

St. Jude Children's Research Hospital

**HAPCORD – NCT02199041**

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Activated:

COMBINED HAPLOIDENTICAL AND UMBILICAL CORD BLOOD  
TRANSPLANTATION IN PATIENTS WITH HEMATOLOGIC MALIGNANCIES USING A  
REDUCED-INTENSITY PREPARATIVE REGIMEN

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## COMMON ABBREVIATIONS

Absolute neutrophil count (ANC)	International Cord Blood Transplant Registry (ICBTR)
Acute lymphocytic leukemia (ALL)	Juvenile Myelomonocytic Leukemia (JMML)
Acute myeloid leukemia (AML)	Killer-immunoglobulin receptors (KIR)
Adverse event (AE)	Matched related/sibling donor (MSD)
Alanine aminotransferase (ALT)	Matched unrelated donor (MURD)
American Association of Blood Banks (AABB)	Minimal Residual Disease (MRD)
Aspartate aminotransferase (AST)	Mycophenolate mofetil (MMF)
Body surface area (BSA)	Myeloid Dysplastic Syndrome (MDS)
Bone marrow (BM)	National Cancer Institute (NCI)
Bone marrow transplantation (BMT),	National Marrow Donor Program (NMDP)
Bone Marrow Transplantation Follow Up (BMTFU)	Natural killer (NK)
Center for International Blood and Marrow Transplant Research (CIBMTR)	New York Blood Center (NYBC)
Central Nervous System (CNS)	Overall survival (OS)
Central Protocol and Data Monitoring Office (CPDMO)	Pharmacokinetics (PK)
Chronic Myeloid Leukemia (CML)	Polymerase chain reaction (PCR)
Clinical Data Update System (CDUS)	Protected health information (PHI)
Common toxicity criteria (CTC)	Pulmonary Function Testing (PFT)
Complete Remission (CR)	Reduced Intensity Conditioning (RIC)
Cord Blood Transplantation (COBLT)	Shortening Fraction (SF)
Creatinine Clearance (CrCL)	Standard operating procedures (SOP)
Cyclosporine (CSA)	T cell receptor excision circle (TREC)
Cytomegalovirus (CMV)	Total body irradiation (TBI)
Dimethyl sulfoxide (DMSO)	Total nucleated cell (TNC)
Disease-free survival (DFS)	Total Research and Knowledge System (TRACKS)
Epstein-Barr virus transformed lymphoblastoid cell lines (EBV-LCL)	Transplant related mortality (TRM)
Event-free survival (EFS)	Umbilical cord blood (UCB)
Forced vital capacity (FVC)	Umbilical cord blood transplantation (UCBT)
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)	

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## STUDY SUMMARY

<b>HAPCORD:</b>
<b>Principal Investigator:</b> Mari Hashitate Dallas, MD
<b>IND/IDE:</b> CliniMACS CD34 <sup>+</sup> Enrichment
<p><b>Brief Overview:</b> 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 an combined haploidentical and umbilical cord blood transplantation (UCBT) using a reduced intensity conditioning (RIC) preparative regimen. Patients who have a suitable KIR ligand mismatched haploidentical donor will be preferentially enrolled in available haploidentical protocol (i.e. HAPNK1). Those with a suitable HLA matched sibling or unrelated donor identified may be eligible for participation if the donor is not available in the necessary time. Participants must be <math>\leq 21</math> years old and with sufficient multi-organ function as specified in protocol.</p> <p>The assessments and follow-up evaluations noted in the protocol follow the St. Jude standard operating procedures (SOP) for all recipients of allogeneic HCT.</p>
<b>Intervention:</b> Combined haploidentical graft with an unrelated umbilical cord blood graft using a reduced intensity preparative regimen.
<p><b>Brief Outline of Treatment Plan:</b> The preparative regimen includes total lymphoid irradiation (TLI) (8 Gy), fludarabine (150 mg/m<sup>2</sup>), cyclophosphamide (60mg/kg), melphalan (140 mg/m<sup>2</sup>) 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<sup>2</sup> 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 infused on day 0 and day +1. Cord Blood infusion will occur on day +2 and G-CSF will start on day +3.</p>
<p><b>Objectives:</b></p> <p>Primary objective</p> <ul style="list-style-type: none"><li>• Estimate the incidence of engraftment by day +42 post-transplant for participants with high-risk hematologic malignancies undergoing a reduced intensity conditioning (RIC) hematopoietic cell transplantation (HCT) using a haploidentical donor combined with a unrelated umbilical cord blood (UCB) donor.</li></ul> <p>Secondary objectives:</p> <ul style="list-style-type: none"><li>• Estimate the incidence of malignant relapse, event-free survival, and overall survival at one-year post-transplantation.</li><li>• Estimate the incidence and severity of acute and chronic graft versus host disease (GVHD).</li><li>• Estimate the incidence of transplant related mortality (TRM) and transplant related morbidity in the first 100 days after transplantation.</li></ul>

### 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 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.<sup>1-4</sup> 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.<sup>3</sup> 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.<sup>5,6</sup> A pilot study in adults that combined haploidentical and an unrelated UCBT using a RIC resulted in a rapid engraftment with minimal GVHD and durable remission.<sup>7</sup> Here, we propose to investigate the outcomes of pediatric patient with high-risk malignancies undergoing a combined haploidentical and UCBT using a RIC regimen at St. Jude Children's Research Hospital. The primary objective of the study is to estimate the rate of engraftment by day +42 post-transplant in patients undergoing a combined haploidentical and UCBT using a reduced intensity preparative regimen. Secondary aims will assess 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 3. 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 Prezpiorik 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  $\geq 4$  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 only if the donor is not available in the necessary time. 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.
  - 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 after failure of prior autologous HCT, or unable to mobilize stem cells for autologous HCT.
  - 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  $\geq 40\%$  or cardiac shortening fraction  $\geq 25\%$ .
  - Creatinine clearance or GFR  $\geq 50$  ml/min/1.73m<sup>2</sup>.
  - Forced vital capacity (FVC)  $\geq 50\%$  of predicted value or pulse oximetry  $\geq 92\%$  on room air.
  - Karnofsky ( $\geq 16$  years) or Lansky ( $<16$  years) performance score  $\geq 50$
  - Bilirubin  $\leq 3$  times the upper limit of normal for age.
  - Alanine aminotransferase (ALT)  $\leq 5$  times the upper limit of normal for age.
  - Aspartate aminotransferase (AST)  $\leq 5$  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 (for HAPNK1) 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 Cancer Center's 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

### 1.1 Primary objective

- 1.1.1 To estimate the incidence of engraftment by day +42 post-transplant for participants with high-risk hematologic malignancies undergoing a reduced intensity conditioning (RIC) hematopoietic cell transplantation (HCT) using a haploidentical donor combined with a unrelated umbilical cord blood (UCB) donor.

### 1.2 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 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.
- 1.3.4 Characterize the recovery of Gamma Delta T cells after HCT, including T cell receptor analysis, phenotyping and functional analysis.
- 1.3.3. 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.<sup>8, 9</sup> Studies have demonstrated that HCT is a potentially curative therapy for patients with CML, AML, ALL and MDS.<sup>10-13</sup> Furthermore, patients with non-Hodgkin or Hodgkin's lymphoma who recur after an autologous HCT may be successfully treated with an allogeneic HCT.<sup>14, 15</sup>

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.<sup>1-4</sup> 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.<sup>3</sup> Recent comparative studies report no significant difference in OS in patients undergoing UCBT compared to MURD HCT.<sup>16-20</sup> 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 recipients of UCB is associated with increase incidences of TRM when compared to recipients of MSD or MURD.<sup>5, 6</sup> A pilot study in adults that combined haploidentical and an unrelated UCBT using a RIC resulted in a rapid engraftment with minimal GVHD and durable remission.<sup>7</sup> St. Jude has gained considerable expertise with haploidentical HCT in children <sup>21-28</sup> and we propose to investigate the outcomes of pediatric patient with high-risk malignancies undergoing a combined haploidentical and UCBT using a RIC 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).<sup>29</sup> 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.<sup>30</sup>

The first successful unrelated UCBT in a pediatric patient occurred at Duke University in 1993.<sup>31, 32</sup> In 1996, Kurtzberg et al and Wagner et al simultaneously published the unrelated UCBT experiences at Duke and Minnesota, respectively.<sup>32, 33</sup> 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.<sup>34</sup>

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.<sup>14-18, 25</sup> Thus, UCBT should be considered as a viable options for patients with high-risk malignancies.<sup>16-20, 35 7-11, 25</sup>

## 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.<sup>36-38</sup> Numerous clinical studies have shown that the total nucleated cell (TNC) dose and the CD34<sup>+</sup> 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.<sup>3, 37, 39, 40</sup> 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.<sup>36, 41</sup> 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 engraftment.<sup>38</sup> Thus, the collective data point to improved outcome of UCBT for patients receiving a larger number of CD34<sup>+</sup> cells. Delayed 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.<sup>42</sup> 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.<sup>34, 43, 44</sup>

## 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 engraftment was achieved more rapidly and in a higher proportion of patients than the previous cohort who received only a single UCB unit.<sup>45</sup> 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.<sup>46, 47, 48</sup> Currently, it is unclear whether double UCBT offers any benefit other than extending the application of UCBT by achieving the cell dose threshold.<sup>49</sup> Until data clearly demonstrate a survival advantage, double UCBT is currently only recommended for those patients who do not have an adequate single unit.<sup>50</sup> 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.<sup>48, 50, 51</sup> Despite adequate cell dose, patients undergoing double UCBT continue to have delayed engraftment compared to MSD and MURD HCT.

#### 2.2.4 UCBT Results

*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 engraftment after single UCBT ranges from 65-92%.<sup>31, 33, 37, 38, 40, 41, 52-60</sup> Studies have consistently demonstrated the rate and incidence of 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.<sup>38</sup> 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. 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%.<sup>37, 38, 57, 59, 60</sup>

*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%.<sup>16, 20, 40, 45, 47, 51, 61-65</sup> 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.<sup>16</sup> These and other studies support that UCB should be considered an acceptable source of HSC grafts in the absence of an HLA-matched donor.<sup>16, 17, 20, 37, 38, 40, 45, 47, 51, 57, 59-66</sup>

### 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.<sup>67-71</sup> Due to the high potential for GVHD with the degree of HLA mismatch seen in haploidentical HCT, most haploidentical grafts are extensively T-cell depleted (TCD) prior to infusion.<sup>72</sup> Significant progress was achieved when high cells dose of CD34<sup>+</sup> cells were infused after high-

intensity conditioning.<sup>70</sup> It has been well established that prompt engraftment with rapid immune reconstitution can be achieved with CD34<sup>+</sup> enriched haploidentical cells, purified by positive (CD34<sup>+</sup>) and negative (CD3<sup>-</sup> lectin agglutination) selection.<sup>73,74</sup>

Building on the initial studies above, St. Jude has gained considerable expertise with haploidentical HCT in children.<sup>21-26, 28, 75</sup> 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.<sup>27</sup>

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.<sup>76-83</sup> 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.<sup>24</sup> Thus, patients with high risk hematologic malignancies who 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  $3 \times 10^4$  T cells/kg can cause GVHD.<sup>84</sup> 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<sup>+</sup> progenitors. St. Jude has had success using either method of TCD in haploidentical donor transplantation.

In this study, CD34<sup>+</sup> 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.<sup>85, 86</sup> 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.<sup>87, 88</sup> 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.<sup>21, 89</sup> This results in an increased rate of opportunistic infections. Indeed, viral infections are the most common cause of death of children receiving haploidentical transplants.<sup>90</sup> The majority of these infections occur within the first 6 months following transplantation, when T cell immunity is the lowest.<sup>91</sup> Reconstitution of immunity can be partially restored by therapeutic infusions of donor cytotoxic lymphocytes.<sup>87, 92-94</sup> 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.<sup>95</sup>

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.<sup>96</sup> 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.<sup>97</sup> Furthermore, memory T cells are capable of more rapidly responding to future infectious challenges.<sup>98</sup>

The isoform of the leukocyte common antigen, CD45RA, selectively identifies naïve T cells. Human studies have shown that sorted donor CD45RA<sup>+</sup> naïve T cells are far more alloreactive than all memory subsets tested.<sup>99</sup> In animal models, naïve T cells, are potent inducers of GVHD.<sup>95</sup> Moreover, in similar models, lymphocyte infusions specifically depleted of CD45RA<sup>+</sup> naïve T cells do not cause GVHD.<sup>100</sup> Conversely, CD45RA<sup>+</sup> memory T cells do not cause physical or histologic evidence of GVHD. Among CD4<sup>+</sup> T cells, the CD45RA<sup>+</sup> subset has equivalent “helper” functions as CD45RA<sup>+</sup> cells in the generation of alloreactive cytotoxic T cells.<sup>101</sup> Further, it is the CD45RA<sup>+</sup> 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<sup>+</sup> 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<sup>+</sup> cells (Data provided by Miltenyi). Furthermore, experiments were performed in the HAL to confirm and qualify the CD45RA<sup>+</sup> depletion procedures. HSC products were obtained from G-CSF mobilized normal donors by apheresis. The HPC, Apheresis products were depleted of CD45RA<sup>+</sup> 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<sup>+</sup> 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<sup>+</sup> cells is also included. We hypothesize combining a CD34<sup>+</sup> enriched product (HPC, Apheresis 1) along with a CD45RA<sup>+</sup> depleted product (HPC, Apheresis 2) will provide a HSC graft with good stem cell content (3-5 x 10<sup>6</sup> CD34<sup>+</sup> cells/kg) and a useful memory T cell fraction without additional GVHD risk to the recipients on this trial.

DEPLETION EXPERIMENT				
	Experiment 1		Experiment 2	
	Pre (10 <sup>6</sup> )	Post (10 <sup>6</sup> )	Pre (10 <sup>6</sup> )	Post (10 <sup>6</sup> )
TNC	29748	10270	26166	10981
CD34 <sup>+</sup>	636	311	693	395
CD3 <sup>+</sup>	12238	4555	6353	1985
CD3 <sup>+</sup> CD45RA <sup>+</sup>	7877	4	3520	2.2
CD3 <sup>+</sup> CD45RA <sup>+</sup> Log Depletion		3.28		3.21

## 2.4 Rationale for RIC Preparative Regimen

RIC regimens have the potential to improve the outcomes of allogeneic HCT by decreasing the acute TRM.<sup>102-104</sup> 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 engraftment and reduces GVHD.<sup>105</sup> 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.<sup>106, 107</sup> 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 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 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 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.<sup>108</sup> Despite most of the patient having haploidentical donors, 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.<sup>109</sup> TLI was given as a single dose at 7Gy. Nine out of 10 patients experienced rapid donor 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.<sup>32, 39, 110</sup> 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.<sup>32, 38, 39, 110</sup> 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 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 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.<sup>24, 25</sup> KIR mismatch is predictive of NK alloreactive effects in haploidentical HCT.<sup>24, 25, 78, 80</sup> Thus, patients who have a KIR mismatch haploidentical donor will be enrolled into a haploidentical HCT with NK infusion (HAPNK1). However, approximately one-third of all patients possess 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,  $p = .05$ ).<sup>111</sup> However, the Minnesota group found no advantage using KIR matching in UCBT.<sup>112</sup> Currently, the effect of KIR matching in UCBT is controversial and UCB selection based on KIR matching is not recommended.

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 engraftment after day +42. One patient engrafted at day 49 and another at day 51. The mean time to neutrophil engraftment is  $28 \pm 13$  days, (range; 12-51). Thus, despite optimizing HLA match and cell dose, engraftment continues to be significantly delayed in patients undergoing UCBT.

In order to abrogate the increased TRM and complication from graft failure and delayed 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, melphalan and ATG for high risk malignancies. The graft consisted of a single UCB with minimum cell dose of  $1.0 \times 10^7$  TNC/kg along with a TCD haploidentical donor graft. GVHD prophylaxis was with MMF and tacrolimus. All patients engrafted and the mean time to neutrophil engraftment was 11 days and platelet engraftment was 19 days. Chimerism analysis demonstrated that the predominant haploidentical graft early after HCT is replaced by the UCB graft by day 100. The risk for GVHD was not increased with the reported cumulative incidence of acute GVHD of 25% and chronic GVHD of 5%.

## 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.<sup>113</sup> 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.<sup>113-115</sup> However, other studies suggest that eradication of all acute leukemia cells may not be a prerequisite for cure.<sup>116, 117</sup>

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.<sup>118, 119</sup> 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.<sup>70, 120-122</sup> 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<sup>+</sup>, CD4<sup>+</sup>, CD8<sup>+</sup>, CD19<sup>+</sup> and CD56<sup>+</sup> 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.

Studies demonstrate that the rate of 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 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 engraftment in the BM and thymus.<sup>123, 124</sup> Data obtained from this study addresses a key question regarding the immunological mechanism for enhanced 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<sup>+</sup> T-cell response against the non-engrafted unit.<sup>125</sup>

### 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 ( $\geq 3$  of 6) family member donor.
- 3.1.4 Has a partially HLA-matched single UCB unit ( $\geq 4$  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 High risk ALL in CR1.  
Examples include, but not limited to t(9;22), 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 t(9;22), 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  $\geq 5\%$  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 after failure of prior autologous HCT, or unable to mobilize stem cells for autologous HCT.
  - 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)  $\geq 40\%$  or shortening fraction (SF)  $\geq 25\%$ .

- 3.1.6.2 Creatinine clearance (CrCL) or glomerular filtration rate (GFR)  $\geq 50$  ml/min/1.73m<sup>2</sup>.
  - 3.1.6.3 Forced vital capacity (FVC)  $\geq 50\%$  of predicted value or pulse oximetry (Pox)  $\geq 92\%$  on room air.
  - 3.1.6.4 Karnofsky or Lansky performance score  $\geq 50$  (See APPENDIX A).
  - 3.1.6.5 Bilirubin  $\leq 3$  times the upper limit of normal for age.
  - 3.1.6.6 Alanine aminotransferase (ALT)  $\leq 5x$  the upper limit of normal for age.
  - 3.1.6.7 Aspartate aminotransferase (AST)  $\leq 5x$  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 (for HAPNK1) 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 ( $\geq 3$  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 1271.

INCLUSION CRITERIA SUMMARY		
<b>Donor</b>	Haploidentical ( $\geq 3$ of 6) and single UCB ( $\geq 4$ of 6) HLA matched donor	
<b>Age</b>	$\leq 21$ years	
<b>Malignancy</b>	ALL	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 <u>OR</u> intolerance to tyrosine kinase inhibitor
	Hodgkins Lymphoma	CR2 or subsequent after failure of auto-HCT <u>OR</u> unable to mobilize HSC for auto-HCT
	Non-Hodgkin Lymphoma	CR2 or subsequent after failure of auto-HCT <u>OR</u> unable to mobilize for auto-HCT
	JMML	
	Refractory disease	ALL, AML, CML in blast crisis, Hodgkin or non-Hodgkin Lymphoma
<b>Pre-Evaluation</b>	Cardiac Function	LVEF $\geq 40\%$ or SF $\geq 25\%$
	Renal Function	CrCL or GFR $\geq 50$ ml/min/1.73m <sup>2</sup>
	Lung Function	FVC $\geq 50\%$ or Pox $\geq 92\%$ on room air
	Performance Score	Karnofsky or Lansky $\geq 50$
	Liver Function	Bilirubin $\leq 3$ x upper limit of normal for age ALT $\leq 5$ x upper limit of normal for age AST $\leq 5$ x upper limit of normal for age
EXCLUSION CRITERIA SUMMARY		
<b>Donor</b>	Has a suitable MSD, volunteer MURD, or KIR mismatched haploidentical donor (for HAPNK1) available in the necessary time for HSC donation	
<b>Malignancy</b>	Other active malignancy other than one being treated	
<b>Pre-Evaluation</b>	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

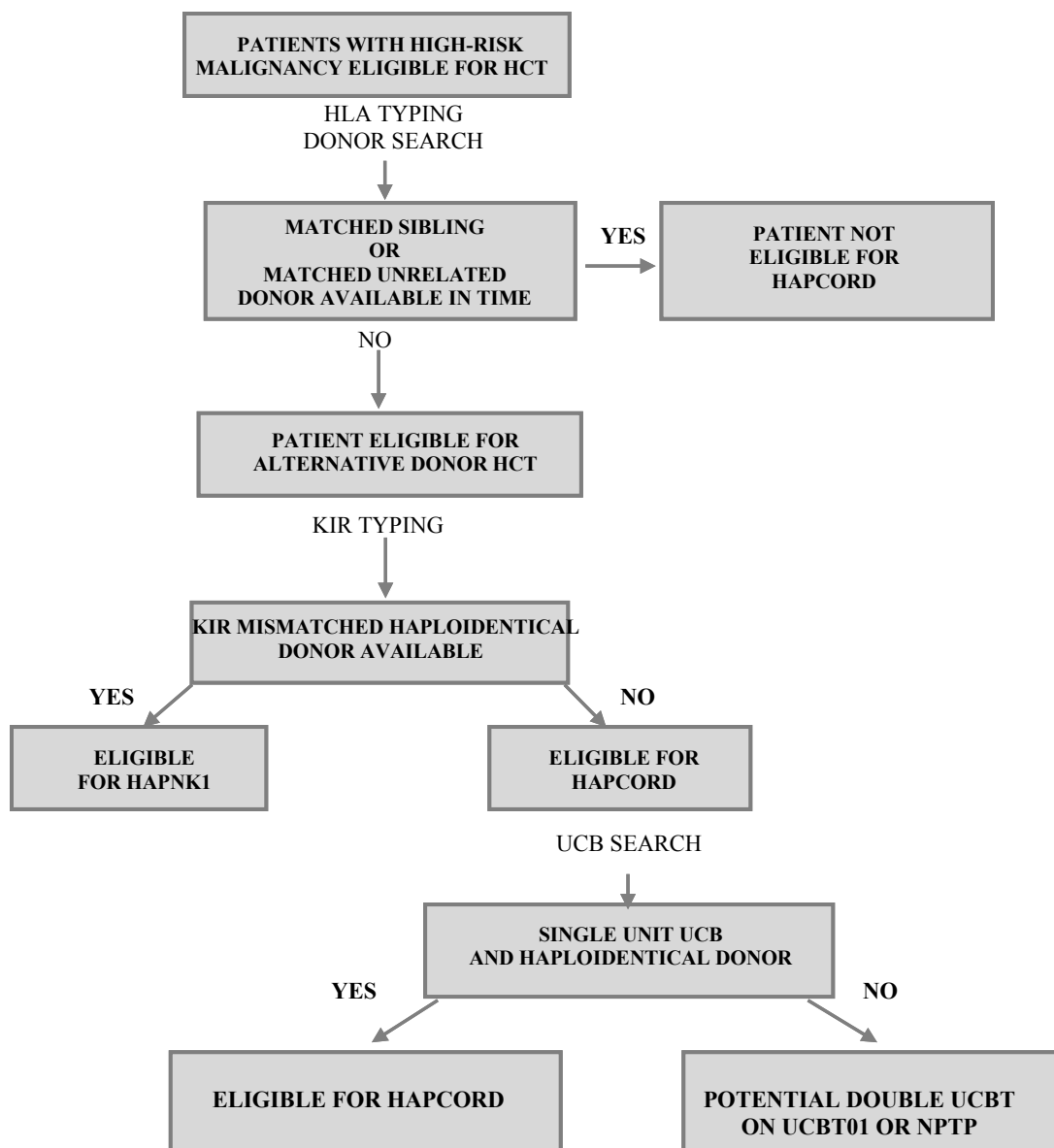
team will enter the eligibility checklist information into the Patient Protocol Manager (PPM) system. Eligibility will be reviewed, and a research participant-specific consent form and assent document (where applicable) will be generated. The complete signed consent/assent document form(s) must be faxed 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 haploidentical and UCBT using a RIC 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)*	2Gy per fraction x 1 fraction	3 of 4
	Fludarabine	30 mg/m <sup>2</sup> intravenous once daily	1 of 5
-7	Total Lymphoid Irradiation (TLI)*	2Gy per fraction x 1 fraction	4 of 4
	Fludarabine	30 mg/m <sup>2</sup> intravenous once daily	2 of 5
-6	Cyclophosphamide (with mesna)	60 mg/kg intravenous once daily	1 of 1
	Fludarabine	30 mg/m <sup>2</sup> intravenous once daily	3 of 5
-5	Fludarabine	30 mg/m <sup>2</sup> intravenous once daily	4 of 5
-4	Fludarabine	30 mg/m <sup>2</sup> intravenous once daily	5 of 5
-3	Thiotepa	5 mg/kg intravenous twice daily	1-2 of 2
-2	Melphalan	70 mg/m <sup>2</sup> intravenous once daily	1 of 2
	Tacrolimus	Maintain until at least 6 months	start
-1	Melphalan	70 mg/m <sup>2</sup> intravenous once daily	2 of 2
0	HPC A Infusion	CD34 <sup>+</sup> selected	HPC A1
	MMF	Maintain until at least 45 days	start
+1	HPC A Infusion	CD45RA depleted	HPC A2
+2	HPC C Infusion		HPC C
+3	G-CSF	5mcg/kg subcutaneous or intravenous daily until ANC >2000 for 3 consecutive days	

## 4.2.1 TLI

A total of 800 cGy of TLI will be administered 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<sup>2</sup>/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<sup>2</sup> total). Fludarabine dose may be reduced per PI if concerns of toxicity (ie CNS).

## 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.

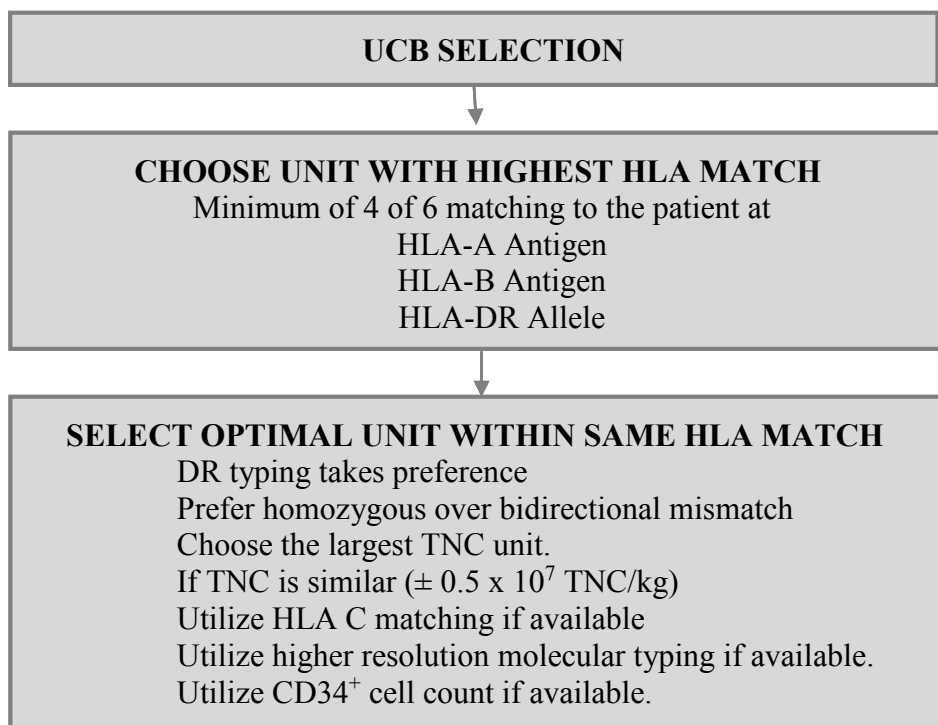
#### 4.2.4 Thiotepea

Thiotepea 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<sup>2</sup> once a day on day -2 and -1 for a total of 2 doses (140 mg/m<sup>2</sup> total).

### 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  $1.0 \times 10^7$  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  $\square$  1 matching, locus of mismatch and cell dose ( $\pm 0.5 \times 10^7$  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<sup>+</sup> 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  $< 1.0 \times 10^7$  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. 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  $3-5 \times 10^6$  CD34<sup>+</sup> 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 5 and 6. 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 be reduced if the donor's WBC is  $>75.0 \times 10^6$ /

\*\*Do not start apheresis if platelet count  $< 50,000/\text{mm}^3$  or hemoglobin of  $< 12.5 \text{ g/dL}$  pre-pheresis

The dose of G-CSF may require modification based on the complete blood counts (CBC). If the donor's white blood count (WBC) is  $>75.0 \times 10^6/\text{ml}$  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/](http://home.web.stjude.org/bone_marrow/)

The daily leukapheresed volumes for HPC, Apheresis collection is generally 3–4 total blood volumes based on  $\text{CD34}^+$  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  $2 \times 10^6 \text{ CD34}^+$  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 4°C. 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<sup>+</sup> cell and CD3<sup>+</sup> T cell content by flow cytometry. The processed HPC, Apheresis 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 <sup>+</sup> cells/kg	$\geq 2 \times 10^6$	$2 \times 10^6$	$50 \times 10^6$
CD3 <sup>+</sup> cells/kg	$\leq 0.5 \times 10^5$	$1 \times 10^3$	$1 \times 10^5$

Once the target dose is obtained for the CD34<sup>+</sup> enriched product, one additional day of apheresis will be performed. This HPC, Apheresis product will be processed for CD45RA<sup>+</sup> 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 4°C. 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<sup>+</sup> enriched product, the target CD34<sup>+</sup> dose will be  $3-5 \times 10^6/\text{kg}$ . There is no CD3<sup>+</sup> cell target dosing on the CD45RA<sup>+</sup> depleted product, however release criteria of the product will include at least a  $\geq 2.00 \log_{10}$  depletion of CD45RA<sup>+</sup> cells.

HPC Graft	Target Dose	Minimum Dose	Maximum Dose
CD34 <sup>+</sup> cells/kg	$3-5 \times 10^6$	$2 \times 10^6$	$50 \times 10^6$
CD45RA <sup>+</sup>	$\geq 2.0 \log_{10}$ depletion (CD45RA <sup>+</sup> 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<sup>+</sup> infusion is  $\geq 5 \times 10^6$  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™ device using either CD34<sup>+</sup> selection (using established SOPs) or CD3<sup>+</sup> depletion methodology may be utilized. The boost target dose for these patients is  $\geq 10 \times 10^6$  CD34<sup>+</sup> cells/kg with a CD3<sup>+</sup> cell/kg dose of  $\leq 0.5 \times 10^5$  CD3<sup>+</sup> cells/kg.

#### 4.7 Donor Lymphocyte Infusions

Donor lymphocyte infusions (DLI) may be administered 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. The initial dose will typically be  $2.5 \times 10^4$  CD3<sup>+</sup>/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	$2.5 \times 10^4$ CD3 <sup>+</sup> /kg	Approximately 2-4 week interval If no moderate or severe GVHD
Dose #2	$5.0 \times 10^4$ CD3 <sup>+</sup> /kg	
Dose #3	$10.0 \times 10^4$ CD3 <sup>+</sup> /kg	

Although this algorithm will be appropriate for a majority, the treating transplant attending physician may alter the dose and/or interval of DLI based on response to previous DLI, the severity of the clinical situation, and the condition of the patient. There is a low threshold for providing an initial dose of  $2.5 \times 10^4$  CD3<sup>+</sup>/kg. DLI at this dose level have not been associated with GVHD. Therefore, DLI may be initiated for any chimerism less than 100% in a patient who is off immune suppression. The risk of GvHD increases with increasing doses. In the last 5 years, no patient has developed moderate or severe GVHD after haploidentical DLI dosed at  $5 \times 10^4$  CD3<sup>+</sup>/kg, though mild GVHD has occasionally occurred. Doses of  $10 \times 10^4$  CD3<sup>+</sup>/kg should be considered to carry a risk of severe GVHD. Therefore dose escalation to this level must incorporate the appropriate risk/benefit analysis by the treating transplant physician.

#### 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, sterility (culture and Gram stain), and the presence of endotoxin. Culture and endotoxin 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 or endotoxin results exceed release limits.

#### 4.9 Immunosuppressive Therapies

##### 4.9.1 EBV post-transplant lymphoproliferative disease (PTLPD) prophylaxis

Rituximab at 375 mg/m<sup>2</sup> 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 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 engraftment among UCB patients, generally between day 7 and 21. 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. 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. Please alert PI if patient is requiring methylprednisolone for > 6 days for engraftment syndrome.

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. Please alert PI if patient is requiring methylprednisolone for > 6 days for engraftment syndrome.

#### 4.11 Growth Factors

##### 4.11.1 Granulocyte colony-stimulating factor (G-CSF)

G-CSF to start on day +1 at 5 mcg/kg/day intravenously or subcutaneously until absolute neutrophil count (ANC)  $\geq 2,000/\text{mm}^3$  for three consecutive days.

#### 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](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 (Cytosan)	
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 aldophosphamide. Aldophosphamide spontaneously splits into nitrogen mustard, which is considered to be the major active metabolite, and acrolein. In addition, 4-hydroxycyclo-phosphamide may be enzymatically metabolized to 4-ketocyclophosphamide and aldophosphamide 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 and Stability	Cyclophosphamide is available in vials containing 100, 200, 500, 1000 and 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
<b>5.2. Thiotepa (Thioplex® by Immunex) (TESPA, TSPA)</b>	
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-phosphoramidate (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 NaHCO <sub>3</sub> . 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

<b>5.3 Fludarabine (Fludara)</b>	
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 <sup>2</sup> /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 <sup>2</sup> .
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-sarcollisin, Alkeran <sup>®</sup> )	
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 <i>Escherichia coli</i> . 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 <i>E. coli</i> 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 <i>Streptomyces tsukubaensis</i> . It inhibits T-lymphocyte activation, although the exact

	<p>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 <math>t_{1/2}</math> 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.</p>
Formulation and Stability	<p>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).</p>
Supplier	Commercially available
Toxicities	<p>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</p>



	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™ 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<sup>+</sup> 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<sup>+</sup> 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 demagnetization. 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 demagnetization 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<sup>+</sup> 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<sup>+</sup> or CD45RA<sup>+</sup>) or selection of a target cell population (e.g. CD34<sup>+</sup> or CD56<sup>+</sup> 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](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.

*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 will include an optional skin punch biopsy 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. During viral infections, or reactivations detected by DNA regardless of copy number, samples to be collected weekly x 4 or until cleared (whichever is less). 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 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. Epstein-Barr virus transformed lymphoblastoid cell lines (EBV-LCL) will be generated from the donor cells obtained from the discarded infusion bag. UCB 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 will also require a 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 of this protocol.

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  $\geq 0.5 \times 10^9/L$  ( $500/mm^3$ ) for three consecutive laboratory values obtained on different days. Date of engraftment is the date of the first of the three consecutive laboratory values.

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

7.5 Primary graft failure will be defined as an ANC never meeting or exceeding  $500/mm^3$  for 3 consecutive measurements after day +42 post-transplant.

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 engraftment.

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

## **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.
- 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 for the first two participants enrolled, then 15% of study enrollees every six 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 6 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 engraftment by day +42. 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.<sup>126</sup>

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.<sup>59</sup> 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.<sup>62</sup> 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.<sup>16</sup> 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 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 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 engraftment of donor graft(s) at a rate of  $\geq 91\%$ . We do not anticipate censoring during the 42 day time period and we can approximate the rate of 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  $H_0: P \leq 0.80$  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 engraftment rate  $P_1 = 0.91$  requires 21 evaluable patients at the first stage and 49 evaluable patients in total<sup>76</sup>. 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 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 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 engraftment but before day +42 will count as having engrafted. Any patient who dies prior to engraftment will count as a graft failure.

After the study is finished, for the first primary objective, the rate of 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.

**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 engraftment, we will closely monitor the trial for early excessive toxicities in terms of severe acute GVHD and therapy related death. Therapy related death is any death in remission and related to protocol therapy. The incidence of 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. The safety endpoints will be monitored independently and a consideration to stopping the trial would be given if there is evidence suggesting that the rate of grades 3-4 acute GVHD is greater than 20%, or the rate of transplant related death is greater than 10%. Then, the planned interim evaluation time points and stopping rules, using group sequential design based on Pocock stopping boundaries after considering five interim analyses (p-value threshold = 0.0158) to ensure an overall type one error of 0.05<sup>127-129</sup>, for each of the two endpoints are provided in Tables 3 and 4:

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

No of of Research Participants Enrolled	No. of Grades 3-4 aGVHD Observed
$\leq 11$	$\geq 6$
$\leq 21$	$\geq 9$
$\leq 31$	$\geq 12$
$\leq 41$	$\geq 15$
$\leq 49$	$\geq 17$

**Table 4. Stopping Rules for Toxicities Based on Therapy Related Death within the first 100 days Post-transplant**

No. of Research Participants Enrolled	No. of Therapy Related Death Observed
$\leq 11$	$\geq 5$
$\leq 21$	$\geq 6$
$\leq 31$	$\geq 8$
$\leq 41$	$\geq 10$
$\leq 49$	$\geq 11$

Based on the above Table 3, if six grades 3-4 acute GVHD occur within the first 100 days post transplantation in the first eleven evaluable research participants treated, then a consideration will be given to temporarily stopping the trial and amending the study to include additional pharmacologic GVHD prophylaxis. Similarly, from Table 4 if we observe five deaths related to therapy within the first 100 days after transplantation in the first eleven evaluable research participants treated, then a consideration will be given to temporarily stopping the trial and amending the study appropriately.

## 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  $OS = \min(\text{date of last follow-up, date of death}) - \text{date of HCT}$  and all participants surviving after 1 year post-transplant will be considered as censored, and  $EFS = \min(\text{date of last follow-up, date of relapse, date of graft failure, date of death due to any cause}) - \text{date of transplant}$ , 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

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 rate of TRM in the first 100 days after HCT.

The cumulative incidence of TRM will be estimated using the same method as used in evaluating Objective 10.2.2. Deaths before day 100 because of other reasons are the competing risk events. 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) analysis, V-beta spectratyping, and phenotype and functional analysis of reconstituted lymphocyte subsets. All immune reconstitution measures will be descriptively analyzed.

#### 11.3.3 Characterize the recovery of Gamma Delta 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](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 18<sup>th</sup> 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.

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APPENDIX A:

## PERFORMANCE STATUS SCALE

<b>KARNOFSKY PERFORMANCE STATUS SCALE</b>	
<b>Score</b>	<b>General Description</b>
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.

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<b>LANSKY PERFORMANCE STATUS SCALE</b>	
<b>Score</b>	<b>General Description</b>
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

## Criteria for Acute GVHD

ORGAN STAGING FOR ACUTE GVHD						
<i>Organ</i>		<i>Stage 0</i>	<i>Stage 1</i>	<i>Stage 2</i>	<i>Stage 3</i>	<i>Stage 4</i>
<i>Skin</i>		No rash	Rash < 25% <sup>a</sup>	Rash 25 < 50%	Rash >50% generalized erythroderma	Plus bullae and desquamation
<i>Gastrointestinal</i>	<i>Lower</i>	Diarrhea <500 mL/day	Diarrhea 501 to 1000 mL/day <sup>c</sup>	Diarrhea 1001 to 1500 mL/day	Diarrhea >1500 mL/day	Severe abdominal pain with or without ileus
	<i>Upper</i>	^	Persistent nausea <sup>d</sup>	^	^	^
<i>Liver</i>		Bilirubin <2.0 mg/dl	Bilirubin 2.1 to 3.0 mg/dl <sup>b</sup>	Bilirubin 3.1 to 6.0 mg/dl <sup>b</sup>	Bilirubin >6.1 to 15 mg/dl <sup>b</sup>	Bilirubin > 15 mg/dl

OVERALL GRADING FOR ACUTE GVHD			
<i>Grade</i>	<i>Skin</i>	<i>Liver</i>	<i>Gut</i>
<i>I</i>	Stage 1-2	None	None
<i>II</i>	Stage 3 or	Stage 1 or	Stage 1
<i>III</i>	-	Stage 2-3 or	Stage 2-4
<i>IV<sup>f</sup></i>	Stage 4 or	Stage 4	-

a. Use rule of Nines<sup>g</sup> or burn chart to determine extent of rash.

b. Range given as total bilirubin. Downgrade one stage if additional cause of elevated bilirubin is documented.

c. Volume of diarrhea applies to adults. For pediatric patients, the volume of diarrhea should be based on body surface area. Downgrade one stage if additional cause of diarrhea has been documented.

d. Persistent nausea with histological evidence of GVHD in stomach or duodenum.

e. Criteria for grading given as minimum degree of organ involvement required conferring that grade.

f. Grade IV may also include lesser organ involvement but with extreme decrease in performance status.

## APPENDIX B (continued)

## CRITERIA FOR GRADING CHRONIC GVHD GRADE

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



## APPENDIX B (continued)

## CRITERIA FOR GRADING CHRONIC GVHD GRADE

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

PFT: Pulmonary Function Testing  
 ECOG, Eastern Cooperative Oncology Group  
 BSA, body surface area  
 AP, alkaline phosphatase  
 ULN, upper limit of normal

LFS, Lung Function Score  
 KPS, Karnofsky Performance Status  
 ADL, activities of daily living  
 ALT, alanine aminotransferase

GVHD, graft versus host disease  
 LPS, Lansky Performance Status  
 LFTs, liver function tests  
 AST, aspartate aminotransferase

APPENDIX B (continued)

## CRITERIA FOR GRADING CHRONIC GVHD GRADE

GLOBAL GRADING OF CHRONIC GVHD<sup>84</sup>

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

## 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 D

## Recommended testing and evaluation schedule

STANDARD OF CARE STUDIES	SAMPLE	VOLUME	PRE	MONTH 1	MONTH 2	MONTH 3	MONTH 6	MONTH 12
<b>Pregnancy test</b>	PB	2 ml	X	As clinically indicated				
<b>Physical exam</b>	N/A	N/A	X	Weekly			X	X
<b>CBC with diff.</b>	PB	0.5 - 2 ml	X	Daily until engrafted, then weekly			X	X
<b>Chemistry</b>	PB	0.25- 2 ml	X	Weekly			X	X
<b>Viral surveillance (BMT-PCR)</b>	PB	4 ml	X	Weekly			As clinically indicated	
<b>Chimerism</b>	PB	1-2 ml		Weekly			Monthly	
	BM	2 ml		X		X		X
<b>Disease Status Evaluation</b>	BM	N/A	X	X		X		X
<b>MRD</b>	PB	5 ml	X <sup>6</sup>	X <sup>6</sup>		X <sup>6</sup>		X <sup>6</sup>
	BM	0.2-5 ml	X	X		X		X
<b>Lymphocyte Subset Study</b>	PB	2.5-4 ml	X	Monthly				
<b>Quantitative Immunoglobulins</b>	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.
- <sup>6</sup> 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 D (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		
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 – CMV+	7.5 ml		WEEKLY x 4 DURING ACTIVE VIRAL REACTIVATION						
	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.
- T-FUNCTION testing in the presence of viral infection and/or reactivation will be obtained as directed above (weekly x4) within the first 100 days post-transplant, however from Day 100 to Month 12 it will be obtained as possible (includes all viruses).
- Skin Biopsy for T-FUNCTION is an optional additional test 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.
- For RESEARCH studies, the posted volumes are the minimum volumes required to perform the respective protocol evaluations.

APPENDIX D (continued)Research testing for HAPLOIDENTICAL DONOR*Prior to initial stem cell collection procedure:*OPTIONAL research immune studies testing schedule

<i>Evaluation</i>	<i>Volume Requirement</i>
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

- 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 D (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:

<b>If the Planned Evaluation Time Point is:</b>	<b>Window</b>
Weekly	$\pm 3$ 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 E

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](http://home.web.stjude.org/bone_marrow/clinicalHome.shtml).