+$ depletion methodology may be utilized. The boost target dose for these patients is $\geq$10 x 10^6 $CD34^+$cells/kg with a $CD3^+$ cell/kg dose of $\leq$ 0.5 x 10^5 $CD3^+$ cells/kg.

4.7 Donor Lymphocyte Infusions

Some patients may have neutrophil engraftment derived from the haploidentical donor. Patients who have engrafted with the haploidentical donor may be eligible for donor lymphocyte infusions (DLI) from the haploidentical donor for decreasing donor chimerism, serious viral reactivation or infection, or any evidence of disease (from any level of MRD to frank relapse). The DLI collected may be collected as a whole blood unit donation or by leukapheresis. If the DLI is collected by standard phlebotomy, the volume to be collected would be approximately 300 ml whole blood. If the DLI is collected by leukapheresis, the amount to be processed would be approximately 2 total blood volumes.

Prior to administration of DLI, the immunosuppression should be withdrawn and the recipient should have no active GVHD. GVHD staging must be reviewed by PI prior to DLI administration. The initial dose will typically be 2.5 x 10^4 $CD3^+$/kg. Subsequent doses will be administered at approximately 2 to 4-week intervals with escalating doses of T cells if no moderate or severe GVHD occurs with the prior DLIs. The typical initial dose escalation for patients on this protocol is presented in the following table:

DLI DOSE AND SCHEDULE		
DLI	Dose	Comments
Initial Dose	2.5 x 10^4 $CD3^+$/kg	Approximately 2-4 week interval If no moderate or severe GVHD
Dose #2	5.0 x 10^4 $CD3^+$/kg	
Dose #3	10.0 x 10^4 $CD3^+$/kg	

4.8 Quality Assurance of Cellular Products

Quality assurance for cell products is overseen by independent Quality Assurance personnel who authorizes release of all products. Only trained stem cell processors will process the cell products. A labeling and product tracking system is in place to ensure that the correct cells are infused into the research participant.

Assays of cell numbers and immunophenotyping will be performed both before cell processing and at critical stages of the process. These values will be recorded according to SOP of the HAL. All TCD products will be tested for viability, and sterility (culture and Gram stain. Culture results are not available before infusion of cell products. If the gram stain is positive, the research participant/parent and/or guardian will be informed of this event and of the risks of proceeding prior to infusion. Positive results will be investigated as per the variance procedures of the HAL. The IRB and FDA will be notified, if at any time after infusion, cell product was determined to be contaminated.

4.9 Immunosuppressive Therapies

4.9.1 EBV post-transplant lymphoproliferative disease (PTLPD) prophylaxis

Rituximab at 375 mg/m^2 may be given intravenously as clinically indicated. For all patients, monitoring peripheral blood EBV DNA and prophylaxis and treatment will be according to BMTCT standard guides and/or at the discretion of the treating physician as clinically indicated based on ongoing EBV copy levels and clinical assessment. If a positive selection methodology would be used for a haploidentical HCT infusion, rituximab would not be administered, unless otherwise clinically indicated.

4.9.2 Tacrolimus

Tacrolimus to start on day -2. Preferably, start tacrolimus as a continuous infusion until patient engrafts and able to reliably obtain therapeutic levels. Maintain therapeutic levels according to institutional guidelines and practice. May modify dose for decreased chimerism or positive MRD. May convert to oral dosing when patient is tolerating oral and has a normal gastro-intestinal transit time. If no evidence of GVHD, begin to wean by approximately 10% every week on day 100, and discontinue around day 180 and no sooner than 6 months post transplant.

4.9.3 MMF

MMF to start on day 0 at 15mg/kg intravenously TID or every 8 hours. May convert to oral dosing when patient is tolerating oral and has a normal gastro-intestinal transit time. Adjust dose as clinically indicated. If no evidence of GVHD, MMF can be discontinued on day +45 or on/about 7 days after neutrophil engraftment, whichever is later. May hold for decreased chimerism or positive MRD. Continue MMF if patient has no evidence of donor engraftment on bone marrow evaluation and contact PI of results.

4.9.4 Methylprednisolone*

Methylprednisolone may be used for treatment of acute GVHD*. (2mg/kg divided every 12 hours intravenously is suggested as initial therapy for patient with active acute GVHD requiring systemic therapy) If no response after 7 days, treat with a second line agent according to SOP. Methylprednisolone at 1-5 mg/kg/day can be used for management of pre-engraftment immune reaction as described below in 4.10*

**Use of methylprednisolone and dosing are recommendations and variance in medication, dosing and frequency can occur due to the participant's current clinical condition and will not be noted as protocol deviations.*

4.10 Management of Pre-engraftment Immune Reaction

A well-recognized clinical entity consisting of skin rash, fever, loose stools and respiratory distress has been noted to occur prior to neutrophil engraftment among UCB patients, generally between day 7 and 21.[127-129] This clinical syndrome likely involves cytokine activation, and though clinically similar to acute or hyperacute GVHD, 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, patients may be treated with steroids. Recommendation of methylprednisolone is provided.

The usage of methylprednisolone described here is a recommendation, and variations in medication, dosing and/or frequency can occur due to the participant's current clinical condition and will not be noted as protocol deviations.

4.10.1 For patients not on steroid therapy when the syndrome occurs: methylprednisolone should be given at 1 mg/kg intravenously once a 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. <u>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. Must alert PI if patient is requiring methylprednisolone for > 6 days for engraftment syndrome.</u>

4.10.2 For patients already on steroids for other reasons when the syndrome occurs: methylprednisolone should be given at a dose of 3-5 mg/kg intravenously (max dose 500 mg) every 12 hours for maximum of 48 hours, followed by a rapid taper to 1 mg/kg intravenously every 12 hours. Patients should be weaned after response as tolerated. <u>Please alert PI if patient is requiring methylprednisolone for > 6 days for engraftment syndrome.</u>

Other syndromes of eosinophila and hyperimmune syndrome have not been well described in the UCB field. Thus, patients presenting with the described pre-engraftment syndrome should be diagnosed and treated accordingly.

4.11 Growth Factors

4.11.1 Granulocyte colony-stimulating factor (G-CSF)

G-CSF may start on day +3 at 5 mcg/kg/day intravenously or subcutaneously until absolute neutrophil count (ANC) ≥ 2,000/mm^3 for three consecutive days. G-CSF may be held at the discretion of the attending physician

4.12 Treatment and Conditioning Regimen Related Notes

The HSC infusion may be delayed by approximately 24 hours in order to accommodate HAL as well as the research participant clinical condition.

The term "every" used in tables is an approximate term meaning that these medications noted will be administered approximately "every" 12 hours (or 8 hours as applicable). The drug administration timing in these cases may be modified as clinically indicated such as in the case of surgical procedures or to accommodate other necessary medication, blood product delivery, or procedures. The term "day" does not refer to an absolute calendar day. It refers to a general 24-hour period.

Dosing for all protocol treatment medications may be modified for research recipients based upon actual body weight or adjusted ideal body weight and/or for infants weighing less than 10kg, when clinically indicated. Mesna will be administered for prevention of hemorrhagic cystitis from the medication cyclophosphamide.

Criteria for medication calculations based on body weight/body surface area and other medication related information can be found in the St. Jude Formulary (http://www.crlonline.com/crlsql/servlet/crlonline) or the St. Jude Department of Pharmaceutical Sciences intranet website http://home.web.stjude.org/pharmaceutical_ser/drugInfo.shtml. Medication doses may be rounded to the nearest integer or to the nearest appropriate quantity when clinically or pharmaceutically indicated as per the MD and PharmD.

5.0 MEDICATION INFORMATION

5.1 Cyclophosphamide (Cytoxan)	
Source & Pharmacology	Cyclophosphamide is a nitrogen mustard derivative. It acts as an alkylating agent that causes cross-linking of DNA strands by binding with nucleic acids and other intracellular structures, thus interfering with the normal function of DNA. It is cell cycle, phase non-specific. Cyclophosphamide is well absorbed from the GI tract with a bioavailability of >75%. It is a prodrug that requires activation. It is metabolized by mixed function oxidases in the liver to 4-hydroxycyclo-phosphamide, which is in equilibrium with aldophosfamide. Aldofosfamide spontaneously splits into nitrogen mustard, which is considered to be the major active metabolite, and acrolein. In addition, 4-hydroxycy-clophosphamide may be enzymatically metabolized to 4-ketocyclophosphamide and aldophosfamide may be enzymatically metabolized to carboxyphosphamide that is generally considered inactive. Cyclophosphamide and its metabolites are excreted mainly in the urine. Dose adjustments should be made in patients with a creatinine clearance of <50 ml/min.
Formulation	Cyclophosphamide is available in vials containing 100, 200, 500, 1000 and

and Stability	2000mg of lyophilized drug and 75 mg mannitol per 100 mg of cyclophosphamide. Both forms of the drug can be stored at room temperature. The vials are reconstituted with 5, 10, 25, 50 or 100 ml of sterile water for injection, respectively, to yield a final concentration of 20 mg/ml. Reconstituted solutions may be further diluted in either 5% dextrose or 0.9% NaCl containing solutions. Diluted solutions are physically stable for 24 hours at room temperature and 6 days if refrigerated, but contain no preservative, so it is recommended that they be used within 24 hours of preparation.
Supplier	Commercially available
Toxicities	Dose limiting toxicities of cyclophosphamide includes BM suppression and cardiac toxicity. Cardiac toxicity is typically manifested as congestive heart failure, cardiac necrosis or hemorrhagic myocarditis and can be fatal. Hemorrhagic cystitis may occur and necessitates withholding therapy. The incidence of hemorrhagic cystitis is related to cyclophosphamide dose and duration of therapy. Forced fluid intake and/or the administration of mesna decreases the incidence and severity of hemorrhagic cystitis. Other toxicities reported commonly include nausea and vomiting (may be mild to severe depending on dosage), diarrhea, anorexia, alopecia, immunosuppression and sterility. Pulmonary fibrosis, SIADH, anaphylaxis and secondary neoplasms have been reported rarely.
Route	Intravenous infusion
5.2. Thiotepa (Thioplex® by Immunex) (TESPA, TSPA)	
Source & Pharmacology	Thiotepa is a cell-cycle nonspecific polyfunctional alkylating agent. It reacts with DNA phosphate groups to produce cross-linking of DNA strands leading to inhibition of DNA, RNA and protein synthesis. Thiotepa is extensively metabolized in the liver to metabolites that retain activity, primarily triethylene-phosphoramide (TEPA). The main route of elimination is via the urine, mainly as metabolites; the elimination half-life of the thiotepa is 2.5 hours, and that of TEPA is 17.6 hours.
Formulation and Stability	Thiotepa is supplied in single-use vials containing 15 mg of lyophilized thiotepa, 80 mg NaCl and 50 mg NaHCO3. The intact vials should be stored under refrigeration and protected from light. Each vial should be reconstituted with 1.5 ml of sterile water for injection to yield a concentration of 10 mg/ml. Further dilution with sterile water for injection to a concentration of 1 mg/ml yields an isotonic solution; if larger volumes are desired for intracavitary, intravenous infusion, or perfusion therapy, this solution may then be diluted with 5% dextrose or 0.9% NaCl containing solutions. The 10 mg/ml reconstituted solution is chemically stable when stored in the refrigerator for up to 5 days, however, it is recommended that solutions be prepared just prior to administration since they do not contain a preservative. Reconstituted solutions should be clear to slightly opaque: the solutions may be filtered through a 0.22 micron filter to eliminate haze.
Supplier	Commercially available; manufactured by Immunex.

Toxicities	Dose limiting toxicity is myelosuppression. The leukocyte nadir may occur at any time from 10 to >30 days. Other toxicities include pain at the injection site, nausea and vomiting, anorexia, mucositis, dizziness, headache, amenorrhea, interference with spermatogenesis, and depigmentation with topical use. Allergic reactions, including skin rash and hives, have been reported rarely. Rare cases of apnea, hemorrhagic cystitis, and renal failure have occurred. Thiotepa is mutagenic, carcinogenic, and teratogenic in animals. Pregnancy category D.
Route	Intravenous
5.3 Fludarabine (Fludara)	
Source & Pharmacology	Fludarabine phosphate is a synthetic purine nucleoside analog and acts by inhibiting DNA polymerase, ribonucleotide reductase and DNA primase by competing with the physiologic substrate, deoxyadenosine triphosphate, resulting in inhibition of DNA synthesis. It can also be incorporated into growing DNA chains as a false base and interfere with chain elongation and halt DNA synthesis. Fludarabine is rapidly dephosphorylated in the blood and transported intracellularly by a carrier-mediated process. It is then phosphorylated intracellularly by deoxycytidine kinase to the active triphosphate form. ~23% of the dose is excreted as the active metabolite in the urine (with dosages of 18-25 mg/m^2/day for 5 days). Renal clearance appears to become more important at higher doses, with approximately 41-60% of the dose being excreted as the active metabolite in the urine with dosages of 80-260 mg/m^2.
Formulation and Stability	Fludarabine is supplied in single-dose vials containing 50 mg fludarabine as a white lyophilized powder and 50 mg of mannitol. The intact vials should be stored under refrigeration. Each vial can be reconstituted by adding 2 ml of sterile water for injection resulting in a final concentration of 25 mg/ml. Because the reconstituted solution contains no antimicrobial preservative, the manufacturer recommends that it should be used within 8 hours of preparation. The solution should be further diluted in 5% dextrose or 0.9% NaCl prior to administration.
Supplier	Commercially available.
Toxicities	The major dose-limiting toxicity of fludarabine is myelosuppression. Nausea and vomiting are usually mild. Side effects reported commonly include anorexia, fever and chills, alopecia and rash. Neurotoxicity can be manifested by somnolence, fatigue, peripheral neuropathy, mental status changes, cortical blindness and coma and is more common at high doses. Neurotoxicity is usually delayed, occurring 21-60 days after the completion of a course of therapy and may be irreversible. Side effects reported less commonly include diarrhea, stomatitis, increased liver function tests, liver failure, chest pain, arrhythmias and seizures. Pulmonary toxicity includes allergic pneumonitis characterized by cough, dyspnea, hypoxia and pulmonary infiltrates. Drug induced pneumonitis is a delayed effect, occurring 3-28 days after the administration of the third or later course of therapy. Administration of corticosteroids usually results in resolution of these symptoms.

Route	Intravenous
5.4 Melphalan (L-phenylalanine mustard, phenylalanine mustard, L-PAM, L-sarcolysin, Alkeranâ)	
Source & Pharmacology	Melphalan, a derivative of nitrogen mustard, is a bifunctional alkylating agent. Its chemical name is 4-[bis(2-chloroethyl)amino]-L-phenylalanine, and it has a molecular weight of 305.20. Melphalan is active against tumor cells that are actively dividing or at rest. Its cytotoxicity is thought to be due to inter-strand cross-linking with DNA, probably by binding at the N7 position of guanine. Melphalan is highly protein bound and does not penetrate well into the cerebral spinal fluid. Elimination half-life after intravenous administration in adults is approximately 75 minutes. Elimination appears to be primarily by chemical hydrolysis, but caution should be used in patients with renal impairment. Plasma concentrations of melphalan after oral administration are highly variable, possibly due to incomplete absorption, variable "first pass" hepatic metabolism or rapid hydrolysis. Area under the plasma concentration-time curves for orally administered melphalan is approximately 60% of intravenously administered melphalan in adult studies.
Formulation and Stability	Available as 2 mg tablets for oral administration. This medication is stable at room temperature until expiration date on the packaging. Intravenous formulation is supplied as 50 mg freeze dried glass vial. Each 50 mg vial is supplied in a carton containing a 10 ml vial of sterile diluent. Lyophilized melphalan should be stored at controlled room temperature and protected from light. Each vial is marked with its expiration date. The melphalan for injection must be reconstituted immediately prior to infusion by rapidly adding the contents of the diluent vial (10 ml) to the freeze dried powder with a 20 gauge or larger sterile needle and immediately shaking vigorously until a clear solution is obtained. This results in a 5 mg/ml solution. The dose should then be diluted in 0.9% sodium chloride for injection to a final concentration of not greater than 0.45 mg/ml. The resulting admixture should be infused over a minimum of 15 minutes. The infusion should be completed within 60 minutes of reconstitution. Do Not Refrigerate the Reconstituted Melphalan.
Supplier	Commercially available
Toxicities	Melphalan is cytotoxic and caution should be used in handling and preparing the solution or administering the tablets. Use of gloves is recommended, and if contact with skin or mucosa occurs, immediately wash thoroughly. Second cancers such as acute non-lymphocytic leukemia, myeloproliferative syndrome, and carcinoma have been reported in patients taking melphalan alone or in combination with other chemotherapy or radiation. Melphalan causes suppression of ovarian function in premenopausal women, with a significant number of patients having amenorrhea. Testicular suppression (reversible and irreversible) has been reported. The most common adverse reaction is myelosuppression. Irreversible bone marrow failure has been reported. Gastrointestinal side effects reported include nausea/vomiting,

	diarrhea and oral mucosa ulceration. Hepatic toxicity has occurred, including veno-occlusive disease. Acute hypersensitivity reactions occur in about 2.4% of patients, and can include anaphylaxis. Hypersensitivity reactions were characterized by urticaria, pruritus, and edema. Some patients exhibited tachycardia, bronchospasm, dyspnea and hypotension that responded to antihistamines and corticosteroids. Other side effects that have been reported include skin ulceration or necrosis at injection site, vasculitis, alopecia, hemolytic anemia, pulmonary fibrosis, and interstitial pneumonitis.
Route	Intravenous infusion
5.5 Mesna (Mesnex)	
Source & Pharmacology	Mesna is a synthetic sulfhydryl (thiol) compound. Mesna contains free sulfhydryl groups that interact chemically with urotoxic metabolites of oxaza-phosphorine derivatives such as cyclophosphamide and ifosfamide. Oral bioavailability is 50%. Upon injection into the blood, mesna is oxidized to mesna disulfide, a totally inert compound. Following glomerular filtration, mesna disulfide is rapidly reduced in the renal tubules back to mesna, the active form of the drug. Mesna and mesna disulfide are excreted primarily via the urine.
Formulation and Stability	Mesna is available in 2 ml, 4 ml and 100 ml amps containing 100 mg/ml of mesna solution. The intact vials can be stored at room temperature. Mesna may be further diluted in 5% dextrose or 0.9% NaCl containing solutions. Diluted solutions are physically and chemically stable for at least 24 hours under refrigeration.
Supplier	Commercially available
Toxicities	Mesna is generally well tolerated. Nausea and vomiting, headache, diarrhea, rash, transient hypotension and allergic reactions have been reported. Patients may complain of a bitter taste in their mouth during administration. Mesna may cause false positive urine dipstick readings for ketones.
Dosage and Administration	Mesna is generally dosed at approximately 25% of the cyclophosphamide dose. It is generally given intravenously prior to and again at 3, 6 and 9 hours following each dose of cyclophosphamide.
Route	Intravenous
5.6 G-CSF (Filgrastim, Neupogen®)	
Source & Pharmacology	G-CSF is a biosynthetic hematopoietic agent that is made using recombinant DNA technology in cultures of *Escherichia coli.* G-CSF stimulates production, maturation and activation of neutrophils. In addition, endogenous G-CSF enhances certain functions of mature neutrophils, including phagocytosis, chemotaxis and antibody--dependent cellular cytotoxicity.
Formulation and Stability	G-CSF is supplied in vials containing 300 and 480 mcg of G-CSF at a concentration of 300mcg/ml. The intact vials should be stored under refrigeration. The vials can be left out of refrigeration for 24 hours, but should be discarded if left at room temperature for longer periods of time. G-CSF can be drawn up into tuberculin syringes for administration and stored under refrigeration for up to 7 days prior to usage. G-CSF can be further

	diluted for intravenous infusion in 5% dextrose. Do not dilute in saline---precipitate may form. If the final concentration of this product is <15 mcg/ml, it is recommended that albumin be added to a final concentration of 2mg/ml (0.2%) to minimize adsorption of the drug to infusion containers and equipment.
Supplier	Commercially available.
Toxicities	G-CSF causes marked leukocytosis. Common adverse reactions include bone pain, thrombocytopenia, diarrhea, nausea, rash, alopecia, fever, anorexia and pain or bruising at the injection site. Allergic reactions, MI, atrial fibrillation, and splenomegaly have been reported rarely. G-CSF is contraindicated in participants with allergy to *E. coli* derived products.
Route	Intravenous or subcutaneous.

5.7 Mycophenolate mofetil (MMF, CellCept®)	
Source & Pharmacology	MMF is hydrolyzed to mycophenolic acid (MPA), an immunosuppressive agent. MPA inhibits B and T-cell proliferation, T-cell synthesis, and antibody secretion by potent, noncompetitive reversible inhibition of inosine monophosphate dehydrogenase (IMPDH) in the purine biosynthesis pathway. Inhibition of IMPDH results in a depletion of guanosine triphosphate and deoxyguanosine triphosphate, important intermediates in the synthesis of lymphocyte DNA, RNA, proteins and glycoproteins. Oral formulations of MMF are rapidly and extensively absorbed when given on an empty stomach. Aluminum and magnesium-containing antacids and food decrease absorption of MMF. MMF is rapidly hydrolyzed to the active metabolite (MPA) after oral or intravenous administration. Free MPA is conjugated in the liver by glucuronyl transferase to inactive mycophenolic acid glucuronide (MPAG) that is excreted in the urine and feces. Time to peak plasma concentration is 0.8–1.3 hours, and the mean elimination half-life is 17.9 hours. Enterohepatic recirculation of MPA contributes to plasma concentrations. Administration of cholestyramine interrupts the enterohepatic recirculation and can decrease bioavailability by as much as 40%. Patients with renal insufficiency have increased plasma concentrations of MPA and MPAG. Acyclovir and ganciclovir may compete with MPAG for renal tubular secretion, resulting in increased plasma concentrations of both drugs.
Formulation and Stability	MMF is commercially available as 250 mg capsules, 500 mg tablets, 200 mg/ml powder for oral suspension, and 500 mg vials of powder for injection.
Supplier	Hoffmann La Roche, Inc.
Toxicities	AE seen in patients taking MMF include hypertension, hypotension, peripheral edema, leukopenia, anemia, thrombocytopenia, hypochromic anemia, leukocytosis, headache, insomnia, dizziness, tremor, anxiety, paresthesia, hyperglycemia, hypercholesterolemia, hypokalemia, diarrhea, hyperkalemia, hypophosphatemia, constipation, nausea, vomiting, anorexia, abdominal pain, dyspepsia, urinary burning or frequency, renal tubular necrosis, hematuria, increase serum creatinine and BUN, a variety of infections due to immunosuppression, rash, acne, ocular changes (cataracts,

	blepharitis, keratitis, glaucoma, and macular abnormalities) occasional leg cramps or pain, bone pain, myalgias, and hand cramps. Intravenous infusions have been reported to cause thrombosis and phlebitis. There have been occasional reports of gastrointestinal hemorrhage. High dose therapy with mycophenolate in adults with psoriasis has been associated with the following neoplasms: adenocarcinoma of the breast and colon, basal cell carcinoma, carcinoma of the gallbladder, histiocytic lymphoma, glioblastoma multiforme, and squamous cell carcinoma of the epiglottis.
Route	Oral or intravenous
5.8 Tacrolimus (FK506, Prograf®, Protopic®)	
Source & Pharmacology	Tacrolimus is a macrolide immunosuppressant produced by *Streptomyce tsukubaensis*. It inhibits T-lymphocyte activation, although the exact mechanism of action is not known. Tacrolimus activity is primarily due to the parent drug. The plasma protein binding of tacrolimus is approximately 99% and is independent of concentration over a range of 5-50 ng/mL. The t½ in adult patients ranges from 11-19 hours. Whole blood trough concentrations from 31 patients less than 12 years old showed that pediatric patients needed higher doses than adults to achieve similar tacrolimus trough concentrations. It is extensively metabolized by the mixed-function oxidase system, primarily the cytochrome P-450 system (CYP3A) in the liver and to a lesser extent in the intestinal mucosa. The main route of elimination is via the biliary tract and excretion in feces. A retrospective comparison of Black and Caucasian kidney transplant patients indicated that Black patients required higher tacrolimus doses to attain similar trough concentrations; there were no gender-based differences. The absorption of tacrolimus from the gastrointestinal tract is incomplete and variable exhibiting large intra- and inter-patient variability. Administration with food significantly decreases the rate and extent of absorption. Drugs that stimulate or inhibit hepatic p-450 enzymes will alter clearance of tacrolimus and close attention to potential drug interactions is crucial.
Formulation and Stability	Intravenous formulation**:** Tacrolimus is available as a sterile solution (tacrolimus injection) containing the equivalent of 5 mg anhydrous tacrolimus in 1 mL Each mL contains polyoxyl 60 hydrogenated castor oil (HCO-60), 200 mg, and dehydrated alcohol, USP, 80% v/v. Store between 5°C and 25°C (41°F and 77°F). Oral formulations: It is available for oral administration as capsules containing the equivalent of 0.5 mg, 1 mg or 5 mg of anhydrous tacrolimus. Inactive ingredients include lactose, hydroxypropyl methylcellulose, croscarmellose sodium, and magnesium stearate. The 0.5 mg capsule shell contains gelatin, titanium dioxide and ferric oxide, the 1 mg capsule shell contains gelatin and titanium dioxide, and the 5 mg capsule shell contains gelatin, titanium dioxide and ferric oxide. Store at 25°C (77°F); excursions permitted to 15°-30°C (59°-86°F).
Supplier	Commercially available
Toxicities	Immunosuppression results in increased susceptibility to infection and possible development of lymphoma and other malignancies, particularly of

	the skin. After intravenous administration, monitor closely for an acute allergic reaction for the first 30 minutes and at frequent intervals thereafter. Fetal toxicity has been noted in animals. Common adverse effects are headache, hypertension, GI toxicities, fever, immunosuppression, tremor, renal dysfunction, hematological abnormalities, CNS abnormalities, electrolyte abnormalities, clotting abnormalities, alopecia. Late effects can include skin disorders, delayed wound healing, and hirsutism. Insulin-dependent post-transplant diabetes mellitus (PTDM) was reported in 11-22% of tacrolimus treated transplant patients without pretransplant history of diabetes mellitus, several human studies. Mild to severe hyperkalemia is reported in 8-45% of transplant patients receiving tacrolimus, so serum potassium levels should be monitored and potassium-sparing diuretics should not be used. To avoid excess nephrotoxicity, tacrolimus should not be used simultaneously with cyclosporine or other drugs that may be associated with renal dysfunction. Tacrolimus or cyclosporine should be discontinued at least 24 hours prior to initiating the other. In the presence of elevated tacrolimus or cyclosporine concentrations, dosing with the other drug usually should be further delayed. It is not recommended that sirolimus and tacrolimus be given concomitantly, as serious increases in wound healing complications, renal function impairment and insulin-dependent post-transplant diabetes mellitus have been observed. Drugs that stimulate or inhibit p-450 enzymes will alter clearance of tacrolimus and close attention to potential drug interactions is crucial.
Route	Oral or intravenous
5.9 Methylprednisolone (Medrol®, Solu-Medrol)	
Source & Pharmacology	Adrenal corticosteroid – anti-inflammatory, immunosuppressant decreases inflammation by suppression of migration of polymorphonuclear leukocytes and reversal of increased capillary permeability.
Formulation and Stability	Tablet (4mg), Sodium Succinate powder for injection 125 mg, 500 mg.
Supplier	Commercially available.
Toxicities:	Toxicities include edema, hypertension, vertigo, seizures, psychoses, headache, pseudotumor cerebri, acne, skin atrophy, impaired wound healing, Cushing's syndrome, pituitary adrenal axis suppression, growth suppression, glucose intolerance, hypokalemia, alkalosis, peptic ulcer, nausea, vomiting, transient leukocytosis, muscle weakness, osteoporosis, fractures, cataracts, glaucoma, and increased risk of infection.
Route	Intravenous or oral.

5.10 CliniMACS^{TM} System

The mechanism of action of the CliniMACS Cell Selection System is based on magnetic-activated cell sorting (MACS). The CliniMACS device is a powerful tool for the isolation of many cell types from heterogeneous cell mixtures, (e.g. apheresis products). These can

then be separated in a magnetic field using an immunomagnetic label specific for the cell type of interest, such as $CD3^+$ human T cells.

The cells to be isolated are specifically labeled with super-paramagnetic particles by an anti-body directed toward a cell surface antigen. After magnetic labeling, the cells are separated using a high-gradient magnetic separation column as described below. The magnetically labeled cells are retained in the magnetized column while the unlabeled cells flow through the column for collection. The retained cells are eluted by removing the magnetic field from the column, washing the cells out and collecting them in a separate container from the unlabeled cells.

The super-paramagnetic particles are small in size (about 50 nm in diameter) and are composed of iron oxide/hydroxide and dextran conjugated to monoclonal antibodies. These magnetic particles form a stable colloidal solution and do not precipitate or aggregate in magnetic fields. The antibody conjugated beads used in this system are highly specific (e.g. CD3+ cells via OKT3 conjugated beads). High-gradient MACS technology has been shown to achieve rapid and highly specific depletion or enrichments of large numbers of target cells from BM, cord blood, and normal peripheral blood mononuclear cells.

The CliniMACS device incorporates a strong permanent magnet and a separation column with a ferromagnetic matrix to separate the cells labeled with the magnetic particles. The high-gradient system allows the application of strong magnetic forces and a rapid de-magnetization. Small ferromagnetic structures, such as the column matrix, placed in a magnetic field concentrate this homogenous field and thereby produce high magnetic gradients. In their immediate proximity, the ferromagnetic structures generate magnetic forces 10,000-fold greater than in the absence of those structures enabling the retention of magnetically labeled cells. After removing the column from the magnet, the rapid de-magnetization of the column matrix allows the release of retained cells.

The CliniMACS device is comprised of a computer controlled instrument incorporating a strong permanent magnet, a closed-system sterile tubing set containing columns with a coated ferromagnetic matrix and a paramagnetic, cell specific, labeling reagent. The instrument will separate the cells in a fully automated process yielding a cell population highly depleted of $CD3^+$ cells. The CliniMACS device is not licensed by the FDA and therefore is investigational.

The CliniMACS device has separate programs that allow cell selection procedures optimized for either depletion (e.g. $CD3^+$ or $CD45RA^+$) or selection of a target cell population (e.g. $CD34^+$ or $CD56^+$ cells). The basic mechanism is the same for either application; target cells are "tagged" with super-paramagnetic particles and eventually separated from the unlabeled cells using the CliniMACS device as described above. The desired target cells can either be infused or discarded appropriately.

6.0 REQUIRED OBSERVATIONS AND EVALUATIONS

6.1 Standard Pre/Peri/Post-Transplant Evaluations

All pre/peri/posttransplant and long-term follow-up evaluations for these participants will be carried out as guided by the SOPs of the SJCRH, Department of BMTCT, for recipients of allogeneic HCT. Copies of these SOPs and ongoing updates can be found at the following site: http://home.web.stjude.org/bone_marrow/clinicalHome.shtml. A schedule of these for these assessments is outlined in Appendix D.

6.2 Long-term Follow-up Evaluations

In general, recipients of allogeneic HCT at St. Jude are seen at least annually until 10 years post-transplant in the Department of BMTCT outpatient clinic. For the purpose of this study, research participants will be followed to year 1 post-transplantation. At that time, transplant recipients will be eligible for enrollment in the institutional long-term follow-up protocol for children and young adults who have received stem cell transplantation at St. Jude Children's Research Hospital (BMTFU protocol).

6.3 Research Testing.

Timing for the required research tests are summarized in Appendix D. Furthermore, to accommodate the studies, flexibility in the date is allowed without a deviation from protocol. The degree of flexibility in the timing is also provided in Appendix D.

6.3.1 Immune Reconstitution

Standard measures of post-HCT immune reconstitution will be performed as delineated in Appendix D. Research testing of Immune Reconstitution will include:

6.3.1.1 VBETA/TREC Research: Thymic output and T cell repertoire.

6.3.1.2 Lymphocyte Phenotypes Research: T cell and NK cell number and function.

6.3.1.3 IR-PHENOTYPE

Donor: Donor UCB cells necessary for research studies will be harvested from the discarded bag post infusion, ensuring that UCB cells will not be diverted from patient care. Immunophenotypic evaluation of the UCB unit prior to infusion will be performed to enumerate the content of naïve, memory T cells, regulatory T cells, NK cells, and naïve B cells and memory B cells. Samples from haploidentical donors will be obtained if they choose to participate in the optional research testing (described below).

Host: Immunophenotypic evaluation of immune reconstitution in the patient will be performed for the enumeration of naïve, memory T cells, regulatory T cells and naïve B cells and memory B cells.

6.3.1.4 T-FUNCTION

Immune function studies include antigen-specific T-lymphocyte response to viral infections, such as herpes viruses (CMV, HSV and VZV), intracellular cytokine and cytokine secretion assays. Studies may include an optional skin punch biopsy, at the discretion of the PI, to be performed either at the time of the pre-transplant BM biopsy, during sedation for radiation treatment or at the time of the day 28 BM biopsy. In order to follow viral infections,

and/or reactivations detected by DNA regardless of copy number, additional samples will be collected weekly for the first 100 days post-transplant when possible. If infection(s) are detected in non-blood samples (nasal wash, BAL etc), leftover samples may be used for research purposes. Studies to be performed in the laboratory of Dr. Paul Thomas in collaboration with Dr. Dallas.

6.3.1.5 HOST-DONOR INTERACTION

Donor: Donor Haploidentical samples are optional and UCB cells necessary for research studies will be minimized. UCB cells will also be harvested from the discarded bag post infusion. . Epstein-Barr virus transformed lymphoblastoid cell lines (EBV-LCL) will be generated from the donor cells obtained from the discarded infusion bag. Donor KIR typing and HLA-typing will be performed for high resolution HLA-A, HLA-B, HLA-DR, HLA-C and HLA-DQ if not available.

Host: Host and donor immunologic interaction studies may also include an optional skin punch biopsy to be performed with the pre-transplant BM biopsy to generate fibroblasts. Prior to the start of conditioning, peripheral blood samples will be collected to generate EBV-LCL from the patient for research studies evaluating donor/host immunologic reactions.

6.4 Research Testing on Haploidentical Donor (optional)

Donors will be offered the option for participation in research studies of immune reconstitution of T cells, B cells, and NK cells. These tests will be obtained after consent and preferably prior to growth factor administration. Lymphocyte subset analysis of the donor appears to allow for the prediction of the reconstitution of the lymphocyte subsets in the research participant after transplantation. Data in larger donor/research participant pairs will help to verify these observations. A list of these optional research studies are noted in Appendix D and detailed below:

6.4.1 Lymphocyte Subset Study: Flow cytometry enumeration

6.4.2 VBETA/TREC Research: Thymic output and T cell repertoire

6.4.3 Lymphocyte Phenotypes Research: T cell and NK cell number and function

7.0 EVALUATION CRITERIA

7.1 Adverse event (AE) monitoring for on-study research participants will be assessed using the NCI Common Toxicity Criteria Version 3.0. The specific criteria for adverse event monitoring are noted in APPENDIX C.

7.2 GVHD scoring (acute and chronic) will be evaluated and graded using the criteria found in APPENDIX B and Cof this protocol. The COG stem cell committee consensus guidelines for establishing organ stage and overall grade of acute GVHD has been adopted as the standard GVHD diagnostic guidelines for the Department of BMTCT, and will be applied to patients on this protocol (see Appendix B). In addition, acute GVHD will be assessed at least once a week for the first 100 days per BMTCT SOP 20.01.

Appendix C contains a summary of the NIH consensus development project on criteria for clinical trials in chronic GVHD. This table will be used for staging/grading of chronic GVHD.

7.3 Performance status will be assessed by Karnofsky/Lansky Performance Scores (age-dependent) (see APPENDIX A).

7.4 Hematologic recovery post-transplant will be determined using the engraftment criteria as follows:

(1) Neutrophil engraftment is defined as absolute neutrophil count (ANC) recovery of ≥ 0.5 x 10^9/L (500/mm^3) for three consecutive laboratory values obtained on different days (derived from either donor). Date of engraftment is the date of the first of the three consecutive laboratory values.

(2) Platelet engraftment will be defined as platelet count ≥ 20,000/mm^3 for three consecutive laboratory values obtained on different days with no platelet transfusions in the preceding 7 days. Date of platelet engraftment is the date of the first of the three consecutive laboratory values.

7.5 Primary graft failure will be defined as donor -derived ANC (either donors) never meeting or exceeding 500/mm^3 for 3 consecutive measurements. Neutrophil engraftment occurring after day +42 post-transplant is defined as delayed engraftment.

7.6 Secondary graft failure or graft rejection will be defined as no evidence of donor chimerism by UCB and/or haploidentical donor (<10%), or too few cells to perform adequate chimerism analysis, in research participants with prior neutrophil engraftment.

7.7 Mixed hematopoietic chimerism will be defined as between 10% and 95% donor chimerism in the absence of immunosuppressive therapy.

7.8 Engraftment syndrome, characterized by fever, rash, pulmonary edema, weight gain, liver and renal dysfunction, and/or encephalopathy is an early complication of hematopoietic stem cell transplantation that occurs around neutrophil engraftment time and is attributed to the sudden cytokine discharge associated with robust engraftment of transplanted cells. When diagnosed, the following grading will apply:

- Grade I (mild): transient (<48 hours) low grade fever with or without limited rash
- Grade II (moderate): sustained fever above 39.0˚C (> 48 hours), rash >25% body surface area and/or evidence of pulmonary injury (infiltrate or hypoxia), requiring corticosteroids and/or intermittent oxygen and responding to interventions
- Grade III (severe): not rapidly responding to interventions, evidence of multiple organ dysfunction/failure (e.g. hypoxia requiring continuous oxygen, renal impairment requiring HD or CVVH, evidence of encephalopathy)
- Grade 4 (life threatening): pressor or ventilator support indicated

8.0 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF-STUDY CRITERIA

8.1 Recipient Off-therapy Criteria – Recipient participants may remain on-study, but considered off therapy, for monitoring if one of the following occurs (off therapy participants monitored for disease status and survival only):

8.1.1 Experiences graft failure/rejection.

8.1.2 Noncompliance with the protocol.

8.1.3 Positive pregnancy test after the HSC infusions.

8.1.4 Recipient requires an additional HSC infusion, and unable to receive these cells due to donor issues.

8.1.5 Physician decides that it is in the best medical interest of the participant.

8.2 Recipient Off-study Criteria: Recipient participants will remain on study until one of the following occurs:

8.2.1 Withdrawal of consent.

8.2.2 Death.

8.2.3 Lost to follow-up or inadequate follow-up per discretion of PI

8.2.4 Requires an additional transplant procedure using a different allogeneic donor.

8.2.5 Requires enrollment on another therapeutic study or non-protocol therapy for disease.

8.2.6 Study evaluations are complete (i.e. has completed the month 12 post-HSC infusion evaluations, and first annual post-transplant evaluation).

8.2.7 Development of a significant change in health status at any point of therapy, which would render receipt of the transplantation procedure or continuation in the study no longer in the participant's best interest.

8.2.8 Unable to undergo the primary HCT procedure due to donor and/or donor center inability to provide the HSC product.

8.3 Donor Off-study Criteria: Donor participants will remain on-study until one of the following occurs:

8.3.1 Withdrawal of consent.

8.3.2 Death.

8.3.3 Development of a significant change in health status at any point of therapy, which would render the donor medically ineligible to serve (or continue to serve) as donor; or renders the donor's continuation in the study no longer in his/her best interest.

8.3.4 Date of corresponding recipient's off-study date.

9.0 SAFETY AND ADVERSE EVENT REPORTING REQUIREMENTS

9.1 Reporting adverse experiences and deaths

The following definitions apply:

Serious event – any event, in which the outcome is fatal or life-threatening, results in permanent disability, causes inpatient hospitalization or prolongs existing hospitalization, or is a congenital anomaly, cancer, or overdose.

Unanticipated adverse event – any event, not identified in their nature, severity, or frequency in the current risk documents (e.g., investigator's brochure), or consistent with the investigational plan.

9.2 Reporting to St. Jude Institutional Review Board (IRB)

Principal investigators are responsible for promptly reporting to the IRB any adverse events that are unanticipated, serious, and that may represent potential harm or increased risk to research participants. When an unanticipated death occurs, the PI should report it to the Director of Human Subject's Protection immediately, by phone: [redacted], cell: [redacted], fax: [redacted], or e-mail: [redacted]. A reportable event entry into TRACKS should follow within 48 hours of notification of the event.

Serious, unanticipated, and related or possibly related events must be reported within 10 working days of notification of the event. At the same time, the investigator will notify the study sponsor and/or the FDA, as appropriate. All other SAEs and captured AEs will be reported to the IRB in the continuing review, with the following exceptions:

- Any grade III-IV infusion reactions will be reported as soon as possible but every effort should be made to assure reporting is no more than within 7 business days of the event.
- Any episodes of overall grade III or IV acute GVHD in participants will be reported to the IRB as soon as possible but no more than within approximately 10 days of the PI's confirmation of the diagnosis/grade of the event.
- Clinical diagnosis of PTLPD will be reported to the IRB as soon as possible but no more than within 10 days of the PI's determination of the disorder.

The principal investigator is responsible for reviewing the aggregate toxicity reports and reporting to the IRB if the frequency or severity of serious toxicities exceed those expected as defined in the protocol or based on clinical experience or the published literature. Any proposed changes in the consent form or research procedures resulting from the report are to be prepared by the study team and submitted with the report to the IRB for approval.

Recipient participants will be followed for NCI Grade III-V, and clinically significant I-II, adverse events from the start of conditioning and throughout the first year post HCT, regardless of their relationship to the treatment given. However, all GVHD events will be captured on an ongoing basis regardless of stage or grade.

Haploidentical donor participants will be followed for any serious AE (SAE) and any clinically significant AEs (per judgment of PI) that are deemed related to the mobilization and/or apheresis procedure from the time of initiation of mobilization growth factors to 7-days post last day of the apheresis procedure. If the transplant recipient requires a second HSC infusion, meaning that the donor is required to undergo the mobilization and apheresis procedure again, collection of this donor safety data will restart upon the initiation of the subsequent mobilization procedure and continue until 7-days post the last

day of this apheresis procedure. Timelines for reporting of these donor events to the institutional and federal governing agencies will be according to the same timelines noted in the following sections. A listing of the captured donor safety data will be provided in a separate table from the transplant recipients within each respective continuing review report. Continuing review reports to all regulatory authorities will be structured in a manner so that any infusion toxicities or stem cell product related variances will be reported in separate listings from all other required elements.

9.3 Reporting to St. Jude Institutional Biosafety Committee (IBC)

Continuing review reports will be sent to the IBC on at least an annual basis using the most current version of the continuing review form found on the IBC website. The safety reports, sent to the IRB for both the donors and stem cell recipients, will be simultaneously forwarded to the IBC. Therefore, reporting for safety events to this committee will be according to the same timelines as reporting to the IRB. This includes notification of achievement of MTD (if/when applicable). As per the direction of the IBC, only those protocol revisions and amendments directly related to the CliniMACS processing and related reagent(s) will require review and consideration by the IBC. Other revisions/amendments will be noted in the IBC continuing review report.

9.4 Reporting to FDA

The FDA will be notified in writing (IDE safety report) of any serious and unexpected AE associated with an investigational treatment or device; or any results from laboratory animal tests that suggest a significant risk for human subjects including reports of mutagenicity, teratogenicity, or carcinogenicity.

Each notification to the FDA should be made as soon as possible and no later than 15 calendar days after the sponsor's initial receipt of the information. The FDA may require additional data to be submitted. In each written IND safety report, the sponsor shall identify all safety reports previously filed with the IND concerning a similar adverse experience, and shall analyze the significance of the adverse experience in light of the previous, similar reports where applicable.

The sponsor shall also notify the FDA by telephone or by facsimile transmission of any unexpected fatal or life-threatening experience associated with the use of the investigational medication as soon as possible but no later than 7 calendar days after the sponsor's initial receipt of the information. Any grade III-IV infusion reactions will be reported as soon as possible but every effort should be made to assure reporting is no more than within 7 business days of the event. Follow-up information to a safety report must be submitted as soon as the relevant information is available.

If the results of further investigation show an AE that was not initially determined to be reportable should later be deemed reportable, the sponsor shall inform the FDA of the event in a written safety report as soon as possible, but no later than 15 calendar days after the determination is made. Results of the investigation of other safety information shall be submitted, as appropriate, in an information amendment or annual report. Continuing review reports, which will include the up-to-date clinical and safety data, will be submitted to the FDA at least annually.

9.5 Reporting to St. Jude Office of Regulatory Affairs

Copies of all correspondence to the St. Jude IRB, including SAE reports are provided to the St. Jude Regulatory Affairs Office. All FDA related correspondence and reporting will be conducted through the Regulatory Affairs Office. Adverse event reporting and annual reporting will be in accord with the FDA Title 21 CFR312.32 and Title 21 CFR312.33, respectively. The Regulatory Affairs Office can be reached at [redacted] (secondary contact: St. Jude Vice President of Clinical Trials Administration [redacted]).

9.6 Continuing review reports

Continuing review reports of protocol progress and summaries of adverse events will be filed with the St. Jude IRB, and IBC at least annually.

9.7 Reporting to the St. Jude Data Safety Monitoring Board

This study has been referred to the St. Jude Data and Safety Monitoring Board (DSMB) for regular monitoring. The DSMB is charged with advising the Director and other senior leaders of St. Jude on the safety of clinical protocols being conducted by St. Jude investigators and on their continuing scientific validity. DSMB monitoring and review for this study will be conducted in accordance with the NCI guidance for DSMBs on an approximate semiannual basis. The St. Jude DSMB is responsible for ongoing review of the protocol and related information such as enrollment, issues related to participant safety (specifically toxicities and the risk:benefit ratio of the trial), interim analyses, and the overall study conduct necessary to accomplish the primary protocol objectives. This includes evaluation of the accrual rate, adherence to the study design, outcome measures, and review of protocol related primary outcome data. The PI will meet with DSMB during the semiannual visits to review the information submitted and discuss the status of the protocol. The DSMB may recommend that the trial be modified, suspended to accrual, and/or stopped based on their review.

9.8 Data submission to Miltenyi Biotec

Clinical and safety related data will be provided to Miltenyi Biotec, the manufacturer of the CliniMACS system. Data will include but is not limited to the transplant research participant's age and diagnosis, donor product(s) related information including donor type, the stem cell mobilization, selection, and infusion procedure. Outcome data including lymphohematopoietic reconstitution, immunological response, disease response and transplant complications will be shared with Miltenyi Biotec. Representatives from Miltenyi Biotec will be able to review the participant's (donor and transplant research recipient) laboratory and medical record for data verification purposes. Copies of all reports to the institutional and governing regulatory bodies will also be accessible upon request. In the event that the protocol is placed on a clinical hold by the PI or governing regulatory authorities, representatives from Miltenyi Biotec will be notified as soon as possible.

9.9 Reporting to the Center for International Blood and Marrow Transplant Research

The Transplant Program at St. Jude is required by the federal government to report transplant information to the Center for International Blood and Marrow Transplant Research (CIBMTR). The CIBMTR is a research partnership of the International Bone Marrow Transplant Registry, the National Marrow Donor Program (NMDP), and the

Foundation for the Accreditation of Cellular Therapy (FACT). This organization is responsible for the collection and maintenance of a standardized data warehouse registry of autologous and all allogeneic (related and unrelated donor) transplants performed in the United States.

The Office of General Counsel, U.S. Department of Health and Human Services, had deemed the CIBMTR not a covered entity under the Privacy Rule (45 CFR 164.512), 45 CFR Parts 160 and 164, and the Health Insurance Portability and Accountability Act (HIPPA) of 1996. For this reason, the submission and disclosure of certain protected health information (PHI), including that required for CIBMTR, is allowable without the individual's authorization (i.e. consent is waived) when such disclosure is made to public health authorities authorized by law for the purpose of preventing or controlling disease, injury, or disability.

Data resulting from this transplant procedure will be sent for general registry purposes to comply with the federal government requirements. This information for both donor and recipient is submitted using a unique participant identification number. The information submitted for haploidentical recipients is less extensive than recipients of other donor products. For this reason, variables submitted may include but are not limited to the transplant recipient's date of birth, country/state of current residence, diagnosis, basic lympho-hematopoietic reconstitution (e.g. date of ANC and platelet engraftment), post-HCT disease status, and basic AEs (e.g. GVHD- yes or no), survival status, date/cause of death.

10.0 DATA COLLECTION, STUDY MONITORING, AND CONFIDENTIALITY

10.1 Data collection

The St. Jude Cancer Center Clinical Research Associates assigned to the Department of BMTCT will assure protocol compliance, and conduct all clinical and safety data collection. Data will be entered into an institutional database. The PI will be responsible for review of data for accuracy and completeness once entered into the secure departmental database.

10.2 Study monitoring

This protocol will be monitored for safety and data as per the St. Jude Data and Safety Monitoring Plan for Clinical Trials approved by the NCI in 2010, and is considered to be in the High Risk III category. The Central Protocol and Data Monitoring Office (CPDMO) will verify 100% of the informed consent documentation on all participants and verify 100% of St. Jude participants' eligibility status. The study team will meet at appropriate intervals to review case histories and data quality summaries on all participants. The St. Jude Clinical Research Monitor will assess protocol and regulatory compliance as well as the accuracy and completeness of all data points 100% of study enrollees every three months. The protocol will be tracked continuously for the accrual of donors and recipients. All AE and SAE reports will be reviewed by the study Principal Investigator for type, grade, attribution, duration, timeliness and appropriateness on all study participants. All SAE reports will be reviewed by the monitor every 3 months.

Protocol compliance monitoring will include participant status, eligibility, the informed consent process, demographics, staging, study objectives, subgroup assignment, treatments, evaluations, responses, participant protocol status, off-study, and off-therapy

criteria. The Monitor will generate a formal report which is shared with the Principal Investigator (PI), study team and the Internal Monitoring Committee (IMC). Monitoring may be conducted more frequently if deemed necessary by the CPDMO or the IMC. Continuing reviews by the IRB and CT-SRC will occur at least annually. In addition, SAE reports in TRACKS (Total Research and Knowledge System) are reviewed in a timely manner by the IRB. The Regulatory Affairs Office will assist the PI in reporting to the FDA and other external oversight agencies, as necessary.

10.3 Confidentiality

Unique participant numbers will be used in place of an identifier such as a medical record number when reporting any data to outside agencies. No research participant names will be recorded on the data collection forms. The list containing the unique participant numbers and the medical record number will be maintained in a locked file accessible only to the study team.

The medical records of study participants may be reviewed by the St. Jude IRB, FDA, clinical research monitors, etc.

11.0 STATISTICAL CONSIDERATIONS

11.1 Statistical design and analysis for the primary objectives and stopping rules

This study is designed as a phase II study. The primary objective of this study is to evaluate the rate of neutrophil engraftment by day +42. Neutrophil engraftment for the purposes of this objective will be as defined in section 7.

As detailed in section 2, the use of alternative donors such as haploidentical donors and unrelated UCB are frequently needed and largely successful. However, the use of these alternative donors is typically complicated with additional problems, particularly poor hematopoietic recovery and graft failure.[130]

The majority of published experience with alternative (HLA-mismatched) donor transplantation in children is with UCB grafts. The COBALT study included 191 children with hematologic malignancies who received UCB transplantation with TBI based (1350Gy) myeloablative conditioning.[60] The cumulative incidence of neutrophil engraftment by day 42 was 80%. The New York Blood Center published outcomes on 1061 patients (78% pediatric) with hematologic malignancies who received myeloablative UCBT with units from their bank.[63] The cumulative incidence of neutrophil engraftment was 74% by day 77. The CIBMTR published a comparison of UCB transplantation with HLA-matched unrelated donor transplantation, in which 503 children with acute leukemia received UCBT.[17] They confirmed that although the leukemia-free survival was similar, the rate of neutrophil engraftment (and TRM) were significantly worse with HLA-mismatched UCB grafts than with HLA-matched BM grafts. For UCB recipients, the best neutrophil engraftment was obtained in the rare 6/6 matched UCB recipients with 85% of the 35 patients achieving neutrophil recovery at day +42. In addition, recipients who received a single antigen mismatch UCB unit with an appropriately high cell dose (n=154) had a neutrophil engraftment rate of 80% at day +42. Recipients of lower cell dose or two antigen mismatch units fared worse.

Given the data, we consider a rate of successful neutrophil engraftment of less than 80% by day +42 to be unacceptable for alternative donor transplantation. The goal of our study

is to develop a novel preparative regimen to facilitate successful neutrophil engraftment of donor graft(s) at a rate of ≥91%. We do not anticipate censoring during the 42 day time period and we can approximate the rate of neutrophil engraftment by day +42 using a Binomial distribution. In order to keep the validity of Binomial distribution approximation, patients lost for follow up will be counted as a failure to engraft. Therefore, in this study, we propose to test the null hypothesis H_0: P≤0.80 versus H_1: P>0.80, where P is the proportion of research participants who engrafted by day +42 after HCT. With type I error of 10% and type II error of 20%, Simon's two stage optimum design powered at alternative successful neutrophil engraftment rate P_1=0.91 requires 21 evaluable patients at the first stage and 49 evaluable patients in total[76]. The stopping rules are provided in Table 1, with the understanding that stopping the trial early would be suggestive of the proposed transplant strategy not being an effective treatment option for this group of patients. The interpretation is that if we observe 17 or fewer participants engrafted by day +42 in the first 21 participants, then we would stop the trial for lack of efficacy. However, if we observe 18 or more patients engrafted in the first 21 participants by day +42 in stage one, then 28 more patients will be enrolled in stage two. If we observe 43 or more participants engrafted by day +42 upon completion of the trial, then we conclude that the true rate of neutrophil engraftment is at least 80% and our novel regimen will be proposed for further development and phase III clinical trial.

Table 1: Stopping rules for lack of efficacy based on the Simon's 2-stage optimum design (unacceptable low rate of successful neutrophil engraftment by day +42)

Accept H_0 if the number of research participants engrafted					
P_0	P_1	($\leq r_1/n_1$)	($\leq r/n$)	EN(P_0)	PET(P_0)
0.80	0.91	17/21	42/49	31	0.63

Note: r_1 and r denote the number of patients successfully engrafted by day +42; EN(P_0) denotes the expected sample size under P_0; PET(P_0) denotes the probability of early termination at stage I under P_0.

All participants who receive the prescribed transplant will be evaluable for the primary outcome. In addition, any patient who starts the conditioning regimen but stops prior to receiving the graft will count as a failure, if the reason for stopping therapy is toxicity from the conditioning regimen. Patients who enroll but do not initiate treatment due to withdraw of patient, withdraw of donor or health status change to make the treatment not in the patient's best medical interest, etc., will be replaced. Any patient who dies from toxicity after neutrophil engraftment but before day +42 will count as having engrafted. Any patient who dies prior to neutrophil engraftment will count as a graft failure.

After the study is finished, for the first primary objective, the rate of neutrophil engraftment by day +42 and its 95% Blyth-Still-Casella confidence interval will be estimated based on the binomial approximation.

Table 2 shows the number of haploidentical HCT and the estimated number of KIR mismatch cases performed at our institution in last 5 years. With the initiation of UCB protocols at SJCRH, the number of referrals for patients requiring alternative donor HCT has increased. Furthermore, HAPCORD will be a high priority protocol. Based on this table and PI's estimation, it is expected that approximately 10 patients per year will be

enrolled in this study. Therefore, the expected accrual period for this study is maximally 5 years. Adequate enrollment will be monitored every 6 month and if the accrual is less than 50% projected, protocol revision or closure will be considered.

Table 2: The number of haploidentical HCT performed at St. Jude 2007 to 2012

Year	2007	2008	2009	2010	2011	2012
Number haploidentical HCT	20	17	22	19	24	22
Estimated KIR mismatch	14	12	15	13	17	15
Estimated KIR matched	6	5	7	6	7	7
Number of UCBT	3	2	0	0	2	8

Stopping Rules for Toxicities

In addition to the stopping rules based on successful neutrophil engraftment, we will closely monitor the trial for early excessive toxicities in terms of secondary graft failure, severe acute grade III/IV GVHD and therapy related death/mortality (TRM). Secondary graft failure is defined in section 7.0. TRM is any death in remission and related to protocol therapy. The incidence of secondary graft failure, acute GVHD and transplant related deaths will be monitored for 100 days from the date of transplantation for application of the stopping rules. Toxicities secondary to non-protocol therapy for post-transplant persistent or recurrent disease will not count towards the toxicity stopping rules.

In 2007, Eapen et al published the CIBMTR and National Cord Blood Program outcomes of 503 pediatric patients undergoing UCBT for acute leukemia in the United States. The study reports a rate of 40% (188/468) for TRM and 23% (110/486) for acute grade III/IV GVHD. The rate for graft failure was 18% (89/500). Similar rates were reported for patients aged 16 years or over who underwent a transplant for acute leukemia in 2010.

The safety endpoints will be monitored independently and if there is evidence suggesting that the rate of secondary graft failure is greater than 20% or the rate of stage III/IV acute GVHD is greater than 30% (type I error rate 20%), or the rate of TRM is greater than 25%, stopping the trial or amending the therapy will be considered. The planned interim evaluation time points and stopping rules based on the exact upper 90% Blyth-Still-Casella confidence bounds for each of the three endpoints are provided in Tables 3-5

Table 3. Stopping Rules for Toxicities Based on Grades 3-4 acute GVHD within the First 100 Days Post-transplant

No. of Research Participants Enrolled	No. of Grades 3-4 acute GVHD Observed	Exact Upper Confidence Bounds
≤ 21	≥ 5	0.3199
≤ 35	≥ 9	0.3199
≤ 49	≥ 12	0.3023

Table 4. Stopping Rules for Toxicities Based on TRM within the first 100 days Post-transplant

No. of Research Participants Enrolled	No. of Therapy Related Death Observed	Exact Upper Confidence Bounds
≤ 21	≥ 3	0.2778
≤ 35	≥ 6	0.2647
≤ 49	≥ 9	0.2695

Table 5. Stopping Rules for Secondary Graft Failure within the First 100 Days Post-transplant

No. of Research Participants Enrolled	No. of Secondary Graft Failure Observed	Exact Upper Confidence Bounds
≤ 21	≥ 2	0.2178
≤ 35	≥ 4	0.2103
≤ 49	≥ 6	0.2011

Based on the above Table 3, if five grades 3-4 acute GVHD occur within the first 100 days post transplantation in the first twenty-one evaluable research participants treated, then stopping the trial and/or amending the study will be considered. Similarly, if we observe three TRMs (Table 4) or two secondary graft failures (Table 5) in the the first 100 days after transplantation in the first twenty-one evaluable research participants, then temporarily stopping the trial and amending the study will be considered. It may be noted that the above stopping rules are "ad hoc" in nature.

11.2 Statistical analysis for secondary objectives

11.2.1 Estimate the incidence of malignant relapse, EFS and OS at one-year post-transplantation

The estimate of cumulative incidence of relapse will be estimated using Kalbfleisch-Prentice method. Death is the competing risk event. The analysis will be implemented using SAS macro (bmacro252-Excel2007\cin) available in the St. Jude Department of Biostatistics. The Kaplan-Meier estimate of OS and EFS with relapse, death due to any cause and graft failure as events along with their standard errors will be calculated using the SAS macro (bmacro251-Excel2007\kme) available in the Department of Biostatistics at St. Jude, where OS = min (date of last follow-up, date of death) – date of HCT and all participants surviving after 1 year post-transplant will be considered as censored, and EFS = *min* (date of last follow-up, date of relapse, date of graft failure, date of death due to any cause) – date of transplant, and all participants surviving at the time of analysis without events will be censored. The analysis for this objective will be performed when the last evaluable participant has been followed for one-year post transplant.

11.2.2 Estimate the incidence and severity of acute and chronic GVHD in the first 100 days after HCT.

The cumulative incidence of acute and chronic GVHD will be estimated using Kalbfleisch-Prentice method. Death is the competing risk event. The SAS macro (bmacro252-Excel2007\cin) available in the Department of Biostatistics at St. Jude will be used for such analysis. The severity of acute GVHD and chronic GVHD will be described. The analysis for this objective will be performed when the last evaluable participant has been followed for 100 days post transplant.

11.2.3 Estimate the incidence of secondary graft failure, transplant related mortality (TRM) and transplant related morbidity in the first 100 days after HCT.

The cumulative incidence of TRM, transplant related mortality, and secondary graft failures will be estimated using the same method as used in evaluating Objective 11.2.2. Deaths before day 100 because of other reasons are the competing risk events for TRM and transplant related mortality. Deaths due to toxicity and relapse before day 100 are the competing risk events for secondary graft failure. The analysis for this objective will be performed when the last evaluable participant has been followed for 100 days post transplant.

11.3 Analysis for exploratory objectives

The final results of these exploratory objectives are expected to be available when the last evaluable participant has been followed for one-year post transplant.

11.3.1 Assess the relationship between pre-transplant MRD with transplant outcomes.

The relationship of pre-transplant MRD and transplant outcomes will be examined through Cox proportional hazard model or generalized linear model. The model can also be used to adjust for other confounding factors such as patient's age at transplant.

11.3.2 Record immune reconstitution parameters, including chimerism analysis, quantitative lymphocyte subsets, T cell receptor excision circle (TREC) and spectratyping. Immunophenotyping and functional assays of T, B, and NK cells and lymphocytes will also be evaluated.. All immune reconstitution measures, immunophenotyping and functional assay measures will be descriptively analyzed.

11.3.3 Characterize the recovery of Gamma Delta (γδ) T cells after HCT, including T cell receptor analysis, phenotyping and functional analysis. All Gamma Delta T cell measures will be descriptively analyzed.

11.3.4 Characterize influenza infection during HCT by monitoring viral isolates and key host factors associated with influenza susceptibility. All Influenza infection monitoring will be descriptively analyzed.

12.0 OBTAINING INFORMED CONSENT

The ongoing informed consent process will be carried out per the policies and procedures put forth in the St. Jude Investigator's Handbook for Clinical Research (http://home.web.stjude.org/clinical_research/administration/doc/handbook.pdf). The PI or physician sub-investigator will conduct the signature authorization portion of the consent process.

12.1 Informed consent prior to research interventions

For protocol required research interventions (research samples for baseline immune reconstitution evaluation); informed consent must be obtained prior to performing these research interventions.

12.2 Consent at age of majority

The age of majority in the state of Tennessee is 18 years old. Research participants must be consented at the next St. Jude clinic visit after their 18th birthday, and prior to performing any research interventions during that visit.

12.3 Consent when English is not the primary language

When English is not the patient, parent, or legally authorized representative's primary language, the Social Work department will determine the need for an interpreter. This information will be documented in the participant's medical record. Either a certified interpreter or the telephone interpreter's service will be used to translate the consent information. The process for obtaining an interpreter and for the use of an interpreter is outlined on the Interpreter Services, OHSP, and CPDMO websites.

13.0 REFERENCES

1. Westoff CF. Fertility in the United States. *Science* 1986; **234**(4776): 554-9.
2. Kernan NA, Bartsch G, Ash RC, et al. Analysis of 462 transplantations from unrelated donors facilitated by the National Marrow Donor Program. *N Engl J Med* 1993; **328**(9): 593-602.
3. Barker JN, Wagner JE. Umbilical-cord blood transplantation for the treatment of cancer. *Nat Rev Cancer* 2003; **3**(7): 526-32.
4. Beatty PG, Clift RA, Mickelson EM, et al. Marrow transplantation from related donors other than HLA-identical siblings. *N Engl J Med* 1985; **313**(13): 765-71.
5. Ottinger HD, Ferencik S, Beelen DW, et al. Hematopoietic stem cell transplantation: contrasting the outcome of transplantations from HLA-identical siblings, partially HLA-mismatched related donors, and HLA-matched unrelated donors. *Blood* 2003; **102**(3): 1131-7.
6. Szydlo R, Goldman JM, Klein JP, et al. Results of allogeneic bone marrow transplants for leukemia using donors other than HLA-identical siblings. *J Clin Oncol* 1997; **15**(5): 1767-77.
7. Liu H, Rich ES, Godley L, et al. Reduced-intensity conditioning with combined haploidentical and cord blood transplantation results in rapid engraftment, low GVHD, and durable remissions. *Blood* 2011; **118**(24): 6438-45.
8. Jagasia MH, Greinix HT, Arora M, et al. National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: I. The 2014 Diagnosis and Staging Working Group report. *Biol Blood Marrow Transplant* 2015; **21**(3): 389-401 e1.
9. Thomas E, Storb R, Clift RA, et al. Bone-marrow transplantation (first of two parts). *N Engl J Med* 1975; **292**(16): 832-43.
10. Thomas ED, Buckner CD, Banaji M, et al. One hundred patients with acute leukemia treated by chemotherapy, total body irradiation, and allogeneic marrow transplantation. *Blood* 1977; **49**(4): 511-33.
11. Appelbaum FR, Fisher LD, Thomas ED. Chemotherapy v marrow transplantation for adults with acute nonlymphocytic leukemia: a five-year follow-up. *Blood* 1988; **72**(1): 179-84.
12. Butturini A, Gale RP. Chemotherapy versus transplantation: II. Acute lymphoblastic leukemia. *Haematologica* 1989; **74**(4): 337-9.
13. McGlave PB, Haake RJ, Bostrom BC, et al. Allogeneic bone marrow transplantation for acute nonlymphocytic leukemia in first remission. *Blood* 1988; **72**(5): 1512-7.
14. Barrett AJ, Horowitz MM, Gale RP, et al. Marrow transplantation for acute lymphoblastic leukemia: factors affecting relapse and survival. *Blood* 1989; **74**(2): 862-71.
15. Sarina B, Castagna L, Farina L, et al. Allogeneic transplantation improves the overall and progression-free survival of Hodgkin lymphoma patients relapsing after autologous transplantation: a retrospective study based on the time of HLA typing and donor availability. *Blood* 2010; **115**(18): 3671-7.
16. Gross TG, Hale GA, He W, et al. Hematopoietic stem cell transplantation for refractory or recurrent non-Hodgkin lymphoma in children and adolescents. *Biol Blood Marrow Transplant* 2010; **16**(2): 223-30.
17. Eapen M, Rubinstein P, Zhang MJ, et al. Outcomes of transplantation of unrelated donor umbilical cord blood and bone marrow in children with acute leukaemia: a comparison study. *Lancet* 2007; **369**(9577): 1947-54.
18. Laughlin MJ, Eapen M, Rubinstein P, 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.

19. Rocha V, Cornish J, Sievers EL, et al. Comparison of outcomes of unrelated bone marrow and umbilical cord blood transplants in children with acute leukemia. *Blood* 2001; **97**(10): 2962-71.
20. Rocha V, Labopin M, Sanz G, 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.
21. Rocha V, Kabbara N, Ionescu I, Ruggeri A, Purtill D, Gluckman E. Pediatric related and unrelated cord blood transplantation for malignant diseases. *Bone Marrow Transplant* 2009; **44**(10): 653-9.
22. Chen X, Hale GA, Barfield R, et al. Rapid immune reconstitution after a reduced-intensity conditioning regimen and a CD3-depleted haploidentical stem cell graft for paediatric refractory haematological malignancies. *Br J Haematol* 2006; **135**(4): 524-32.
23. Woodard P, Cunningham JM, Benaim E, et al. Effective donor lymphohematopoietic reconstitution after haploidentical CD34+-selected hematopoietic stem cell transplantation in children with refractory severe aplastic anemia. *Bone Marrow Transplant* 2004; **33**(4): 411-8.
24. Handgretinger R, Klingebiel T, Lang P, Gordon P, Niethammer D. Megadose transplantation of highly purified haploidentical stem cells: current results and future prospects. *Pediatr Transplant* 2003; **7 Suppl 3**: 51-5.
25. Leung W, Iyengar R, Turner V, et al. Determinants of antileukemia effects of allogeneic NK cells. *J Immunol* 2004; **172**(1): 644-50.
26. Leung W, Iyengar R, Triplett B, et al. Comparison of killer Ig-like receptor genotyping and phenotyping for selection of allogeneic blood stem cell donors. *J Immunol* 2005; **174**(10): 6540-5.
27. Handgretinger R, Chen X, Pfeiffer M, et al. Feasibility and outcome of reduced-intensity conditioning in haploidentical transplantation. *Ann N Y Acad Sci* 2007; **1106**: 279-89.
28. Leung W, Campana D, Yang J, et al. High success of hematopoietic cell transplantation regardless of donor source in children with very high-risk leukemia. *Blood*.
29. Hale G, Chen X, Benaim E, et al. Haploidentical transplantation for children with refractory haematologic malignancies. *Bone Marrow Transplant* 2006; **37**(Supplement 1): S250.
30. Ende M, Ende N. Hematopoietic transplantation by means of fetal (cord) blood. A new method. *Va Med Mon (1918)* 1972; **99**(3): 276-80.
31. Almici C, Carlo-Stella C, Mangoni L, et al. Density separation of umbilical cord blood and recovery of hemopoietic progenitor cells: implications for cord blood banking. *Stem Cells* 1995; **13**(5): 533-40.
32. Gluckman E, Broxmeyer HA, Auerbach AD, et al. Hematopoietic reconstitution in a patient with Fanconi's anemia by means of umbilical-cord blood from an HLA-identical sibling. *N Engl J Med* 1989; **321**(17): 1174-8.
33. Kurtzberg J, Laughlin M, Graham ML, 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.
34. Wagner JE, Rosenthal J, Sweetman R, 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.
35. Smith AR, Baker KS, Defor TE, Verneris MR, Wagner JE, Macmillan ML. Hematopoietic cell transplantation for children with acute lymphoblastic leukemia in second complete remission: similar outcomes in recipients of unrelated marrow and umbilical cord blood versus marrow from HLA matched sibling donors. *Biol Blood Marrow Transplant* 2009; **15**(9): 1086-93.

36. Tomblyn MB, Arora M, Baker KS, et al. Myeloablative hematopoietic cell transplantation for acute lymphoblastic leukemia: analysis of graft sources and long-term outcome. *J Clin Oncol* 2009; **27**(22): 3634-41.
37. Gluckman E, Rocha V, Boyer-Chammard A, 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.
38. Rocha V, Gluckman E. Improving outcomes of cord blood transplantation: HLA matching, cell dose and other graft- and transplantation-related factors. *Br J Haematol* 2009; **147**(2): 262-74.
39. Rubinstein P, Carrier C, Scaradavou A, et al. Outcomes among 562 recipients of placental-blood transplants from unrelated donors. *N Engl J Med* 1998; **339**(22): 1565-77.
40. Rubinstein P, Rosenfield RE, Adamson JW, Stevens CE. Stored placental blood for unrelated bone marrow reconstitution. *Blood* 1993; **81**(7): 1679-90.
41. Wagner JE, Barker JN, DeFor TE, 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.
42. Gluckman E, Rocha V, Chastang C. European results of unrelated cord blood transplants. Eurocord group. *Bone Marrow Transplant* 1998; **21 Suppl 3**: S87-91.
43. Gluckman E, Rocha V. Cord blood transplantation for children with acute leukaemia: a Eurocord registry analysis. *Blood Cells Mol Dis* 2004; **33**(3): 271-3.
44. Delaney C, Gutman JA, Appelbaum FR. Cord blood transplantation for haematological malignancies: conditioning regimens, double cord transplant and infectious complications. *Br J Haematol* 2009; **147**(2): 207-16.
45. Brunstein CG, Gutman JA, Weisdorf DJ, et al. Allogeneic hematopoietic cell transplantation for hematological malignancy: relative risks and benefits of double umbilical cord blood. *Blood* 2010.
46. Barker JN, Weisdorf DJ, DeFor TE, 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.
47. MacMillan ML, Weisdorf DJ, Brunstein CG, et al. Acute graft-versus-host disease after unrelated donor umbilical cord blood transplantation: analysis of risk factors. *Blood* 2009; **113**(11): 2410-5.
48. Rodrigues CA, Sanz G, Brunstein CG, et al. Analysis of risk factors for outcomes after unrelated cord blood transplantation in adults with lymphoid malignancies: a study by the Eurocord-Netcord and lymphoma working party of the European group for blood and marrow transplantation. *J Clin Oncol* 2009; **27**(2): 256-63.
49. Beck JC, Wagner JE, DeFor TE, et al. Impact of cytomegalovirus (CMV) reactivation after umbilical cord blood transplantation. *Biol Blood Marrow Transplant* 2010; **16**(2): 215-22.
50. Verneris MR, Miller JS. The phenotypic and functional characteristics of umbilical cord blood and peripheral blood natural killer cells. *Br J Haematol* 2009; **147**(2): 185-91.
51. Smith AR, Wagner JE. Alternative haematopoietic stem cell sources for transplantation: place of umbilical cord blood. *Br J Haematol* 2009; **147**(2): 246-61.
52. Delaney M, Cutler CS, Haspel RL, et al. High-resolution HLA matching in double-umbilical-cord-blood reduced-intensity transplantation in adults. *Transfusion* 2009; **49**(5): 995-1002.

53. Ballen K, Broxmeyer HE, McCullough J, et al. Current status of cord blood banking and transplantation in the United States and Europe. *Biol Blood Marrow Transplant* 2001; **7**(12): 635-45.
54. Wagner JE, Jr. Umbilical cord and placental blood transplantation: analysis of the clinical results. *J Hematother* 1993; **2**(2): 265-8.
55. Wagner JE, Broxmeyer HE, Byrd RL, et al. Transplantation of umbilical cord blood after myeloablative therapy: analysis of engraftment. *Blood* 1992; **79**(7): 1874-81.
56. Wagner JE, Kernan NA, Steinbuch M, Broxmeyer HE, Gluckman E. Allogeneic sibling umbilical-cord-blood transplantation in children with malignant and non-malignant disease. *Lancet* 1995; **346**(8969): 214-9.
57. Gluckman E, Rocha V, Chastang CL. Umbilical cord blood hematopoietic stem cell transplantation. Eurocord-Cord Blood Transplant Group. *Cancer Treat Res* 1999; **101**: 79-96.
58. Gluckman E, Rocha V, Ionescu I, et al. Results of unrelated cord blood transplant in fanconi anemia patients: risk factor analysis for engraftment and survival. *Biol Blood Marrow Transplant* 2007; **13**(9): 1073-82.
59. Gluckman E, Wagner J, Hows J, Kernan N, Bradley B, Broxmeyer HE. Cord blood banking for hematopoietic stem cell transplantation: an international cord blood transplant registry. *Bone Marrow Transplant* 1993; **11**(3): 199-200.
60. Kurtzberg J, Prasad VK, Carter SL, et al. Results of the Cord Blood Transplantation Study (COBLT): clinical outcomes of unrelated donor umbilical cord blood transplantation in pediatric patients with hematologic malignancies. *Blood* 2008; **112**(10): 4318-27.
61. Rocha V, Wagner JE, Jr., Sobocinski KA, et al. Graft-versus-host disease in children who have received a cord-blood or bone marrow transplant from an HLA-identical sibling. Eurocord and International Bone Marrow Transplant Registry Working Committee on Alternative Donor and Stem Cell Sources. *N Engl J Med* 2000; **342**(25): 1846-54.
62. Ballen KK, Spitzer TR, Yeap BY, et al. Double unrelated reduced-intensity umbilical cord blood transplantation in adults. *Biol Blood Marrow Transplant* 2007; **13**(1): 82-9.
63. Barker JN, Scaradavou A, Stevens CE. Combined effect of total nucleated cell dose and HLA match on transplantation outcome in 1061 cord blood recipients with hematologic malignancies. *Blood* 2010; **115**(9): 1843-9.
64. Brunstein CG, Baker KS, Wagner JE. Umbilical cord blood transplantation for myeloid malignancies. *Curr Opin Hematol* 2007; **14**(2): 162-9.
65. Herr AL, Kabbara N, Bonfim CM, et al. Long-term follow-up and factors influencing outcomes after related HLA-identical cord blood transplantation for patients with malignancies: an analysis on behalf of Eurocord-EBMT. *Blood* 2010.
66. Locatelli F, Rocha V, Chastang C, et al. Factors associated with outcome after cord blood transplantation in children with acute leukemia. Eurocord-Cord Blood Transplant Group. *Blood* 1999; **93**(11): 3662-71.
67. Laughlin MJ, Barker J, Bambach B, 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.
68. Aversa F. Haploidentical haematopoietic stem cell transplantation for acute leukaemia in adults: experience in Europe and the United States. *Bone Marrow Transplant* 2008; **41**(5): 473-81.

69. Aversa F, Terenzi A, Tabilio A, et al. Full haplotype-mismatched hematopoietic stem-cell transplantation: a phase II study in patients with acute leukemia at high risk of relapse. *J Clin Oncol* 2005; **23**(15): 3447-54.
70. Ciceri F, Labopin M, Aversa F, et al. A survey of fully haploidentical hematopoietic stem cell transplantation in adults with high-risk acute leukemia: a risk factor analysis of outcomes for patients in remission at transplantation. *Blood* 2008; **112**(9): 3574-81.
71. Handgretinger R, Klingebiel T, Lang P, et al. Megadose transplantation of purified peripheral blood CD34(+) progenitor cells from HLA-mismatched parental donors in children. *Bone Marrow Transplant* 2001; **27**(8): 777-83.
72. Bethge WA, Faul C, Bornhauser M, et al. Haploidentical allogeneic hematopoietic cell transplantation in adults using CD3/CD19 depletion and reduced intensity conditioning: an update. *Blood Cells Mol Dis* 2008; **40**(1): 13-9.
73. Goldman FD, Rumelhart SL, DeAlacron P, et al. Poor outcome in children with refractory/relapsed leukemia undergoing bone marrow transplantation with mismatched family member donors. *Bone Marrow Transplant* 2000; **25**(9): 943-8.
74. Aversa F, Terenzi A, Felicini R, et al. Mismatched T cell-depleted hematopoietic stem cell transplantation for children with high-risk acute leukemia. *Bone Marrow Transplant* 1998; **22 Suppl 5**: S29-32.
75. Handgretinger R, Schumm M, Lang P, et al. Transplantation of megadoses of purified haploidentical stem cells. *Ann N Y Acad Sci* 1999; **872**: 351-61; discussion 61-2.
76. Leung W, Campana D, Yang J, et al. High success rate of hematopoietic cell transplantation regardless of donor source in children with very high-risk leukemia. *Blood*; **118**(2): 223-30.
77. Karre K. Immunology. A perfect mismatch. *Science* 2002; **295**(5562): 2029-31.
78. Long EO. Regulation of immune responses through inhibitory receptors. *Annu Rev Immunol* 1999; **17**: 875-904.
79. Moretta A, Pende D, Locatelli F, Moretta L. Activating and inhibitory killer immunoglobulin-like receptors (KIR) in haploidentical haemopoietic stem cell transplantation to cure high-risk leukaemias. *Clin Exp Immunol* 2009; **157**(3): 325-31.
80. Ruggeri L, Mancusi A, Burchielli E, et al. NK cell alloreactivity and allogeneic hematopoietic stem cell transplantation. *Blood Cells Mol Dis* 2008; **40**(1): 84-90.
81. Vilches C, Parham P. KIR: diverse, rapidly evolving receptors of innate and adaptive immunity. *Annu Rev Immunol* 2002; **20**: 217-51.
82. Ruggeri L, Capanni M, Casucci M, et al. Role of natural killer cell alloreactivity in HLA-mismatched hematopoietic stem cell transplantation. *Blood* 1999; **94**(1): 333-9.
83. Ruggeri L, Capanni M, Urbani E, et al. Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. *Science* 2002; **295**(5562): 2097-100.
84. Moretta A, Bottino C, Mingari MC, Biassoni R, Moretta L. What is a natural killer cell? *Nat Immunol* 2002; **3**(1): 6-8.
85. Lang P, Handgretinger R. Haploidentical SCT in children: an update and future perspectives. *Bone Marrow Transplant* 2008; **42 Suppl 2**: S54-9.
86. Godder KT, Hazlett LJ, Abhyankar SH, et al. Partially mismatched related-donor bone marrow transplantation for pediatric patients with acute leukemia: younger donors and absence of peripheral blasts improve outcome. *J Clin Oncol* 2000; **18**(9): 1856-66.

87. Champlin RE, Passweg JR, Zhang MJ, et al. T-cell depletion of bone marrow transplants for leukemia from donors other than HLA-identical siblings: advantage of T-cell antibodies with narrow specificities. *Blood* 2000; **95**(12): 3996-4003.
88. Amrolia PJ, Muccioli-Casadei G, Huls H, et al. Adoptive immunotherapy with allodepleted donor T-cells improves immune reconstitution after haploidentical stem cell transplantation. *Blood* 2006; **108**(6): 1797-808.
89. Ciurea SO, Mulanovich V, Jiang Y, et al. Lymphocyte Recovery Predicts Outcomes in Cord Blood and T Cell–Depleted Haploidentical Stem Cell Transplantation. *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation* 2011; **17**(8): 1169-75.
90. Novitzky N, Davison GM. Immune reconstitution following hematopoietic stem-cell transplantation. *Cytotherapy* 2001; **3**(3): 211-20.
91. Maltezou HC, Kafetzis DA, Abisaid D, Mantzouranis EC, Chan KW, Rolston KV. Viral infections in children undergoing hematopoietic stem cell transplant. *The Pediatric infectious disease journal* 2000; **19**(4): 307-12.
92. Andre-Schmutz I, Dal Cortivo L, Fischer A, Cavazzana-Calvo M. Improving immune reconstitution while preventing GvHD in allogeneic stem cell transplantation. *Cytotherapy* 2005; **7**(2): 102-8.
93. Walter EA, Greenberg PD, Gilbert MJ, et al. Reconstitution of cellular immunity against cytomegalovirus in recipients of allogeneic bone marrow by transfer of T-cell clones from the donor. *N Engl J Med* 1995; **333**(16): 1038-44.
94. Leen AM, Christin A, Myers GD, et al. Cytotoxic T lymphocyte therapy with donor T cells prevents and treats adenovirus and Epstein-Barr virus infections after haploidentical and matched unrelated stem cell transplantation. *Blood* 2009; **114**(19): 4283-92.
95. Perruccio K, Tosti A, Burchielli E, et al. Transferring functional immune responses to pathogens after haploidentical hematopoietic transplantation. *Blood* 2005; **106**(13): 4397-406.
96. Anderson BE, McNiff J, Yan J, et al. Memory CD4+ T cells do not induce graft-versus-host disease. *The Journal of clinical investigation* 2003; **112**(1): 101-8.
97. Chao NJ. Are there effective new strategies for the treatment of acute and chronic GvHD? *Best practice & research Clinical haematology* 2008; **21**(1): 93-8.
98. Jameson SC, Masopust D. Diversity in T cell memory: an embarrassment of riches. *Immunity* 2009; **31**(6): 859-71.
99. Ochsenbein AF, Pinschewer DD, Sierro S, Horvath E, Hengartner H, Zinkernagel RM. Protective long-term antibody memory by antigen-driven and T help-dependent differentiation of long-lived memory B cells to short-lived plasma cells independent of secondary lymphoid organs. *Proceedings of the National Academy of Sciences of the United States of America* 2000; **97**(24): 13263-8.
100. Distler E, Bloetz A, Albrecht J, et al. Alloreactive and leukemia-reactive T cells are preferentially derived from naive precursors in healthy donors: implications for immunotherapy with memory T cells. *Haematologica* 2011; **96**(7): 1024-32.
101. Chen BJ, Cui X, Sempowski GD, Domen J, Chao NJ. Hematopoietic stem cell dose correlates with the speed of immune reconstitution after stem cell transplantation. *Blood* 2004; **103**(11): 4344-52.
102. Yamashita N, Bullington R, Clement LT. Equivalent helper functions of human "naive" and "memory" CD4+ T cells for the generation of alloreactive cytotoxic T lymphocytes. *Journal of clinical immunology* 1990; **10**(5): 237-46.

103. Chen YB, Spitzer TR. Current status of reduced-intensity allogeneic stem cell transplantation using alternative donors. *Leukemia* 2008; **22**(1): 31-41.
104. Aversa F, Terenzi A, Felicini R, et al. Haploidentical stem cell transplantation for acute leukemia. *Int J Hematol* 2002; **76 Suppl 1**: 165-8.
105. Aversa F, Velardi A, Tabilio A, Reisner Y, Martelli MF. Haploidentical stem cell transplantation in leukemia. *Blood Rev* 2001; **15**(3): 111-9.
106. Kohrt H, Lowsky R. Total lymphoid irradiation for graft-versus-host disease protection. *Curr Opin Oncol* 2009; **21 Suppl 1**: S23-6.
107. Kohrt HE, Turnbull BB, Heydari K, et al. TLI and ATG conditioning with low risk of graft-versus-host disease retains antitumor reactions after allogeneic hematopoietic cell transplantation from related and unrelated donors. *Blood* 2009; **114**(5): 1099-109.
108. Lowsky R, Takahashi T, Liu YP, et al. Protective conditioning for acute graft-versus-host disease. *N Engl J Med* 2005; **353**(13): 1321-31.
109. Heinzelmann F, Lang PJ, Ottinger H, et al. Immunosuppressive total lymphoid irradiation-based reconditioning regimens enable engraftment after graft rejection or graft failure in patients treated with allogeneic hematopoietic stem cell transplantation. *Int J Radiat Oncol Biol Phys* 2008; **70**(2): 523-8.
110. Palma J, Salas L, Carrion F, et al. Haploidentical stem cell transplantation for children with high-risk leukemia. *Pediatr Blood Cancer*.
111. Gluckman E. History of cord blood transplantation. *Bone Marrow Transplant* 2009; **44**(10): 621-6.
112. Willemze R, Rodrigues CA, Labopin M, et al. KIR-ligand incompatibility in the graft-versus-host direction improves outcomes after umbilical cord blood transplantation for acute leukemia. *Leukemia* 2009; **23**(3): 492-500.
113. Brunstein CG, Wagner JE, Weisdorf DJ, et al. Negative effect of KIR alloreactivity in recipients of umbilical cord blood transplant depends on transplantation conditioning intensity. *Blood* 2009; **113**(22): 5628-34.
114. Coustan-Smith E, Behm FG, Sanchez J, et al. Immunological detection of minimal residual disease in children with acute lymphoblastic leukaemia. *Lancet* 1998; **351**(9102): 550-4.
115. Coustan-Smith E, Sancho J, Behm FG, et al. Prognostic importance of measuring early clearance of leukemic cells by flow cytometry in childhood acute lymphoblastic leukemia. *Blood* 2002; **100**(1): 52-8.
116. Coustan-Smith E, Sancho J, Hancock ML, et al. Clinical importance of minimal residual disease in childhood acute lymphoblastic leukemia. *Blood* 2000; **96**(8): 2691-6.
117. Nucifora G, Larson RA, Rowley JD. Persistence of the 8;21 translocation in patients with acute myeloid leukemia type M2 in long-term remission. *Blood* 1993; **82**(3): 712-5.
118. Roberts WM, Estrov Z, Ouspenskaia MV, Johnston DA, McClain KL, Zipf TF. Measurement of residual leukemia during remission in childhood acute lymphoblastic leukemia. *N Engl J Med* 1997; **336**(5): 317-23.
119. Knechtli CJ, Goulden NJ, Hancock JP, et al. Minimal residual disease status before allogeneic bone marrow transplantation is an important determinant of successful outcome for children and adolescents with acute lymphoblastic leukemia. *Blood* 1998; **92**(11): 4072-9.
120. Langebrake C, Creutzig U, Dworzak M, et al. Residual disease monitoring in childhood acute myeloid leukemia by multiparameter flow cytometry: the MRD-AML-BFM Study Group. *J Clin Oncol* 2006; **24**(22): 3686-92.

121. Uchida N, Tsukamoto A, He D, Friera AM, Scollay R, Weissman IL. High doses of purified stem cells cause early hematopoietic recovery in syngeneic and allogeneic hosts. *The Journal of clinical investigation* 1998; **101**(5): 961-6.
122. Chen X, Barfield R, Benaim E, et al. Prediction of T-cell reconstitution by assessment of T-cell receptor excision circle before allogeneic hematopoietic stem cell transplantation in pediatric patients. *Blood* 2005; **105**(2): 886-93.
123. Weinberg K, Blazar BR, Wagner JE, et al. Factors affecting thymic function after allogeneic hematopoietic stem cell transplantation. *Blood* 2001; **97**(5): 1458-66.
124. Langfitt D, Wallace N, Dallas MH. Dendritic Cell Facilitate Thymic Recovery And Enhance Immune Reconstitution After Hematopoietic Stem Cell Transplant. *Biology of Blood and Marrow Transplantation* 2010; **16**(2, Supplement 1): S173-S.
125. Langfitt D, Wallace N, Gaines S, Dallas M. Dendritic cells and Regulatory T cells facilitate donor engraftment after hematopoietic stem cell transplantation. *J Immunol* 2010; **184**(1_MeetingAbstracts): 145.35-.
126. Gutman JA, Turtle CJ, Manley TJ, et al. Single-unit dominance after double-unit umbilical cord blood transplantation coincides with a specific CD8+ T-cell response against the nonengrafted unit. *Blood* 2010; **115**(4): 757-65.
127. Frangoul H, Wang L, Harrell Jr FE, Ho R, Domm J. Preengraftment Syndrome after Unrelated Cord Blood Transplant Is a Strong Predictor of Acute and Chronic Graft-versus-Host Disease. *Biology of Blood and Marrow Transplantation* 2009; **15**(11): 1485-8.
128. Park M, Lee SH, Lee YH, et al. Pre-Engraftment Syndrome after Unrelated Cord Blood Transplantation: A Predictor of Engraftment and Acute Graft-versus-Host Disease. *Biology of Blood and Marrow Transplantation* 2013; **19**(4): 640-6.
129. Schmid I, Stachel D, Pagel P, Albert MH. Incidence, Predisposing Factors, and Outcome of Engraftment Syndrome in Pediatric Allogeneic Stem Cell Transplant Recipients. *Biology of Blood and Marrow Transplantation* 2008; **14**(4): 438-44.
130. Ballen KK, Koreth J, Chen YB, Dey BR, Spitzer TR. Selection of optimal alternative graft source: mismatched unrelated donor, umbilical cord blood, or haploidentical transplant. *Blood*; **119**(9): 1972-80.

APPENDIX A:

PERFORMANCE STATUS SCALE

KARNOFSKY PERFORMANCE STATUS SCALE	
Score	**General Description**
100	Normal. No complaints. No evidence of disease.
90	Able to carry on normal activity. Minor signs or symptoms of disease.
80	Normal activity with effort. Some signs or symptoms of disease.
70	Care of self. Unable to carry out normal activity or to do active work.
60	Requires occasional assistance, but is able to care for most of his needs.
50	Requires considerable assistance and frequent medical care.
40	Disabled. Requires special care and assistance.
30	Severely disabled. Hospitalization is indicated although death is not imminent.
20	Hospitalization necessary, very sick, active support treatment necessary.
10	Moribund. Fatal processes progressing rapidly.
0	Dead.

LANSKY PERFORMANCE STATUS SCALE	
Score	**General Description**
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 activity
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 but 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	No play; does not get out of bed
0	Unresponsive

APPENDIX B

COG STEM CELL COMMITTEE CONSENSUS GUIDELINES FOR ESTABLISHING ORGAN STAGE AND OVERALL GRADE OF ACUTE GRAFT VERSUS HOST DISEASE (GVHD)

Table 1 outlines standard criteria for GVHD organ staging. However, confounding clinical syndromes (such as non-GVHD causes of hyperbilirubinemia) may make staging GVHD in a given organ difficult. In addition, timing of organ specific symptoms affects whether that symptom is more or less likely to be true GVHD. Please refer to ***Tables 2 and 3*** to assist you in deciding whether to attribute these clinical findings to GVHD, especially in situations where a biopsy is not possible. For additional help, please see the text which follows the tables. ***Table 4*** reviews the approach to assessing GVHD as acute, chronic, or the overlap between the two.

Finally, ***engraftment syndrome*** will be reported separately from the GVHD scoring presented below.

Engraftment Syndrome

A clinical syndrome of fever, rash, respiratory distress, and diarrhea has been described, just prior to engraftment in patients undergoing unrelated cord blood and mismatched transplantation. If, in the judgment of the treating physician, a patient experiences this syndrome, details of the event will be recorded in the medical record.

Modified Glucksberg Staging Criteria for Acute Graft versus Host Disease

Table 1: Organ Staging (See tables and text below for details)

Stage	Skin	Liver (bilirubin)	Gut (stool output/day)
0	No GVHD rash	< 2 mg/dL	Adult: < 500 mL/day Child: < 10 mL/kg/day
1	Maculopapular rash < 25% BSA	2-3 mg/dL	Adult: 500-999 mL/day Child: 10-19.9 mL/kg/day. ***Or persistent nausea, vomiting, or anorexia, with a positive upper GI biopsy.***
2	Maculopapular rash 25-50% BSA	3.1-6 mg/dL	Adult: 1000-1500 mL/day Child: 20-30 mL/kg/day
3	Maculopapular rash > 50% BSA	6.1-15 mg/dL	Adult: > 1500 mL/day Child: > 30 mL/kg/day
4	Generalized erythroderma plus bullous formation and desquamation > 5% BSA	>15 mg/dL	Severe abdominal pain with or without ileus, or grossly bloody stool (regardless of stool volume).

For GI staging: The "adult" stool output values should be used for patients > 50 kg in weight. Use 3 day averages for GI staging based on stool output. If stool and urine are mixed, stool output is presumed to be 50% of total stool/urine mix (see 3.2 below).

For Stage 4 GI: the term "severe abdominal pain" will be defined as:

a) Pain control requiring institution of opioid use, or an increase in on-going opioid use, PLUS
b) Pain that significantly impacts performance status, as determined by the treating MD.

If colon or rectal biopsy is +, but stool output is < 500 mL/day (< 10 mL/kg/day), then consider as GI stage 0.

There is no modification of liver staging for other causes of hyperbilirubinemia.

Overall Clinical Grade (based on the highest stage obtained):
Grade 0: No stage 1-4 of any organ
Grade I: Stage 1-2 skin and no liver or gut involvement
Grade II: Stage 3 skin, or Stage I liver involvement, or Stage l GI
Grade III: Stage 0-3 skin, with Stage 2-3 liver, or Stage 2-3 GI
Grade IV: Stage 4 skin, liver or GI involvement

Table 2 Evaluating Liver GVHD in the Absence of Biopsy Confirmation (See Table 3.0 below)

Establishing liver GVHD with no skin or GI GVHD

No Skin/GI GVHD Day 0-35	Assume no liver GVHD, unless proven by biopsy	
No Skin/GI GVHD Day 36-100	If NO other etiology identified, NO improvement with stopping hepatotoxic medications/TPN: **Stage as liver GVHD**	If other etiology identified or improves with stopping hepatotoxic drugs/TPN: **Do not stage as liver GVHD**

Establishing liver GVHD with skin or GI GVHD and other cause of hyperbilirubinemia

Skin and/or GI GVHD present	Worsening bilirubin level (includes worsening just prior to onset of skin or GI tract GVHD) OR stable elevated bilirubin despite resolution of non-GVHD cause of increased bilirubin: **Stage as liver GVHD**	Stable or improving bilirubin after diagnosis of skin or GI GVHD, irrespective of treatment: **Do not stage as liver GVHD**

Changing liver GVHD stage with other cause of hyperbilirubinemia

Skin and GI GVHD stable, improving, or absent	Liver GVHD staging is carried forward without increase in stage until other disease process resolves (e.g., if TTP is diagnosed in the presence of stage 2 liver GVHD, the liver GVHD stage 2 is carried forward despite rising bilirubin level until TTP is resolved. If there is no liver GVHD – stage 0 – and new onset TTP, the stage 0 is carried forward until TTP is resolved).
Skin and/or GI GVHD worsening	**Liver GVHD is staged according to the Glucksberg criteria. The elevated bill is attributed to GVHD alone**. Thus, when skin or GI GVHD is worsening, there is no downgrading of liver GVHD staging for other causes of hyperbilirubinemia. (e.g., if TTP is diagnosed in the presence of stage 2 liver GVHD and worsening skin or GI GVHD, the liver is staged according to the actual bilirubin level even if some of the rise in bilirubin is attributed to TIP). Similarly, even if there is no liver GVHD at onset of a new process, (such as TPN cholestasis), but skin or GI GVHD worsen during that process, then liver GVHD is diagnosed and staged according to the height of the bilirubin. **There is one exception to this**: the diagnosis of TTP, with high LDH and **unconjugated** bilirubin precludes the diagnosis and staging of new liver GVHD in the absence of a confirmatory liver biopsy.

Table 3 Evaluating GI GVHD in the Absence of Biopsy Confirmation (See Table 4.0 below)

Establishing GI GVHD with new onset diarrhea and no skin or liver GVHD

No skin/liver GVHD Day 0 through engraftment	Assume no GI GVHD, unless proven by biopsy	
No skin/liver GVHD engraftment through Day 100	NO other etiology of diarrhea identified: **Stage as GI GVHD**	Any other etiology of diarrhea identified: **Do not stage as GI GVHD**

Establishing GI GVHD with pre-existing diarrhea and skin or liver GVHD

Skin and/or liver GVHD present	Worsening diarrhea (includes worsening just prior to onset of skin or liver GVHD) OR persistent diarrhea despite resolution of non-GVHD cause: **Stage as GI GVHD**	Improving diarrhea after the diagnosis of skin or liver GVHD (irrespective of treatment) OR persistent diarrhea without resolution of underlying non-GVHD cause: **Do not stage as GI GVHD**

Differentiating Acute GVHD, Chronic GVHD, and Overlap Syndrome:
There is often confusion differentiating acute from chronic GVHD, especially in the setting of reduced intensity transplants, DLI and new prophylactic treatments. The NIH Working Group recently published new classifications for GVHD:

Table 4 Acute GVHD, Chronic GVHD, and Overlap Syndrome

Category	Time of Symptoms after HCT or DLI	Presence of Acute GVHD features	Presence of Chronic GVHD features
Acute GVHD			
Classic acute GVHD	<100 d	Yes	No
Persistent, recurrent, or late-onset acute	>100 d	Yes	No
Chronic GVHD			
Classic chronic GVHD	No time limit	No	Yes
Overlap syndrome	No time limit	Yes	Yes

- Scoring of acute GVHD may need to occur past day 100. In particular, patients should continue to be scored for acute GVHD when classic acute GVHD symptoms (maculopapular rash, nausea, vomiting, anorexia, profuse diarrhea - particularly if bloody and ileus) persist past day 100 or if identical symptoms previously scored as acute GVHD resolve and then recur within 30 days during immunosuppression taper but past day 100.

- Those patients being scored as having acute GVHD should NOT have diagnostic or distinctive signs of chronic GVHD.

- **Patients with both acute and chronic symptoms should be diagnosed as having Overlap Syndrome and scored according to their chronic GVHD score**.

Further Explanation of Criteria presented in Tables 2 and 3

1.0 Assessment of Skin GVHD

1.1 Presence or Absence of Skin GVHD: Skin GVHD will be considered present if a rash characteristic of acute GVHD develops after allogeneic marrow transplantation involving more than 25% of the body surface not clearly attributable to causes such as drug administration or infection. The extent of the body surface area involved can be estimated by the "Rule of Nines". In estimating the extent of skin GVHD, the area involved is calculated for individual anatomic areas, such as the arm or leg, and then the total is derived from a simple summation. Areas that are non-blanching should not be considered involved regardless of the overlying color of the rash (red, brown, etc.). Limited distribution erythema (with the exception of palms and soles) in the absence of associated rash elsewhere on the body will not be considered GVHD.

2.0 Assessment of Liver GVHD

2.1 Assessing for the Presence or Absence of Liver GVHD

A. Hyperbilirubinemia (total bilirubin ≥ 2.0 mg/dL) in the **absence** of other signs of acute GVHD in the skin or GI tract:

   i) Day 0-35: If hyperbilirubinemia alone is present with no other signs of acute GVHD in other organ systems, acute GVHD will not be diagnosed based solely on laboratory abnormalities.

      Acute GVHD will be diagnosed if findings on histopathology studies of liver from a biopsy or autopsy are confirmatory.

   ii) Day 35-100: If hyperbilirubinemia (must be conjugated bilirubin) is not improving or is exacerbated (especially if serum alkaline phosphatase is increased), in the absence of acute GVHD in other organ systems, no other etiologies are identified, and does not improve with discontinuation of hepatotoxic drugs, acute GVHD will be diagnosed. However, it is distinctly unusual to develop ascites or a coagulopathy in the early stages of acute GVHD of the liver alone. In the absence of histopathology studies of liver from a biopsy or autopsy specimen, ascites or a coagulopathy secondary to liver dysfunction will be considered to indicate the presence of another disease process (e.g., veno-occlusive disease). Recommended non-invasive studies to define an etiology for hyperbilirubinemia are:

      a. Imaging of liver (CT or ultrasound)
      b. Hepatitis screen (only if ALT is elevated)
      c. PT
      d. Blood cultures
      e. Review of medication list for potentially hepatotoxic drugs
      f. Review of risk factors for viral liver infection (HSV, CMV, VZV, adenovirus, EBV, HBV, and HCV)
      g. Hemolysis screen

B. Pre-existing hyperbilirubinemia clearly attributed to an etiology other than acute GVHD in the presence of signs of acute GVHD in other organ systems.

   i) If pre-existing non-GVHD liver disease (documented clinically, by lab assessment, or by imaging studies) is stable or improving at the onset of signs of acute GVHD in other organs, then acute GVHD of the liver will not be considered to be present unless proven by liver biopsy or autopsy.

   ii) If hyperbilirubinemia worsens several days before or at the time of onset of signs of acute GVHD in other organ systems, GVHD will be considered to be present unless histopathology studies of liver are available and negative on a biopsy during that time interval or autopsy results exclude GVHD.

iii) If hyperbilirubinemia persists and is not improving after resolution of a pre-existing non-GVHD liver disease process (e.g., localized infection of liver, systemic sepsis, biliary tract obstruction) when signs of acute GVHD are present in other organ systems or no other intervening cause has been diagnosed, then acute GVHD will be considered to be present in the absence of a new, clearly identifiable cause of non-GVHD liver disease or unless a liver biopsy or autopsy specimen is negative.

C. Prior acute GVHD in liver with new onset of a disease process that exacerbates pre-existing or recently resolved hyperbilirubinemia:

i) If an etiology other than acute GVHD is clearly identified as causing or exacerbating hyperbilirubinemia and acute liver GVHD has been diagnosed and has been stable, improving, or resolved, then the liver will not be restaged for acute GVHD until the resolution or stabilizing of the concurrent disease process (i.e., the liver stage prior to the onset of the new disease process will be carried forward until the new disease process resolves). Example: Acute GVHD of the liver and gut is diagnosed on day 20. Treatment of acute GVHD results in falling bilirubin levels to liver stage 1. Sepsis or TTP develops with transient worsening of the hyperbilirubinemia. The liver stage is not increased, despite a higher bilirubin level, because the cause of worsening hyperbilirubinemia is attributed to sepsis or TTP.

ii) If an etiology other than acute GVHD is clearly identified as causing or exacerbating hyperbilirubinemia in the presence of already worsening acute liver GVHD **or** GVHD of the skin or GI tract is simultaneously worsening, then the liver GVHD will be staged according to the actual bilirubin level, even though another cause of hyperbilirubinemia is present.

3.0 Assessment of GVHD of the Gastrointestinal Tract

3.1 Assessing for the Presence or Absence of GVHD of the Gastrointestinal Tract

A. Diarrhea (≥500 mL/day in adults or > 10 mL/kg in pediatric patients) in the absence of other signs of acute GVHD in other organ systems

i) Day 0-engraftment: If diarrhea alone is present without other signs of acute GVHD in other organ systems, acute GVHD will not be considered present. Diarrhea will be attributed to acute GVHD if histopathology studies of gastrointestinal tract from a biopsy or autopsy are diagnostic.

ii) Engraftment-day 100: If diarrhea persists and is not improving, is exacerbated, or develops de novo in the absence of acute GVHD in other organ systems, histopathology studies of gut biopsies or from autopsy specimens are not available, and no other etiologies are clearly identified, acute GVHD will be considered to be the cause. A stool specimen should be examined to rule out infectious causes (e.g., rotavirus, adenovirus, and C. difficile toxin). It is recommended, if at all possible, that biopsies be obtained for diagnostic purposes.

B. Pre-existing diarrhea clearly attributed to an etiology other than acute GVHD in the presence of signs of acute GVHD in other organ systems:

   i) If pre-existing diarrhea caused by a process other than GVHD has been documented clinically or by lab assessment and is stable or improving at the onset of signs of acute GVHD in the skin or liver, then acute GVHD of the intestine will not be considered to be present in the absence of biopsy confirmation or autopsy report.

   ii) If diarrhea or gastrointestinal symptoms are already present, but worsen significantly at the time of onset of signs of acute GVHD in the skin or liver, GVHD will be considered present, unless biopsy or autopsy are negative.

   iii) If diarrhea persists after resolution of a pre-existing disease process with signs of acute GVHD present in other organ systems, GVHD will be considered present, unless biopsy or autopsy are negative.

C. Prior or present acute GVHD in other organ systems with new onset of diarrhea:

   If diarrhea is clearly attributable to an etiology other than acute GVHD (e.g., infection) and a history of acute GVHD exists or acute GVHD is present in other organ systems and is stable, then the gastrointestinal tract will not be evaluable for acute GVHD until the resolution or stabilizing of the other disease process (e.g., infection) in the absence of biopsy or autopsy confirmation.

D. Persistent anorexia, nausea or vomiting in the absence of signs of acute GVHD in other organ systems:

   Persistent anorexia, nausea or vomiting in the absence of other known causes of these symptoms will be considered stage I acute GVHD if confirmed by endoscopic biopsy.

   If a biopsy is not possible (e.g. secondary to thrombocytopenia) but the clinical findings are compatible with acute GVHD, then the patient will be treated and recorded as having acute GVHD.

3.2 Staging of the Gastrointestinal Tract for the Severity of Acute GVHD

The severity of gastrointestinal tract GVHD will be staged according to modified Glucksberg criteria. To minimize errors caused by large day-to-day variation, diarrhea volume is measured as an average over 3 days and reported as the volume in milliliters per day. When urinary mixing is noted the stool volume will be considered half of the total volume unless nursing staff is able to give a better estimate from direct observation. Abdominal cramps are considered significant for staging if the severity results in a clinical intervention (e.g. analgesia, fasting, etc.). Blood in the stools is considered significant if the blood is visible or hematochezia/melena is present and not clearly attributed to a cause other than GVHD (e.g., epistaxis/hemorrhoids).

APPENDIX C

CRITERIA FOR GRADING CHRONIC GVHD GRADE

	Score 0	Score 1	Score 2	Score 3
Performance Score: _____ **KPS ECOG LPS**	☐ Asymptomatic and fully active (ECOG 0: KPS or LPS 100%)	☐ Symptomatic, fully ambulatory, restricted only in physically strenuous activity (ECOG 1, KPS or LPS 80-90%)	☐Symtomatic, ambulatory, capable of self-care, >50% of waking hours out of bed (ECOG 2, KPS or LPS 60-70%)	☐Symptomatic limited self-care, >50% of waking hours in bed (ECOG 3-4, KPS or LPS <60%)
SKIN *Clinical features:* ☐Maculopapular rash ☐ Lichen planus-like features ☐ Papuloquamous lesions or ichthyosis ☐Hyperpigmentation ☐Hypopigmentaion ☐Keratosis pilaris ☐Erythema ☐Erythroderma ☐Poikiloderma ☐Sclerotic features ☐Pruritus ☐Hair involvement ☐Nail involvement **% BSA Involved** _____	☐ No symptoms	☐<18% BSA with disease signs but **NO**sclerotic features	☐ 19-50% BSA **OR** involvement with superficial sclerotic features "not hidebound" (able to pinch)	☐>50% BSA **OR**deep sclerotic features "hidebound" (unable to pinch) **OR** impaired mobility, ulceration or severe pruritus
MOUTH	☐No symptoms	☐ Mild symptoms with disease signs but **NOT**limiting oral intake significantly	☐ Moderate symptoms with disease signs **WITH**partial limitation of oral intake	☐ Severe symptoms with disease signs on examination **WITH** major limitation of oral intake
EYES ☐**Mean tear test (mm):** ☐**>10** ☐**6-10** ☐**≥5** ☐**Not done**	☐No symptoms	☐ Mild dry eye symptoms not affecting ADL (requiring eyedrops) ≤3 x per day) **OR** asymptomatic signs of keratoconjunctivitissiccca	☐ Moderate dry eye symptoms partially affecting ADL (requiring drops≥3x per day or punctual plugs), **WITHOUT**vision impairment	☐ Severe dry eye symptoms significantly affecting ADL (special eyeware to relieve pain) **OR**unable to work because of ocular symptoms**OR** loss of vision cause by keratoconjunctivitissicca
GI Tract	☐No symptoms	☐ Symptoms such as dysphagia, anorexia, nausea, vomiting, abdominal pain or diarrhea without significant weight loss (<5%)	☐ Symptoms associated with mild to moderate weight loss) 5-15%)	☐ Symptoms associated with significant weight loss > 15%, requires nutritional supplement for most calorie needs **OR**esophageal dilation
LIVER	☐ Normal LFT	☐ Elevated Bilirubin, AP*, AST or ALT <2 x ULN	☐ Bilirubin >3 mg/dl or Bilirubin, enzymes 2-3 x ULN	☐ Bilirubin or enzymes > 5 x ULN

APPENDIX C (continued)

CRITERIA FOR GRADING CHRONIC GVHD GRADE

	SCORE 0	SCORE 1	SCORE 2	SCORE 3
Lungs† FEV1_____ DLCO_____	☐ No symptoms ☐ FEV1 >80% **OR** LFS=2	☐ Mild symptoms (shortness of breath after climbing one flight of steps) ☐ FEV1 60-79% **OR** LFS 3-5	☐ Moderate symptoms (shortness of breath after walking on flat ground) ☐ FEV1 40-59% **OR** LFS 6-9	Severe symptoms (shortness of breath at rest; requiring 0_2 ☐ FEV1 ≥39% **OR** LFS 10-12
JOINTS AND FASCIA	☐ No symptoms	☐ Mild tightness of arms or legs, normal or mild decreased range of motion (ROM) **AND** not affecting ADL	☐ Tightness of arms or legs **OR** joint contractures, erythema thought due to fasciitis, moderate decrease ROM **AND** mild to moderate limitation of ADL	☐Contractures WITH significant decrease or ROM AND significant limitation of ADL (unable to tie shoes, button shirts, dress self etc.)
GENITAL TRACT	☐ No symptoms	☐ Symptomatic with mild signs on exam **AND** no effect on coitus and minimal discomfort with gynecologic exam	☐ Symptomatic with moderate signs on exam **AND** with mild dyspareunia or discomfort with gynecologic exam	☐ Symptomatic **WITH** advance signs (stricture, labial agglutination or severe ulcerations **AND** severe pain with coitus or inability it insert vaginal speculum

Other indicators, clinical manifestations or complications related to chronic GVHD (check all that apply and assign a score to its severity (0-3) based on its functional impact where applicable (none = 0, mild = 1, moderate =2, severe = 3.

Esophageal stricture or web ____ **Pericarial Effusion ____** **Pleural Effusion(s) ____**

Ascites (serositis) ____ **Nephrotic syndrome ____** **Peripheral Neuropathy ____**

Myasthenia Gravis ____ **Cardiomyopathy ____** **Eosinophilia > 500/µl ____**

Polymyositis ____ Cardiac conduction defects ____ Coronary artery involvement ____

Platelets <100,000/µl ____ Progressive Onset ____

Other: Specify: _____

Organ scoring of chronic GVHD.

***** AP may be elevated in growing children, and not reflective of liver dysfunction.

† Pulmonary scoring should be performed using both the symptom and pulmonary function testing (PFT) scale whenever possible. When discrepancy exists between pulmonary symptoms or PFT scores, the higher value should be used for final scoring. Scoring using the Lung Function Score (LFS**) i**s preferred, but if DLCO is not available, grading using FEV1 should be used. The LFS is a global assessment of lung function after the diagnosis of bronchiolitis obliterans has already been established. The percent predicted FEV1 and DLCO (adjusted for hematocrit but not alveolar volume) should be converted to a numeric score as follows: >80% = 1; 70-79% = 2; 60-69% = 3; 50-59% = 4; 40-49% = 5; <40% = 6. The LFS = FEV1 score + DLCO score, with a possible range of 2-12. GVHD indicates graft versus host disease, ECOG, Eastern Cooperative Oncology Group, KPS, Karnofsky Performance Status; LPS, Lansky Performance Status; BSA, body surface area; ADL, activities of daily living; LFTs, liver function tests; AP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ULN, upper limit of normal.

APPENDIX C (continued)

CRITERIA FOR GRADING CHRONIC GVHD GRADE

GLOBAL GRADING OF CHRONIC GVHD[84]

Final Grade	Number of Organs/Sites*	Maximum Organ Score**	Lung Score
Mild	1 - 2	1	0
Moderate	3 or more	1	1
	At least 1	2	
Severe	At least 1	3	2 - 3

*Determined by adding the total number of organs receiving score > 0 using Figure 1, Appendix B.

**Defined as the maximum score given to any organ system amongst all organs scored using Figure 1, Appendix B.

APPENDIX D

CRITERIA FOR ADVERSE EVENT (AE) EVALUATION AND REPORTING

The St. Jude Department of BMTCT Clinical Research Office standard operating procedure for the documenting and reporting of adverse (SOP 10 Documenting and Reporting of Adverse Events) will provide guidance on the evaluation, collection and reporting of adverse events for this clinical trial. The current version of this document, as well as ongoing updates, can be located at the following website: http://home.stjude.org/bmt/Pages/policies-research.aspx

APPENDIX E

Recommended testing and evaluation schedule

STANDARD OF CARE STUDIES	SAMPLE	VOLUME	PRE	MONTH 1	MONTH 2	MONTH 3	MONTH 6	MONTH 12
Pregnancy test	PB	2 ml	X	As clinically indicated				
Physical exam	N/A	N/A	X	Weekly			X	X
CBC with diff.	PB	0.5 - 2 ml	X	Daily until engrafted, then weekly			X	X
Chemistry	PB	0.25- 2 ml	X	Weekly			X	X
Viral surveillance (BMTPCR)	PB	4 ml	X	Weekly			As clinically indicated	
Chimerism	PB	1-2 ml		Weekly			Monthly	
	BM	2 ml		X		X		X
Disease Status Evaluation	BM	N/A	X	X		X		X
MRD	PB	5 ml	X[&]	X[&]		X[&]		X[&]
	BM	0.2-5 ml	X	X		X		X
Lymphocyte Subset Study	PB	2.5-4 ml	X	Monthly				
Quantitative Immunoglobulins	PB	2.0 ml	X			Every 3 months after off IVIG for minimum of one month		

- The information derived from or noted on the physical examinations, standard tests, and other assessments that comprise standard of care for recipients are not required to be transcribed onto case report forms and/or entered into the database. In reference to section 6.1 Evaluations, the above-indicated follow-up regimen for these evaluations is guided by the SOPs of the Department of BMTCT, for recipients of allogeneic stem cell transplantation. As these evaluations are considered standard clinical care (non-research), variations in frequency (more or less frequent) of these evaluations can occur due to the participant's current clinical condition and will not be noted as protocol deviations.
- Disease status evaluations/BM testing results obtained prior to enrollment may be used for the baseline/pre-infusion assessments.
- [&] MRD PB required for T-Cell ALL only
- Lymphocyte subset studies may be omitted without variance when the absolute lymphocyte count (ALC) is zero.
- In the event of graft failure/rejection, the post failure/rejection time period BM, chimerism and several of the applicable immune studies will be held, as these blood/marrow tests would not be clinically indicated.

APPENDIX E (continued)

Immune reconstitution testing and evaluation schedule for RECIPIENT

RESEARCH STUDIES	SAMPLE	VOL.	PRE	MONTH 1	MONTH 2	MONTH 3	MONTH 6	MONTH 12	LABORATORY
LYMPHOCYTE PHENOTYPES RESEARCH	PB	17.0 ml	X	X	X	X	X	X	LEUNG LAB
VBETA/TREC RESEARCH	PB	17.0 ml	X			X	X	X	LEUNG LAB
HOST-DONOR	UCB	-	X						DALLAS LAB
	PB	7.5 ml	X	X	X	X	X	X	
	HAPLO*	17.0ml	X						
IR-PHENOTYPE	UCB	-	X						DALLAS LAB
	PB	5 ml	X	X	X	X	X	X	
T-FUNCTION	SKIN	BIOPSY	X						DALLAS LAB
	PB	7.5 ml	X	X	X	X	X	X	
	PB – VIRAL	7.5 ml		WEEKLY					
	BM	1ml	X	X		X			

- VBETA/TREC Research and Lymphocyte Phenotypes Research results will be maintained in a secured database in the Leung laboratory.
- HOST-DONOR, IR-PHENOTYPE, T-FUNCTION testing results will be maintained in a secured database in the Dallas laboratory database. Donor sample from haploidentical donor is optional and details provides in next table (optional donor studies)
- The pre-transplant Skin Biopsy & Bone Marrow for T-FUNCTION are optional additional tests that may be omitted at the discretion of the PI. Skin Biopsy may be performed during sedation during procedures (i.e. radiation) or at Month 1 without a protocol variation.
- Weekly VIRAL T-FUNCTION studies will be collected Monday –Thursday ONLY (as participant is available), preferred days being Monday & Wednesday.
- For RESEARCH studies, the posted volumes are the minimum volumes required to perform the respective protocol evaluations.

APPENDIX E (continued)

Research testing for HAPLOIDENTICAL DONOR

Prior to initial stem cell collection procedure:

OPTIONAL research immune studies testing schedule

Evaluation	*Volume Requirement*
Flow cytometry enumeration	Lymphocyte Subset Study = 4 mL
Thymic output and T cell repertoire	VBETA/TREC Research = 17 mL
T cell and NK cell number and function	Lymphocyte Phenotypes Research = 17 mL
Donor Immune Function in Host-Donor Interaction	HOST-Donor = 17 mL

- All donor research testing to be collected prior to stem cell collection – preferably prior to growth factor administration. These optional research tests may be collected at separate times.

APPENDIX E (continued)

Research Study Evaluation Target Windows

Several laboratory tests can only be processed on weekdays; therefore, if the scheduled evaluation falls on a weekend, or during a holiday period, an adjustment in the follow-up visit is expected and would not be noted as a protocol variation. Additionally, in order to accommodate such logistical constraints, evaluation/collection dates of all protocol assessments (required and optional research), may be performed within a reasonable window of the intended date following the guidelines provided in the table below:

If the Planned Evaluation Time Point is:	**Window**
Weekly	± 3 Days
Month 1	Week 2 to Week 6
Month 2	Week 7 to Week 11
Month 3	Week 12 to Month 4
Month 6	Month 5 to Month 7
Month 9	Month 8 to Month 10
Month 12	Month 10 to Month 14

APPENDIX F

The St. Jude Department of BMTCT Clinical SOPs for standard of care for all allogeneic stem cell infusion recipients and stem cell donors will provide guidance on the evaluation, ongoing clinical care and follow up for this clinical trial. The current versions of these SOPs, as well as ongoing updates, of these documents can be located at the following website: http://home.web.stjude.org /bone_marrow/clinicalHome.shtml.