A Phase II Study Using the CliniMACS® Device for CD34+ Cell Selection and T Cell Depletion for Graft-versus-Host Disease Prophylaxis in Alternative Donor Stem Cell Transplant Recipients

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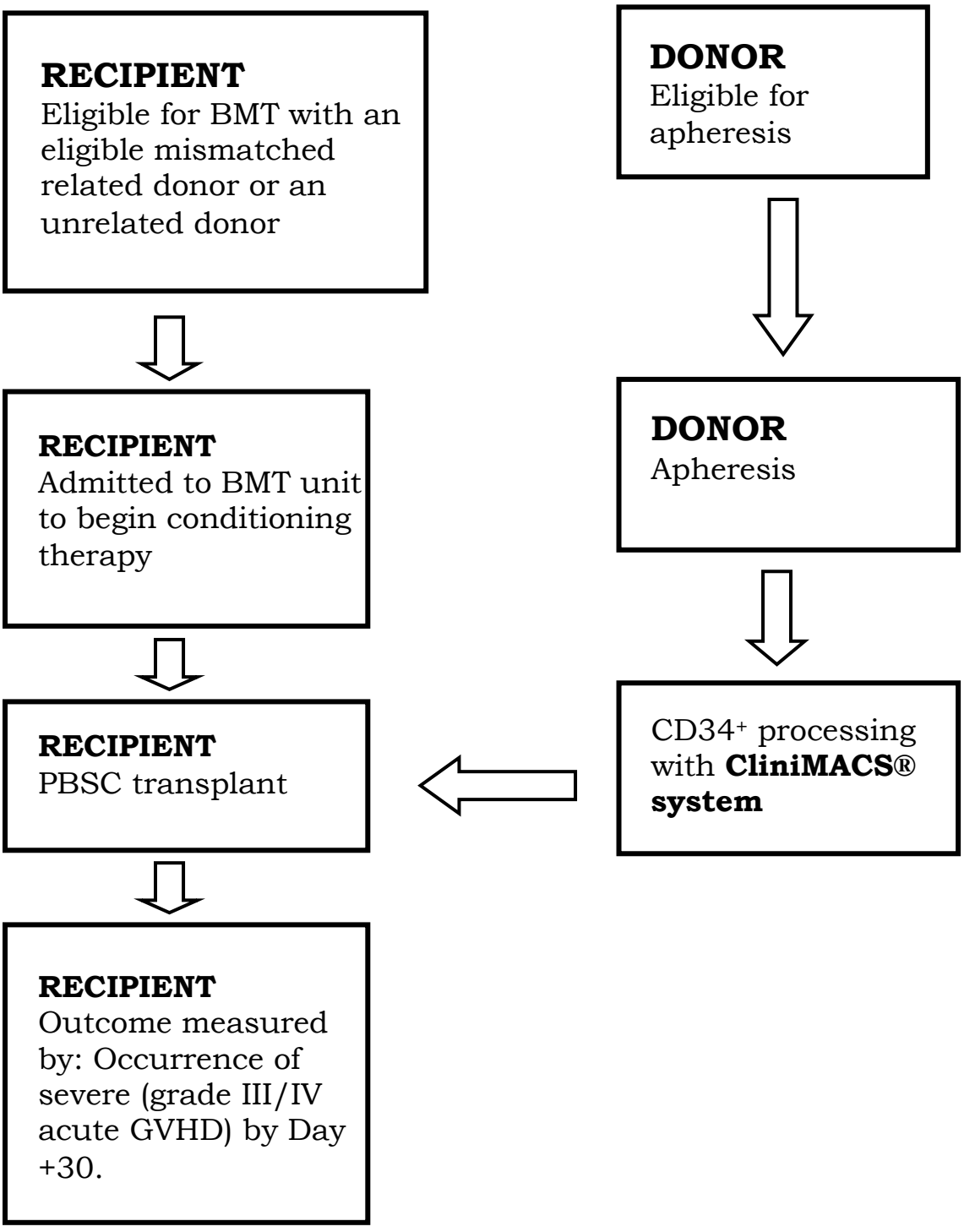
SHORT ABSTRACT

A major issue in alternative donor (mismatched related and unrelated donor) transplantation is the development of graft-versus-host disease (GVHD). Several clinical trials have shown that the use of T-cell depleted peripheral blood stem cells (PBSC) reduces GVHD in alternative donor transplants. The purpose of this study is to determine the ability of CD34 positive selection and T cell depletion using the CliniMACS® Device as the only GVHD prophylaxis to prevent severe acute GVHD in recipients of an alternative donor PBSC transplant. Mismatched related donors will match at least 4 of 8 HLA antigens (haplocompatible) and unrelated donors will match at least 6 out of 8 HLA antigens with the transplant recipient. The patients will receive conditioning therapy based on their diagnosis that may include chemotherapy, anti-thymocyte globulin (ATG), +/- total body irradiation (TBI). The transplant recipients will be followed for 5 years post-transplant for the development of GVHD, engraftment, post-transplant infections, disease relapse, and overall survival. In addition, this study will serve as a platform for a companion study of therapy to accelerate immune recovery after transplant.

SCHEMA


RECIPIENT
Eligible for BMT with an eligible mismatched related donor or an unrelated donor
DONOR
Eligible for apheresis
DONOR
Apheresis
RECIPIENT
Admitted to BMT unit to begin conditioning therapy
CD34+ processing with **CliniMACS® system**
RECIPIENT
PBSC transplant
RECIPIENT
Outcome measured by: Occurrence of severe (grade III/IV acute GVHD) by Day +30.

LIST OF ABBREVIATIONS

AE	Adverse Event
ALL	Acute Lymphocytic Leukemia
AML	Acute Myelogenous Leukemia
ANC	Absolute Neutrophil Count
APC	Antigen Presenting Cells
ASCO	American Society of Clinical Oncology
BMT	Bone Marrow Transplant
BSA	Body Surface Area
CB	Cord Blood
CIBMTR	Center for International Blood and Marrow Transplant Research
CML	Chronic Myelogenous Leukemia
CMV	Cytomegalovirus
CR	Complete Response
CRF	Case Report Form
CTC	Common Toxicity Criteria
DFS	Disease-Free Survival
DLI	Donor Lymphocyte Infusion
DLT	Dose Limiting Toxicity
EBV	Epstein Barr Virus
EFS	Event-Free Survival
FA	Fanconi Anemia
FDA	Food and Drug Administration
FHCRC	Fred Hutchinson Cancer Research Center
GCP	Good Clinical Practice
G-CSF	human Granulocyte-Colony Stimulating Factor
GVHD	Graft versus Host disease
HLA	Human Leukocyte Antigen
HSC	Hematopoietic Stem Cell
ICH	International Conference on Harmonization
IND	Investigational New Drug
IEC	Independent Ethics Committee
IRB	Institutional Review Board
JMML	Juvenile Myelomonocytic Leukemia
KIR	Killer cell Immunoglobulin-like Receptor
MDS	Myelodysplastic Syndrome
MRI	Magnetic Resonance Imaging
MUD	Matched Unrelated Donor
PBSC	Peripheral Blood Stem Cells
PCR	Polymerase Chain Reaction
PI	Principal Investigator
PTLD	Post-transplant Lymphoproliferative Disorder
PRC	Protocol Review Committee, Lineberger Comprehensive Cancer Center
rATG	polyclonal rabbit Anti-Thymocyte Globulin
SAE	Serious Adverse Event
SCT	Stem Cell Transplant
TBI	Total Body Irradiation
TMP/SMX	Trimethoprim-sulfamethoxazole
TRM	Transplant Related Mortality

1.0 GOALS AND OBJECTIVES

1.1 Primary objective

To determine the ability of $CD34^+$ cell selection using the CliniMACS® device as the sole GVHD prophylaxis to prevent severe (grade III-IV) acute GVHD in recipients of alternative donor (mismatched related donor and unrelated donor) hematopoietic stem cell transplants.

1.2 Secondary objectives

1.2.1 To assess the ability of this approach to serve as a platform for strategies to accelerate post-transplant immunological recovery

1.2.2 To evaluate the rate of engraftment in recipients of $CD34^+$ cell selected, T cell-depleted transplants from alternative donors

1.2.3 To evaluate post-transplant infections

1.2.4 To evaluate the rate of EBV-related post-transplant lymphoproliferative disorder (PTLD)

1.2.5 To evaluate post-transplant leukemia relapse

1.2.6 To evaluate transplant-related mortality

1.2.7 To evaluate transplant-related toxicities

1.2.8 To evaluate overall survival

1.2.9 To monitor device performance:

- **1.2.9.1** Purity of selected product
- **1.2.9.2** Yield of $CD34^+$ cells
- **1.2.9.3** $CD3^+$ cell depletion
- **1.2.9.4** Viability
- **1.2.9.5** Sterility

1.2.10 To evaluate the correlation of NK alloreactivity with relapse, engraftment, transplant-related mortality, and survival

2.0 BACKGROUND AND RATIONALE

2.1 Stem cell transplantation

Stem cell transplantation (SCT) can cure some children with marrow failure syndromes, inherited immunodeficiencies, myelodysplastic syndrome (MDS), acute lymphocytic leukemia (ALL), acute myelogenous leukemia (AML), chronic myelogenous leukemia (CML), lymphoma, white blood cell disorders (chronic granulomatous disease, osteopetrosis), and red blood cell disorders (sickle cell disease, thalassemia). The preferred donor for transplantation is a HLA identical sibling. However, less than 20% of patients requiring a transplant will have such a donor. Alternative stem cell sources include volunteer matched unrelated donors (MUD) and unrelated donor cord blood (CB). Approximately 90% of Caucasians and 60% of African-Americans will have a 5/6 or 6/6-matched unrelated marrow donor. Approximately 80% of Caucasians and 40% of African-Americans will have a 5/6 or 6/6-matched unrelated cord blood donor. Most patients will have a 4/6-matched cord blood available (1).

The largest experience using unrelated donors is with unrelated donor bone marrow transplants. Single center data for children include a report from Fred Hutchinson Cancer Research Center (FHCRC) that described 47% 2 yr event-free survival (EFS) for ALL in CR1/2 and 46% for AML in CR1/2 (2). The incidence of severe, life-threatening (grade III-IV) acute GVHD was 37% and 62% of HLA-matched and mismatched recipients, respectively. Data from the Center for International Blood and Marrow Transplant Research (CIBMTR) from 1996-2001 shows a 50% 2 yr EFS for ALL in CR1 and 42% 2 yr EFS for ALL in CR2+. This compares to CIBMTR data for matched sibling

transplants over the same time period that shows a 70% 2 yr EFS for ALL in CR1 and 60% 2 yr EFS for ALL in CR2+ (3).
For unrelated donor cord blood transplants, the University of Minnesota reported a 55% 2 yr EFS for ALL in CR1, 32% 2 yr EFS for ALL in CR2+, and 33% for AML in CR2+. The incidence of grade III-IV acute GVHD was 11%. The use of a 4/6-matched donor was associated with a relative risk of 2.4 for death compared to a 5/6 or 6/6-matched donor (p=0.01) (4). A larger review (562 patients) by Rubinstein et al. reported a 23% incidence of grade III-IV acute GVHD. The cumulative incidence of transplant-related events increased as the number of HLA disparities increased (5).

A comparison of MUD and CB donor sources in 541 children transplanted for acute leukemia showed a 2 year event-free survival of 43% for unmanipulated MUD transplant, 37% for T cell-depleted MUD transplant, and 31% for cord blood transplant. The incidence of grade III-IV acute GVHD was 29%, 8%, and 21% in the respective groups (6). A recent retrospective review of MUD and CB donor transplant for 503 children with leukemia transplanted in the US showed the following results (7):

- 2 yr EFS ~ 40% after matched and mismatched unrelated donor bone marrow transplant,
- 2 yr EFS ~ 40% after 4/6 matched unrelated donor cord blood, ~ 45% after 5/6, ~ 65% after 6/6 matched (there were only 35 patients with a 6/6 matched donor),
- Severe (Grade III/IV) acute GVHD 18% and 32% after matched and mismatched unrelated donor bone marrow transplant, respectively,
- Severe (Grade III/IV) acute GVHD 27% after 4/6 matched unrelated donor cord blood, 20% after 5/6, 9% after 6/6 matched,
- Relapse 39% and 31% after matched and mismatched unrelated donor bone marrow transplant, respectively,
- Relapse 19% after 4/6 matched unrelated donor cord blood, 27% after 5/6, 31% after 6/6 matched.

2.2 Mismatched (haplocompatible) related donor stem cell transplant

Another donor source that has been reported is a mismatched (haplocompatible or sharing only one of two haplotypes) related donor. In the past, the major challenges have been engraftment, graft-versus-host disease (GVHD), and an increased incidence of infection and relapse. Outcomes have improved markedly in recent years with the availability of cell selection devices that allow the administration of a large number of stem cells with a low dose of T cells and the development of more intensely immunosuppressive conditioning regimens. In order to reduce the high risk of fatal GVHD associated with these mismatched donors, the stem cells need to be processed to significantly reduce the number of donor T cells present in the graft. CD34 is a receptor that is expressed on early hematopoietic stem cells (HSC). Monoclonal antibodies are available that efficiently bind to the CD34 antigen, and studies have shown that positive selection of $CD34^+$ marrow or blood cells results in a significant (> 4 log) depletion of T cells from the preparation (8-10). This approach is less time-consuming and may be more efficient than earlier approaches because it specifically targets the HSC. A cell separation system for clinical use is available for evaluation in the US after extensive use in Europe (11,12). The **Miltenyi Biotec Inc.** CliniMACS® **$CD34^+$ Reagent System** has the advantages of the best T cell depletion efficiency achievable and a very high efficiency of $CD34^+$ cell recovery so that fewer apheresis are necessary. The CliniMACS® device uses a sterile, closed magnetic sorting system to isolate $CD34^+$ stem cells. The peripheral blood stem cell product obtained from the donor is incubated with a murine anti-CD34 monoclonal antibody conjugated to small super-paramagnetic beads composed of iron dextran (commercially available to treat iron deficiency). The murine antibody has been used in clinical trials in humans. The dose administered to the

transplant after processing is 100x lower than therapeutic levels. Following incubation, the product passes through a strong magnet. The $CD34^+$ cells remain at the level of the magnet and other cells (T, B, and NK cells, monocytes, and neutrophils) pass through into a waste bag. The magnet is then turned off and the $CD34^+$ cells are released into a bag containing the final product.

In addition, it has been well established that allogeneic peripheral blood stem cells (PBSC) recruited into the blood by administering G-CSF can successfully and durably engraft in the matched (13,14) or mismatched (15,16) relative. PBSC recruited with cytokines engraft earlier and contain a larger number of HSC compared to marrow HSC (14,15).

Much of the work that has been done with this approach has been reported by a group in Perugia, Italy. They used a conditioning regimen of total body irradiation (TBI), thiotepa, fludarabine, and rabbit ATG for 101 patients. They used the CliniMACS® device to lower the T cell content of the donor PBSC to a median of 1×10^4/kg (range $0.04 - 3 \times 10^4$/kg). Ninety-one percent of patients had primary engraftment. Six of seven patients who did not have primary engraftment were successfully engrafted after a second transplant, making the overall engraftment rate 99%. Grade III-IV acute GVHD occurred in 2% of patients. The transplant-related mortality was 37%, with the majority of deaths due to infection (bacterial, viral, and fungal). Relapse occurred in 16% of 66 patients who were in remission at the time of transplant. The 2 year EFS for patients in remission was 48% for AML and 46% for ALL (17).

The results of haplocompatible transplantation in children have been encouraging. Handgretinger reported a 46% 2 yr EFS in patients with ALL in CR1-3 (18) Ortin et al. reported 75% EFS of patients with ALL in CR2/3 with median follow-up of 18 mo (range 6-29) (19) The international experience was reviewed at a conference in Naples in 2004. Lang updated the Tubingen, Germany experience when he reported a 44% 2 yr EFS for 21 children with ALL in CR1-3 (20) Advantages of haplocompatible transplantation are rapid engraftment and the very low incidence of severe acute GVHD. Ortin et al. reported a median time to ANC > 500 and to platelet count > 50,000 of 12 and 20 days, respectively, with the incidence of grade III-IV acute GVHD being 5% (19). Lang reported a 1% incidence of grade III-IV acute GVHD (21).

In a retrospective review of the experience of 3 investigators in the U.S. (Cowan, Gilman, Sleight) with alternative (haplocompatible) donor transplant using $CD34^+$ cell selection for T cell depletion, 13/18 (72%) were surviving with follow-up of 7 mo – 7 yrs (median 31 mo)(22). The median pt age was 8 yrs (range 1-20). Patient with malignancy (n=13) included: AML - CR1 (primary induction failure, failed cord blood transplant) [1], CR2 [3]; MDS - RA/RARS [2], RAEB [2] AML (and Fanconi anemia, FA) [1]; CML - CP2 [1]; ALL - CR3 [2]; NHL - CR2 [1]. Patients with non-malignant (n=5) disease included severe aplastic anemia (1 with prior BMT 3 yrs earlier) [4] and Wiskott-Aldrich syndrome [1]. Fourteen donors were a 3/6 HLA match and 5 were a 4/6 match (one patient had two transplants using different donors). A CD34-positive selection device – Miltenyi CliniMACS® (15), Isolex (3) – was used to select stem cells and deplete T lymphocytes. The conditioning regimen was TBI 12-14 Gy in 6 fractions, thiotepa, fludarabine, and ATG. Fractionated TBI was replaced with single fraction TBI (2 pts) or melphalan (3 pts) as clinically indicated. Cyclophosphamide was used instead of thiotepa for one pt with FA. No post-transplant graft-versus-host disease (GVHD) prophylaxis such as tacrolimus or methotrexate was given. Patients received a median of 18×10^6 $CD34^+$ (stem) cells/kg (range 6-28) and 3×10^4 $CD3^+$ (T) cells/kg (range 0.3-11).

Sustained primary engraftment occurred in 15/18 (83%) patients. Primary graft failure occurred in one patient. Two patients had immunological rejection following HHV-6

reactivation. They both engrafted after a second transplant; therefore the overall engraftment rate was 94%. The median time to an ANC >0.5 x 10^9/L was 12 days (range 9-21). Platelet recovery occurred in 16/18 at a median of 17 days (range 9-22). Primary (occurring after SCT and prior to donor lymphocyte infusion [DLI]) grade II acute GVHD was seen in 4/17 patients (24%). Grade III-IV acute GVHD was seen in 1 pt (6%) with overlap syndrome (acute + chronic GVHD) associated with HHV-6 reactivation. Nine patients received DLI and/or stem cell boosts (boosts for graft rejection); 4 had grade II GVHD (3/4 had a history of acute GVHD) and none had grade III-IV GVHD. After DLI and/or stem cell boost, two patients developed extensive chronic GVHD and one developed overlap syndrome. The Day 100 mortality and 1 year transplant-related mortality were 11% and 19%, respectively. Four patients (of 13 at risk, 31%) have relapsed; 1 pt with cytogenetic relapse is in CR > 1 year later. The 2 yr predicted survival is 64% (60% for 13 patients with malignant disease and 75% for 5 patients with non-malignant disease).

Infections were common. All patients were at risk for CMV reactivation. Seven patients (39%) reactivated CMV. All cases were responsive to anti-viral therapy and/or DLI. No CMV disease was seen. Seven patients had adenovirus reactivation and 6 had HHV-6 reactivation. EBV reactivation occurred in 5/18 (28%) patients, 3 of whom manifested signs of post-transplant lymphoproliferative disorder. Patients received a median of 3 x 10^4 $CD3^+$ cells/kg at the time of transplant. Some patients received additional donor T cells (DLI) for viral reactivation. At 3 months post-transplant, only 4 of the 15 evaluable patients had a CD4 count > 100. By 9 months post-transplant, 10 of the 13 evaluable patients had a CD4 count > 200.

In summary, the use of megadose $CD34^+$ selected PBSC without post-transplant GVHD prophylaxis for children was associated with rapid engraftment, a low 100-day mortality, a very low incidence of severe GVHD, and excellent survival. The overall survival compares favorably with MSD and MUD HSCT. Immune reconstitution was slow and post-transplant infections contributed to morbidity and mortality.

2.3 NK alloreactivity

The risk of relapse with haplocompatible transplantation may be reduced by utilizing both donor NK cells for a graft-versus-leukemia effect and donor lymphocyte infusions (DLI) to accelerate T cell reconstitution. NK alloreactivity has been shown to be present for myeloid leukemias and for lymphoid leukemias (23,24). Alloreactivity can be determined by HLA-C typing with even better results with the addition of NK cell killer immunoglobulin-like receptor (KIR) typing (23,24). In addition to HLA compatibility, the choice of the optimal donor source may depend on the age and size of the recipient, the type of leukemia, disease status, pre-transplant organ dysfunction, and pre-transplant infections.

There is limited experience with unrelated donor transplants for children following $CD34^+$ cell selection and T cell depletion with the CliniMACS device (25). In 30 patients with leukemia, primary engraftment was observed in 84% with the remainder engrafted after a second transplant. Grade III-IV acute GVHD occurred in 7% of patients and followed HHV-6 infection in both patients. The 2 year survival was 44% for patients in remission at the time of transplant (25). The same approach was used for unrelated donor transplantation for 14 children with non-malignant disease. Observed overall survival was 100% (follow-up 1-7 years) and no grade III-IV acute GVHD occurred (21).

In a large prospective study of T cell depletion versus immunosuppressive drugs for GVHD prophylaxis for adults undergoing matched unrelated donor (MUD) transplant, there was no difference in 3 year EFS, 27 vs 34%, respectively (26). However, the T cell depletion used for this trial was much less intense than that achieved with the

CliniMACS® device, as demonstrated by an 18% incidence of grade III-IV acute GVHD (37% in the non-T cell-depleted arm) (26). Post-transplant infections including CMV and aspergillus were more problematic after T cell depletion (27). Interestingly, the outcome for recipients of T cell-depleted bone marrow who did not have a fungal infection was better than that for patients receiving T replete bone marrow. This suggests that successful prevention of post-transplant infection by hastening immune recovery may result in a superior outcome for T cell-depleted transplants.

2.4 Preliminary data

We have collaborated with Dr. Cowan at UCSF for his prospective study of $CD34^+$ selected (with the CliniMACS® device) PBSC from mismatched related donors. Patients received a conditioning regimen including TBI 1200 cGy, thiotepa, fludarabine, and rabbit ATG (3.5 mg/kg). Patients received a fixed T cell dose of 3×10^4/kg at the time of transplant. Seventeen evaluable patients have been enrolled with the following diagnoses: ALL (3), AML (6), bilineage leukemia (1), CML (1), MDS (1), aplastic anemia (2), congenital amegakaryocytic thrombocytopenia (1) combined immunodeficiency (1), and hemophagocytic lymphohistiocytosis (1).

Twelve of the 17 (65%) are alive and well, with follow-up ranging from 3 months to 6.5 years (median follow-up 2.5 years). Survivors include 7/12 (58%) with malignant disease and 5/5 (100%) with non-malignant disease. Of note is that 9/11 patients treated after the last protocol amendment are alive and well, including 5/7 with malignant disease. Both deaths were due to late infection. One patient died at 7 months after transplant due to parainfluenza and Paecilomyces infections and the other died at 23 months after transplant due to disseminated Mucor infection. There was no severe (grade III/IV) acute GVHD. Only 3/17 (18%) of patients achieved > 100 $CD4^+$ T cells/uL by 100 days after transplant and 3/4 of these patients either had received donor T cell infusion for serious viral infections prior to 100 days or had GVHD (in which case the T cells probably represented those causing the GVHD and not providing protection against infection).

2.5 Immune reconstitution

A major challenge of previous approaches to haplocompatible donor transplantation is the prolonged immunodeficiency that follows transplant (28). This results in viral and fungal infections and is the primary cause of transplant-related mortality (29). Patients on this study who meet the eligibility criteria will be offered the opportunity to participate in a companion study, which will investigate the use of a donor lymphocyte (T cell) infusion (DLI) to hasten immune reconstitution. The use of a subsequent intervention will necessitate that the success of this study will be judged by the ability of $CD34^+$ cell selection with the CliniMACS® device to prevent severe, life-threatening acute GVHD while maintaining a satisfactory rate of engraftment.

2.6 Rationale for including alternative conditioning regimens

Several patients have not been eligible for Dr. Cowan's study described above because of the need for an individualized conditioning regimen (ie. contraindication to the total body irradiation (TBI), fludarabine, thiotepa, and ATG regimen) or because of diseases/conditions not allowed in the eligibility criteria (eg. non-Hodgkin's lymphoma, poor lung function). Examples of such patients that we have transplanted include (1) patient with AML who failed to engraft after a cord blood transplant and had invasive fungal infection – alive and well 4 years after transplant, and (2) patient with rare immunodeficiency – NEMO syndrome – who had significant lung dysfunction and was on oxygen at the time of transplant – alive and well 3 years after transplant.

Recent studies using the CliniMACS® device, including Dr. Cowan's, used a $CD3^+$ cell (T cell) dose of $\leq 3 \times 10^4$/kg. Conditioning regimens include the TBI-containing regimen

used by the Perugia group and in Dr. Cowan's study as well as non-TBI containing regimens as indicated by the patient's disease and clinical condition. The substitution of Melphalan for TBI has been reported by several groups (22,30,31). Regimens have been developed for patients in poor clinical condition (31,32) or with DNA repair syndromes (33,34). Because this protocol is designed to look at the ability of the CliniMACS® device to prevent GVHD, the conditioning regimen used will be chosen based on the patient's disease and clinical condition. The conditioning regimen will also include rabbit ATG with the dose based on these factors and prior *in vivo* therapy with anti-T cell antibodies.

3.0 STUDY DESIGN AND ELIGIBILITY CRITERIA

3.1 Study design

Patients will be enrolled with alternative (mismatched/haplocompatible) related donors or unrelated donors. For patients with mismatched related donors, the majority of clinical experience has been with a T cell-depleted PBSC product. The CliniMACS® CD34+ Reagent System from Miltenyi Biotec, Inc. was approved by the FDA in January, 2014 as a Humanitarian Use Device for the processing of PBSC to obtain a CD34-enriched stem cell collection for adults with acute myeloid leukemia in first complete remission undergoing PBSC transplant from a matched related donor without the needs for medications to prevent GVHD. Recent experience with the CliniMACS® device has produced excellent results with a 70-75% survival in children, many of whom were high risk patients (19,22,35).

Patients that receive transplants from unrelated donors usually receive stem cells that are not T cell-depleted. However, this is associated with a high risk of GVHD. The excellent results with mismatched related donor transplants justify expanding this approach to unrelated donor transplant recipients. It is anticipated that the use of the CliniMACS® device will result in a very low risk of GVHD without the need for post-transplant immunosuppression. The outcomes in relatively small studies for children receiving unrelated donor transplants using the CliniMACS® have been comparable to or better than those receiving T replete transplants with post-transplant immunosuppression (21,25).

This protocol will allow the use of patient-specific conditioning regimens. Some patients have contraindications to certain components of the conditioning regimen used for Dr. Cowan's study under BB-IND 8817 (UCSF 01151 protocol). An example is a patient with pre-existing organ dysfunction that would be better served by the use of a reduced intensity conditioning regimen. Another example is a patient for who total body irradiation is contraindicated due to very young age or prior radiation therapy. The target T cell dose that will be given will be $\leq 3 \times 10^4$ /kg. The UCSF 01151 protocol uses a dose of 3×10^4 /kg. The T cell dose in the graft is usually $< 1 \times 10^4$ /kg after processing and T cells need to be added to the product. There is extensive successful experience with this approach (17).

3.2 Recipient inclusion criteria

3.2.1 Age < 30 years

3.2.2 Patient must have a malignant or non-malignant disease that can benefit from alternative stem cell transplantation. Examples include acute and chronic leukemias, myelodysplastic syndrome, lymphoma, severe acquired and congenital cytopenias, white and red blood cell abnormalities, and immunodeficiencies.

3.2.3 Patients with acute lymphoblastic leukemia must be in morphological remission (< 5% blasts) at the time of transplant. Patients with acute

non-lymphocytic leukemia will preferably be in morphologic remission but may be enrolled when aplastic after chemotherapy or with < 20% blasts. Patients with lymphoma must be in complete or close to complete remission (if residual adenopathy, PET scan must be negative or only have slight uptake, eg. SUV < 2).

3.2.4 Patients must lack a healthy HLA-identical related donor of at least one year of age.

3.2.5 Patient must have a mismatched related or an unrelated donor who is:
a) Able to receive G-CSF and undergo apheresis either through placement of catheters in antecubital veins or a temporary central venous catheter,
b) Healthy,
c) Willing.
d) For recipients of an unrelated donor transplant, recipient eligibility will be restricted as follows if in the judgment of the recipients' transplant physician, the recipient cannot receive a transplant with combined positive and negative fractions as described in Section 6.1.3.2 or an unmanipulated PBSC product. The restrictions based on recipient weight and number of apheresis procedures for the donor for these patients will be:
> If donor PBSC are collected in one day, the recipient weight must be 37 kg or less
> If donor PBSC are collected in two days, the recipient weight must be 26 kg or less
See Donor selection criteria below for additional considerations.

3.2.6 If only one mismatched related relative is available, an acceptable unrelated donor must be identified as a backup. If only one acceptable unrelated donor is available, a mismatched related donor must be identified as a backup. Section 3.2.6 can be waived in extraordinary situations when the benefit of transplant outweighs the risk of graft failure in the judgment of the principal investigator.

3.2.7 Patient, parent, or authorized guardian must sign informed consent for this study.

3.3 Recipient exclusion criteria

3.3.1 Patient with an anticipated life expectancy of < 1 month

3.3.2 Active infectious hepatitis or CMV infection

3.3.3 HIV or HTLV-I/II infection

3.3.4 Serious infection (bacterial, fungal, viral) within the last 4 weeks (the interval can be less than 4 weeks under extenuating circumstances)

3.3.5 Cardiac ejection fraction < 45%; can be lower if patient is not in clinical cardiac failure and a reduced intensity conditioning regimen is used.

3.3.6 Creatinine clearance <60 ml/min/1.73 m^2; can be lower if a reduced intensity conditioning regimen is used.

3.3.7 Pulmonary diffusion capacity (adjusted for Hgb), FEV_1, or FVC <60% of predicted or O2 sat < 94% if unable to perform PFTs; can be lower if a reduced intensity conditioning regimen is used.

3.3.8 Serum ALT > 3 x upper limit of normal (can be up to 5 x upper limit of normal if a reduced intensity conditioning regimen is used) or bilirubin > 2. The bilirubin criteria for sickle cell disease patients is direct bilirubin > 2 x upper limit of normal.

3.3.9 Performance score (Lansky/Karnofsky) < 50

3.3.10 Any condition that compromises compliance with the procedures of this protocol, as judged by the principal investigator.

3.4 Donor eligibility criteria

3.4.1 For Related donor: sibling, half-sibling, parent, cousin, aunt, uncle or grandparent will all be considered eligible.

3.4.2 For Related donor: At least a 4 out of 8 HLA antigen genotypic match (haplocompatible). The 8 antigens are at the A, B, C, and DR loci.

3.4.3 For unrelated donor: At least 6 out of 8 HLA antigen match (if two mismatches, they must be at different loci).

3.4.4 Complete medical history, physical and screening for infectious diseases that are acceptable for donation. See Section 7.1.2 for details of evaluation.

3.4.5 If donor is female and of child-bearing age, negative pregnancy test.

3.4.6 Absence of anti-HLA antibodies in recipient directed against donor antigens.

3.4.7 If the recipient is CMV seropositive and has a non-malignant disease that is not anticipated to be fatal in the near term, the donor must be CMV seropositive. If the recipient has a malignant disease or potentially fatal non-malignant disease like HLH, the use of a CMV seronegative donor is discouraged but not prohibited.

3.4.8 Donor, parent, or authorized guardian must be willing to sign informed consent for this study.

3.5 Donor selection

3.5.1 Potential donors will have HLA typing. They will also be offered optional participation in NK cell killer immunoglobulin-like receptor (KIR) typing. KIR typing will not be performed on donors for recipients who express all 3 KIR ligands.

3.5.2 Criteria to consider when choosing among donors are:

a). For haploidentical donors, HLA disparity i.e. 2 Ag mismatch preferred over 3 Ag mismatch; Drβ1 match preferred over class I match

b). KIR mismatch in GVH direction is preferred for patients with malignant disorders and may be helpful in other settings because it has been associated with improved engraftment

c). CMV positive if recipient is CMV positive

d). ABO compatibility

3.6 Patient recruitment

Candidates are referred to study investigators from pediatric hematology/oncology specialists and immunologists. Patients and parents will be seen in the pediatric BMT outpatient clinic or as an inpatient where consent will be presented.

3.7 Patient registration

The patients will be registered by completion of eligibility forms and each patient will be assigned a unique subject study ID.

4.0 INVESTIGATIONAL TREATMENT PLAN

4.1 Dose

The plan is to use the CliniMACS® device to prevent severe (grade III/IV) acute GVHD without the need for post-transplant immunosuppressive medications. The target dose of $CD34^+$ cells is $\geq 20x10^6$/kg for mismatched related donors and $\geq 10x10^6$/kg for matched unrelated donors. The target dose of $CD3^+$ cells is $\leq 3x10^4$/kg. The ClinicMACS® $CD34^+$ Reagent System will be used for donor stem cell selection.

4.2 Duration of therapy

Patients will be followed for outcomes related to the device including acute and chronic GVHD and engraftment. Patients will also be followed long-term for disease-free survival.

5.0 DOSE MODIFICATIONS AND TOXICITIES

These are part of transplant-related issues but not specific to the use of the investigational device (CliniMACS® device).

5.1 Recipient toxicity from the conditioning regimen

5.1.1 **Thiotepa** - Risks include alopecia, mucositis, and hepatic toxicity. There is also cutaneous toxicity which can result in erythema and breakdown of the skin especially in the neck, axilla, and inguinal and perianal areas. This can be painful and can become secondarily infected. Significant skin toxicity can be prevented by frequent baths or showers when the drug is given. In combination with fludarabine and TBI the risk of fatal hepatic toxicity or fatal pulmonary toxicity is <5%. There has also been a multiorgan failure syndrome reported in association with thiotepa containing regimens. The incidence is low (<5%) and was seen when doses greater than 10 mg/kg were used.

5.1.2 **Fludarabine** - The primary toxicity of fludarabine in the doses used has been severe T and B cell immunodeficiency. It has also been associated with the development of immune mediated hemolytic anemias, unlikely to occur as part of an ablative conditioning regimen for BMT. Other side effects include nausea, vomiting and neurotoxicity (seizures, confusion). Neurotoxicity is very unlikely at the doses used on this study.

5.1.3 **Total body irradiation (TBI)** - The total TBI dose is 1200 cGy given in 6 fractions. All fractions will use 50% shielding of the lungs. Potential acute toxicities associated with the use of TBI include nausea, vomiting, and parotitis. Subacute complications or side effects of TBI include hair loss, mucositis, diarrhea, veno-occlusive disease, leukoencephalopathy and interstitial pneumonitis. Chronic complications include cataracts in about 20% of cases, delayed sexual development, short stature, and sterility in >95% of cases, and thyroid dysfunction in 5-10% of cases.

5.1.4 **Rabbit anti-thymocyte globulin (rATG)** - The most important risk associated with rATG is the development of a severe allergic reaction (i.e. anaphylaxis) which is rare. More common side effects of rabbit ATG include fever and rash.

5.1.5 **Melphalan** – common side effects include nausea, vomiting, low blood counts, mucositis, sterility, and hair loss. Much less common side effects include liver and lung damage.

5.1.6 **Cyclophosphamide** – common side effects include nausea, vomiting, low blood counts, sterility, and hair loss. Less common side effects include water retention, damage to the lining of the bladder leading to blood in the urine, and liver damage. Rare side effects include damage to the lungs and heart and secondary cancer (leukemia and lymphoma).

5.1.7 **Busulfan** – common side effects include nausea, vomiting, low blood counts, mucositis, sterility, temporary darkening of the skin, and hair

loss. Less common side effects include liver damage. Rare side effects include lung injury and seizures (the latter are rare when prophylactic anti-convulsants are used).

5.1.8 **Rituximab –** common side effects include fever, chills, nausea, weakness, headache, low blood pressure, itching, rash, bronchospasm, abdominal pain, vomiting, anemia, achy joints and muscles, dizziness, congestion, low blood counts, suppression of B lymphocytes resulting in a greater risk of infections. Rare side effects include angioedema, severe reactivation of hepatitis B infection, liver failure, angioedema involving the skin, mouth, and GI and GU tracts, and progressive multifocal leukoencephalopathy.

5.1.9 **Infection and bleeding** - In general, due to the 1-2 weeks of neutropenia, thrombocytopenia and mucositis, there is a significantly increased risk of infection and bleeding. The mortality associated with these complications is generally <5%.

5.1.10 **Mortality** - The reported transplant related mortality (organ failure, infection, bleeding) is ~30% for patients with high-risk malignancies (25,30,31). In a limited number of children with non-malignant diseases the TRM was lower (10%) but until more patients in this category are evaluated, we will assume it is as high as 30%.

5.1.11 **Secondary malignancy** - There is also an overall reported risk of ~2-10% of a malignancy occurring up to 15 years post-transplant. The majority of these are lymphoproliferative disease related to EBV infection. Because these patients will not receive cyclosporine prophylaxis or post-transplant ATG, and because the processing procedure removes almost all B cells from the donor graft (the source of EBV in these circumstances), this risk is likely to be lower.

5.1.12 **Decreased IQ** - There is about a 30% risk of a decreased IQ (7 points) at 1 year post transplant (36). This occurs regardless of the conditioning regimen and by 3 years there appears to be some recovery of IQ points. Of the remaining patients, 1/3 have no change in their IQ and 1/3 increase their IQ by about 7 points.

5.2 Recipient Toxicity from Transplant

5.2.1 **Failure of engraftment** - Published reports using 1-10x10^6 $CD34^+$ cells/kg haplocompatible stem cell enriched T cell depleted PBSC with TBI-containing regimens have initial engraftment rates of >80% although with second transplants the engraftment rate has been >90%. We believe that the combination of thiotepa, fludarabine, ATG and TBI plus the large number of $CD34^+$ cells in the graft should result in a primary engraftment rate of at least 80%.

5.2.2 **Delayed T-cell reconstitution and increased infections** - The chance of this occurring is likely. In both children and adults who have been heavily pretreated it has taken as long as a year for T cell immunity (i.e. $CD4^+$ cells > 200) to recover. The most commonly reported cause of transplant-related death with this kind of transplant is infection.

5.2.3 **Increased risk of leukemic relapse** - The extent of this problem with PBSC is unknown. The studies of $CD34^+$ PBSC haplocompatible

transplants that have been reported to date do not demonstrate a higher than expected relapse rate.

5.2.4 **Graft vs. Host Disease** - In other studies in which $\leq 7x10^4$ $CD3^+$ cells/kg of haplocompatible PBSC are infused the incidence of grade III-IV acute GVHD has been <5%.

5.3 Toxicity from Miltenyi Biotec Inc. CliniMACS® reagent system processing

5.3.1 **Risk of contamination** of the cell preparation with biologic or other foreign material. The sterility of system components that contact the cell sample and the detailed processing steps are designed to minimize potential contamination.

5.3.2 **Paramagnetic microspheres** - Significant animal and human studies have been done using these super-paramagnetic beads which are small in size (~50 nm in diameter) and are composed of iron oxide and dextran conjugated to murine monoclonal antibodies. These magnetic particles form a stable colloidal suspension and do not precipitate or aggregate in magnetic fields. The concentration of the conjugate is equivalent to 22 µg of antibody protein per ml of reagent, 800 µg/ml of dextran and 800 µg/ml of iron. Detailed toxicity studies have been undertaken to assess the safety of the antibody reagent when delivered to monkeys and rabbits in dosages significantly greater than the projected maximum dosage anticipated in clinical use (CliniMACS® Investigator brochure). There have been more than 300 separations for clinical use of the CliniMACS® system.

5.3.3 **Reaction to CliniMACS® reagent** - (murine monoclonal antibody conjugated to an iron-dextran moiety). Iron dextran is commercially available as a sterile solution of iron dextran complex for the treatment of severe iron-deficient syndromes. It contains 5% iron and 20% dextran, and its safety profile has been well characterized. Iron dextran solution contains 50 mg/ml of elemental iron, most of which is present in the ferric state. A total dose of iron-dextran for the average 70 kg person is calculated to be approximately 2 gm over several days (single dose of 100 mg). The iron dextran exposure from a singe CliniMACS® separation is ~0.5 mg and less than 1 mg dextran, 100x lower than a single dose and 1000x lower than a total dose.

5.3.4 **$CD34^+$ monoclonal antibody** - The other reagent is the murine monoclonal antibody in which there is a risk of an anaphylactic reaction. The anti-CD34 monoclonal antibody, AC101 has been tested for safety in conformance with US standards. Systemic reactions appear related to the dose and rapidity of administration. Therapeutic levels (for cancer therapy or prevention of graft rejection) of mAb appear to be in the range of 2.5-5 mg/ml. The most commonly reported side effects have been myalgia, arthralgia, and flu-like symptoms. The CliniMACS® system results in the administration of a maximum of <15µg of antibody, 100x lower than therapeutic levels. Furthermore, studies have shown that the levels of antibody used in the CliniMACS® system do not induce complement activation in vitro.

5.4 Donor PBSC toxicity

The toxicities listed below are of concern for all donors and not specific to this protocol or the use of the investigational device (CliniMACS® device).

5.4.1 **G-CSF** - For the recruitment of PBSC, G-CSF is known to cause bone pain in most patients as well as other symptoms including headache, bone discomfort or ache, ankle swelling or fluid retention. These symptoms are dose related and generally controlled with analgesics such as Tylenol or ibuprofen (17-19). There is at least a theoretical risk of inducing a malignancy with G-CSF although the extensive experience to date with normal donors does not indicate that this will be a problem. Patients with aplastic anemia or Kostmann's syndrome who have been chronically treated with G-CSF do not have any higher incidence of malignancy than is normally found with these disorders.

5.4.2 **Apheresis** - This has been associated with decreased platelets and temporary hypotension as well as hypocalcemia and some risk of bleeding because of anti-coagulation. There is also pain from the insertion of needles into the antecubital veins and the discomfort of a central line if necessary. All of these are reversible and have been tolerated in previously reported studies. There is the small risk (<1%) that a healthy donor might require a platelet transfusion. There is also the risk that peripheral venous access might be inadequate and placement of a temporary central line under local anesthesia will be necessary (17,18). The risks of inserting a central line include bleeding, infection and/or pneumothorax. There are abnormalities in the circulating white cell populations (T cells and stem cells) that occurs after apheresis in normal donors. Most of these appear to resolve (i.e. numbers return to normal) within the first 3 months post donation, but there may be abnormalities or long-term side effects that at this time have not been identified.

6.0 DEVICE INFORMATION
(Miltenyi Biotec, Inc. $CD34^+$ CliniMACS® reagent system information)

6.1 $CD34^+$ cell processing
The collection will be stem cell enriched and T-cell depleted. The $CD34^+$ cells are positively selected using the CliniMACS® System.

The pheresis product may be stored overnight at 4^oC and at a concentration $\leq 200 \times 10^6$ cells/ml and processed the following morning. Products may be pooled (i.e. first and second collection pooled and third and fourth collection pooled) for processing and cell selection, or the collections may be processed and $CD34^+$cells selected on each day. The determination of whether to store product overnight or select daily will be individualized per patient and be based on patient cell counts and $CD34^+$ column cell capacity. $CD34^+$ cell selection will be performed using the Miltenyi CliniMACS® system. The processing will be performed at University of California-San Francisco.

6.1.1 The target cell doses after processing will be $\geq 20x10^6$ $CD34^+$ cells/kg for mismatched related donors and $\geq 10x10^6$ $CD34^+$ cells/kg for unrelated donors. A dose of $\geq 8x10^6$ $CD34^+$ cells/kg will be acceptable for mismatched related donors and a dose of $\geq 4 x10^6$ $CD34^+$ cells/kg will be acceptable for unrelated donors. The target T cell dose will be $\leq 3x10^4$ $CD3^+$ cells/kg.

At investigator discretion, the CD34+ cell dose for PBSC transplant may be adjusted for overweight patients as follows:
The dose may be reduced to a dose based on adjusted body weight if the patient's actual weight is greater than 120% of the ideal body weight.

The ideal and adjusted body weights will be calculated according to the LCH Blood and Marrow Transplant Program High-dose Chemotherapy Orders policy. The investigator will consider factors such as disease for which the transplant was performed, patient's body frame and build, etc.

6.1.2 The collection doses that correspond with the post-processing doses in 6.1.1 will be higher than these doses because a significant portion of the CD34+ cells can be lost in the processing. Because of the large dose required, it is possible that an inadequate dose will be collected. If this occurs with an unrelated donor, the PI may choose to not process the cells or to combine the positive and negative fractions of a processed graft and give a T replete graft (as in Section 6.1.3.2) or to not utilize these cells and collect from another donor if a T cell-depleted graft is deemed in the patient's best interest. In the latter case, the graft may be cryopreserved to be used in the future if necessary (eg. graft failure). If an inadequate dose is collected from a related donor, then a backup donor will be used. In the situation that a backup donor is used, it is likely and acceptable that the PBSC infusion from the backup donor will occur after the original day 0.

6.1.3 In the unlikely event that the $CD3^+$ cell count is too high in order to achieve the minimum acceptable $CD34^+$ cell dose, there will be the following options:

6.1.3.1 For mismatched related donor and unrelated donor transplants:

a) Patient can be given long course methotrexate.

6.1.3.2 For unrelated donor transplants:

a) Positive and negative fractions can be combined to reconstitute the original product and then the patient can be started on standard tacrolimus and short course methotrexate. Sirolimus and/or ATG may also be used at physician discretion.

6.1.4 The $CD34^+$ selected stem cells will usually be cryopreserved prior to the transplant.

6.1.5 The stem cells will be infused intravenously into the recipient. Prophylactic cefazolin will be given for 24 hours beginning just prior to the infusion unless the patient is receiving IV antibiotics with similar bacterial coverage.

6.1.6 If $>20x10^6$ $CD34^+$ cells/kg are available from a mismatched related donor, then approximately $20\text{-}22x10^6$/kg will be infused and the remainder will be cryopreserved. For an unrelated donor, an aliquot of cells can be cryopreserved at physician discretion if $>10x10^6$ $CD34^+$ cells/kg are available. For an unrelated donor, the infused dose will not exceed approximately $20\text{-}22x10^6$/kg. Depending on the clinical scenario, the treating physician may choose to infuse a smaller dose than above that still exceeds the minimum dose in section 6.1.1 to allow a backup graft to be cryopreserved.

6.1.7 Five aliquots of the negative fraction (<u>non</u> $CD34^+$ selected cells) containing the $CD3^+$ cells will be made and cryopreserved for future DLI. Each aliquot will contain a minimum of $3x10^4$ $CD3^+$ cells/kg body weight of the recipient.

6.2 Release of product for transplant

The studies that will be done in addition to the cell immunophenotyping will be sterility (routine USP culture for bacteria and fungi), endotoxin testing, gram stain, and viability. We already have experience with all of these procedures in other protocols, specifically, BB-IND 8817.

Viability, gram stain and endotoxin testing will be done prior to the release of the product for infusion. If the viability is >70%, the gram stain is negative, and the $CD34^+$ cell and $CD3^+$ cell doses meet the criteria, the product will be released for infusion. If any of these criteria are not met, the PI will need to assess the risk of using the product and to decide if it will be released.

If the culture becomes positive, appropriate antibiotics will be given to the recipient. The donor will undergo a clinical evaluation for infection and the reagents (including the aliquoted reagents) and procedures will be tested and reviewed to try to identify the source. If the gram stain is positive, the cells will be cryopreserved until the culture results are back, and the donor may undergo another leukapheresis providing he or she has no clinical evidence of infection.

6.3 Shipping of $CD34^+$ selected PBSC

USCF will ship the $CD34^+$ selected PBSC to arrive on Day 0 of transplant. A portion of the graft will be retained at UCSF and/or a backup donor will be assigned in case a rare catastrophe (eg. plane crash) leads to loss of the cells.

7.0 SCHEDULE OF ASSESSMENTS AND THERAPY

Timing of protocol therapy administration and response assessment studies are based on schedules derived from the experimental design or on established standards of care. Minor unavoidable deviations (up to 72 hours) from protocol directed therapy and/or disease evaluations for valid clinical, patient or family logistical, or facility or procedure and/or anesthesia scheduling issues are acceptable. The infusion details are intended as guidelines and may be modified as clinically necessary. Necessary minor deviations for common logistical or clinical reasons will not be considered violations at audit. Minor delays as described above will not be construed as prospectively planned or instituted on a routine basis so advance IRB approval will not be required.

7.1 Screening assessments

7.1.1 Recipient pre-transplant evaluation (within 30 days of admission unless specified below):

7.1.1.1 Pulmonary function tests (PFTs) if patient age allows (usually > 5 years of age); if not, the patient will have pulse oximetry. PFTs do not need to be repeated if the patient has had acceptable PFTs in the 3 months prior to admission and has not had chemotherapy with pulmonary toxicity or pneumonia since then.

7.1.1.2 Echocardiogram and ECG. These tests do not need to be repeated if they have been acceptable in the 3 months prior to admission and the patient has not had chemotherapy with cardiac toxicity since then.

7.1.1.3 CXR or chest CT scan

7.1.1.4 Creatinine clearance by Schwartz formula, urine collection, or GFR scan

7.1.1.5 Testing for syphilis, CMV, HIV, EBV, HSV, VZV, Hepatitis B & C, HTLV I/II, and toxoplasmosis. Testing does not need to be repeated if it has been previously positive (ie. CMV positive).

7.1.1.6 ABO typing and antibody screen. The ABO typing does not need to be repeated within 30 days if the patient's ABO typing has been performed. The antibody screen does need to be done within 30 days.

7.1.1.7 Liver function tests, electrolytes, BUN, creatinine, CBC, differential.

7.1.1.8 Bone marrow within 2 weeks of admission for patients with MDS/leukemia. Lumbar Puncture (LP) at this time as indicated.

7.1.1.9 HLA typing. This does not need to be repeated within 30 days if the patient has already had original and verification HLA typing.

7.1.1.10 Complete history and physical exam

7.1.1.11 IgG level

7.1.1.12 Evaluation for anti-HLA antibodies against donor antigens prior to stem cell collection and also within 30 days of admission if collection occurs before this.

7.1.1.13 Pregnancy test if female and of childbearing age. Also, must be performed within 7 days of admission.

7.1.2 Donor pre-apheresis evaluation (must be done within 30 days of donation except as noted below; the following is for related donors, unrelated donors will be evaluated according to the guidelines of the National Marrow Donor Program):

7.1.2.1 Complete history will include:

7.1.2.1.1 Surgical history

7.1.2.1.2 Review of systems

7.1.2.1.3 History of inherited conditions and chronic illness

7.1.2.1.4 History of hematological problems and immunological disorders

7.1.2.1.5 History of cancer

7.1.2.1.6 Donor health questionnaire which includes transfusion, vaccination and travel history

7.1.2.1.7 List of current medications and allergies.

7.1.2.2 Screen for evidence of syphilis, CMV, HIV, EBV, Hepatitis B & C, HTLVI/II, West Nile virus, Chagas' disease, and toxoplasmosis. Testing does not need to be repeated if it has been previously positive (ie. CMV positive).

7.1.2.3 ABO typing and antibody screen. The ABO typing does not need to be repeated within 30 days if the patient's ABO typing has been performed. The antibody screen does need to be done within 30 days.

7.1.2.4 Liver function tests, electrolytes, BUN, creatinine, CBC, diff, PT, PTT

7.1.2.5 HLA typing. This does not need to be repeated within 30 days if the donor has already had original and verification HLA typing.

7.1.2.6 Optional NK cell KIR typing. This does not need to be done within 30 days of donation. KIR typing will not be performed on donors for recipients who express all 3 KIR ligands. Potential donors will be offered participation in KIR phenotyping by flow cytometry and KIR genotyping by PCR in

the laboratory of Dr. Wing Leung at St. Jude Children's Research Hospital. See section 7.8.1 and Appendix V.

7.1.2.7 ECG. The ECG does not need to be repeated if it was acceptable in the 3 months prior to admission and the donor has been well since then.

7.1.2.8 Chest x-ray. The CXR does not need to be repeated if it was acceptable in the 3 months prior to admission and the donor has been well since then.

7.1.2.9 Pregnancy test if female and of child-bearing age.

Donor eligibility will be determined in accordance with 21 CFR 1271.45-.90 and for related donors the LCH BMT Program Hematopoietic Progenitor Cell Donor and Recipient Evaluation Guidelines.

7.2 Therapy

7.2.1 Recipient cytoreductive regimen - this will be patient-specific.

The standard regimen will be:

Total Body Irradiation (TBI) followed by chemotherapy (see table below):

Day – 9	200 cGy TBI x 2
Day – 8	200 cGy TBI x 2
Day – 7	200 cGy TBI x 2
Day – 6	Fludarabine 40 mg/m^2 (1.33 mg/kg if $\leq$12 kg body weight) Thiotepa 10 mg/kg/day divided into 2 doses 12 hours apart
Day – 5	Fludarabine 40 mg/m^2; Rabbit ATG 1.5 mg/kg
Day – 4	Fludarabine 40 mg/m^2; Rabbit ATG 1.5 mg/kg
Day – 3	Fludarabine 40 mg/m^2; Rabbit ATG 1.5 mg/kg
Day – 2	Fludarabine 40 mg/m^2; Rabbit ATG 1.5 mg/kg
Day – 1	Rest
Day 0	Transplant

Drug doses may be reduced to a dose based on adjusted body weight if the patient's actual weight is greater than 120% of the ideal body weight. The ideal and adjusted body weights will be calculated according to the LCH Blood and Marrow Transplant Program High-dose Chemotherapy Orders policy. The investigator will consider factors such as disease for which the transplant was performed, patient's body frame and build, etc.

Example of other conditioning regimens include (see Appendix I for details):

Substitution of melphalan 140 mg/m^2 for TBI

Substitution of busulfan and melphalan for TBI and thiotepa

Substitution of cyclophosphamide for thiotepa in patients with Fanconi anemia

These modifications will be based upon published conditioning regimens (22,30-34) but minor adjustments can be made to adjust for the clinical scenario.

Patients with severe combined immunodeficiency and lack of NK cells or function and no GVHD due to maternal engraftment may be transplanted without conditioning.

Total dose of the TBI (1200 cGy) will be delivered in 200 cGy fractions separated by at least 6 hours. The patient will be treated with AP and PA fields and lungs will be partially blocked such that the dose to the lungs will be 600cGy (**50% clinical shielding of lungs for <u>ALL</u> 6 doses**). Alternatively, 100% shielding of the lungs can be used for 3 doses and no shielding for the remainder if necessary for the clinical circumstances. Also, for patients with ALL, an

additional dose boost to the testes of 400 cGY in a single fraction may be administered.

Thiotepa will be given IV on day –6 at a dose of 5 mg/kg every 12 hrs x 2. Each infusion will be given over 3-4 hrs. Very careful attention to skin care during thiotepa treatment is necessary. Supportive skin care measures will be followed according to LCH Blood and Marrow Transplant Program Guidelines of Use of Thiotepa to prevent skin toxicity.

Fludarabine will be administered IV each day, from–6 to–2, at a dose of 40 mg/m^2 as a 30 minute infusion. The fludarabine dose will be reduced for patients with creatinine clearance less than or equal to 70 ml/min/1.73m^2. A reduced dose of fludarabine (i.e. 30 mg/m2/day for 5 days) will be administered to participants with a history of significant neurologic disease, CNS-directed therapy or toxicity such as CNS irradiation, grade III-IV chemotherapy-induced neurotoxicity, and/or those receiving a CNS radiation boost with this transplant.

Rabbit ATG will be infused each day, from –5 to –2 as six hour infusion. The dose will be 1.5 mg/kg/day.

Patients with a history of EBV infection who will not receive TBI will be given rituximab IV on day -1. The dose will be 375 mg/m^2/dose.

7.2.2 GVHD prophylaxis and therapy

7.2.2.1 There will be no post-transplant GVHD prophylaxis given unless the $CD3^+$ cell dose is too high (see Section 6.1.2).

7.2.2.2 Initial treatment of GVHD will usually be prednisone or methylprednisolone 1-2 mg/kg/d. A rapid taper can be used if there is a quick and complete response. Additional therapy may be added if needed. Alternative therapy can be used depending on the clinical scenario (eg. It may be prudent to avoid steroids in patients with fungal infections).

7.2.3 Infection prophylaxis and pre-emptive therapy

The infection prophylaxis and therapy listed below can be modified if clinically necessary (this includes changing drugs and time period as clinically relevant).

7.2.3.1 Prophylaxis against Herpes viruses:

a) Acyclovir 500 mg/m2/dose IV q8h started on day -1 and continued until day +21 or discharge. The acyclovir dose may be adjusted in patients > 120% of IBW. This will be the first choice for prophylaxis.

b) Foscarnet 60 mg/kg/dose q12h started on day +1 and continued until day +21 or discharge may be given to CMV seropositive transplant recipients.

c) After completing acyclovir IV or foscarnet, valganciclovir 15 mg/kg PO daily (max 900 mg) through day +42 after transplant depending on blood counts. This is particularly important for CMV seropositive transplant recipients.

d) After completing valganciclovir or in place of if counts do not permit, acyclovir approximately 20 mg/kg/dose (max 800 mg) PO TID until the CD4>200 and for a minimum of 6 months. Valacyclovir (Valtrex) 500 mg PO TID may be used as an alternative if patient weight permits.

7.2.3.2 CMV: Patients will have CMV testing by PCR 2x per week (or weekly if donor and recipient are CMV negative) starting on approximately day +10 until day +100 after transplant and then q2 weeks until CD4>200/ul. Monitoring should be more frequent in patients who have prolonged CMV reactivation after transplant. If the PCR test becomes positive, ganciclovir will be started at an induction dose of 5 mg/kg IV q12 x 7-14 days, then a maintenance dose of 5 mg/kg/day 5-7 days per week until PCR is negative x 2 weeks (assuming the patient remains asymptomatic). Alternatively, patients may be treated with foscarnet induction and maintenance. Valganciclovir can be used as prophylaxis following ganciclovir or foscarnet therapy if CMV reactivation occurred when the patient was on acyclovir prophylaxis. Valganciclovir can also be used for therapy at physician discretion.

7.2.3.3 EBV: EBV PCR will be obtained weekly starting on approximately day +10 post SCT and continuing until day +100 after SCT. Monitoring should be more frequent in patients who have EBV reactivation after transplant. Monitoring will be every 1-4 weeks from day +100 to day +180 depending on the clinical scenario. Patients who have no T cell recovery during this time are at greatest risk. If the EBV viral load is positive, rituximab 375 mg/m^2 IV weekly x 4 will be given. CT scan of head, neck, chest, abdomen, and pelvis or PET/CT scan should be considered to evaluate for adenopathy. A therapeutic donor lymphocyte infusion can also be considered.

7.2.3.4 HHV-6: HHV-6 PCR will be obtained 2x/week starting on approximately day +10 until day +60 after transplant and then 1-2x/wk until day +100. HHV-6 may be followed after day +100 in patients who have had reactivation. If the PCR test becomes positive, ganciclovir will be started at an induction dose of 5 mg/kg IV q12 x 7-14 days, then a maintenance dose of 5 mg/kg/day 5-7 days per week until PCR is negative x 2 weeks (assuming the patient remains asymptomatic). Alternatively, patients may be treated with foscarnet induction and maintenance. Valganciclovir can be used as prophylaxis following ganciclovir or foscarnet therapy if HHV-6 reactivation occurred when the patient was on acyclovir prophylaxis. Valganciclovir can also be used for therapy at physician discretion. Reactivation after the initial occurrence will be treated according to the LCH BMT HHV-6 policy and the clinical scenario.

7.2.3.5 Adenovirus: Stool will be monitored weekly for adenovirus until discharge. If the stool test is positive, then a PCR will be done on blood, stool, and urine. Adenovirus PCR on blood will be obtained weekly starting on approximately day +10 post SCT and continuing until day +100 after SCT. For adenovirus in the stool and/or blood by PCR, cidofovir will be given. Treatment of isolated stool positivity may be based on the clinical situation (eg. if patient has renal dysfunction). This will be given as 5 mg/kg IV weekly or 1 mg/kg IV three days per week until the PCR is negative. Testing for adenovirus should be considered in patients with fever, URI symptoms, or diarrhea.

7.2.3.6 Toxoplasma: Toxoplasma PCR will be obtained weekly starting on approximately day +10 post SCT and continuing until day +100 after SCT for patients who are seropositive or have a seropositive donor. Monitoring will be every 1-4 weeks from day +100 to day +180 depending on the clinical scenario. Prophylaxis with trimethoprim/sulfamethoxazole per PCP guidelines is preferred for patients at risk. PCR testing will be continued beyond day +180 for toxo seropositive recipients who have CD4 < 200.

7.2.3.7 Pneumocystis carinii prophylaxis will be trimethoprim/sulfamethoxazole 5 mg/kg/day PO of TMP component divided BID for 3 consecutive days per week until the CD4 > 200 and for a minimum of 6 months after transplant and after discontinuation of immunosuppression. Therapy can be reduced to 2 days per week if needed for low blood counts. If TMP/SMX is not tolerated, alternative prophylaxis will be given per institutional policy.

7.2.3.8 Fungal prophylaxis: Voriconazole 4 mg/kg (max 200 mg) PO (or IV if PO not tolerated) BID or posaconazole 4 mg/kg (max 200 mg) PO TID will be started on Day -1 and continued until CD4 > 200.

7.2.3.9 Bacterial prophylaxis: Ciprofloxacin 10 mg/kg PO BID or 7.5 mg/kg IV starting on day -1 and continuing until ANC > 500 or initiation of antibiotics for fever and neutropenia.

7.2.3.10 IVIG: Gammaglobulin will be administered at a dose of 200 mg/kg IV every 2 weeks while hospitalized and then 400-500 mg/kg IV every 4 weeks as an outpatient. The dose may be rounded to the nearest vial size. IVIG will be given for at least 6 months after transplant. After that time, IVIG may be discontinued when the IgM level is within the normal range within a few days prior to when IVIG is due. An isohemagglutinin (IgM) titer of ≥1:8 and a normal IgA level can be used as additional indications for discontinuation of IVIG at physician discretion. Isohemagglutinin titers are not relevant if the donor's blood type is AB.

7.2.4 Supportive care

7.2.4.1 Post-transplant G-CSF may be started on day +14 if the ANC is <500; G-CSF may be started as early as Day +6 in patients with active fungal infections at the discretion of the treating physician or for other serious infections with PI approval.

7.2.4.2 All transfusions (except the stem cell product) will be irradiated and leukodepleted. In addition, CMV negative PRBC and platelets will be used, when available, for CMV negative recipients.

7.3 Mobilization and collection of donor peripheral blood stem cells

The donor will receive at least 4 days of granulocyte colony stimulating factor (G-CSF, Neupogen®) administered subcutaneously (8 mcg/kg/dose) twice a day. In donors over 120% of IBW, an adjusted G-CSF dose may be considered. The total daily dose may be reduced to 10 mcg/kg and the dose may be given once a day for an unrelated donor if the recommended dosing is not possible. The donor will undergo an outpatient apheresis using antecubital veins for venous access if possible. If the peripheral venous access is inadequate, a temporary central line will be placed as an outpatient. The morning dose of G-CSF will be held on the

day of collection if apheresis will take place in the morning. If the dose is held in the morning, it should be given soon after the completion of the apheresis procedure if another apheresis is planned. G-CSF will be continued until the completion of apheresis. If the donor's white blood count determined on the 4th day and after apheresis on subsequent days of G-CSF (for patients receiving 8 mcg/kg/dose q12) is greater than 60 x 10^9 cells/L, the G-CSF dose should be decreased to 8 ug/kg daily. If the donor's blood count is greater than 80 x 10^9 cells/L, the G-CSF dose should be held.

The preferred apheresis procedure for related donors will process 5 blood volumes (max 25 L). Pheresis performed at Carolinas Medical Center will be performed in accordance with the LCH BMT Program Pediatric PBSC Mobilization and Collection Guidelines. The related donor may require 1-4 apheresis procedures to achieve an adequate cell dose. Apheresis performed on an unrelated donor will be performed according to the guidelines of the donor center and National Marrow Donor Program (NMDP). Unrelated donors will only have 1 large volume (24L in one day) or 2 standard volume (12L/day x 2 days) apheresis procedure(s) according to applicable NMDP protocols and procedures.

The donor PBSC may be collected before the recipient has met all eligibility criteria if the treating physician believes that it is likely that the recipient will meet the criteria. The recipient, parent, or guardian should sign consent for the transplant prior to the donor PBSC collection.

7.4 Treatment of graft failure

If at 4 weeks post-transplant there is no evidence of engraftment based upon blood counts, bone marrow examination and chimerism assays, infusion of a second PBSC transplant will be performed using the same or another donor (the other parent, sibling, or unrelated donor). Additional chemotherapy and immunosuppression will be given prior to the second transplant as needed.

7.4.1 If pancytopenia occurs at a later time point, the same approach may be used. There may be other situations like persistent cytopenias or significantly decreased donor chimerism for which the physician may choose to use this approach.

7.5 Therapeutic donor lymphocyte infusion (DLI)

7.5.1 The following are common indications for DLI. DLI may be given for these indications at physician discretion based on the clinical scenario. For example, patients with malignant and non-malignant diseases may require different approaches. Patients may also receive viral-specific (eg. CMV and EBV-specific) T cells as an alternative.

7.5.2 Indication:

- **7.5.2.1** Mixed Chimerism (< 80% donor) or increasing recipient chimerism
- **7.5.2.2** Relapse
- **7.5.2.3** EBV-related PTLD
- **7.5.2.4** Viral infections that progress despite treatment with anti-virals

7.5.3 Collection of therapeutic donor lymphocytes:

- **7.5.3.1** DLI will usually be obtained from the cryopreserved negative fraction (T cell-containing) of the $CD34^+$ cell selected G-CSF-mobilized PBSC.

7.5.3.2 T cells from the G-CSF-mobilized PBSC may be less alloreactive than T cells collected from blood when G-CSF is not present. G-CSF increases Th2 cells that decrease IL-12 production and delays immune recovery. At the discretion of the treating physician, a new collection can be performed to provide DLI depending on the clinical situation (such as leukemic relapse). For larger recipients, the stem cell donor will undergo lymphocytopheresis to collect several $CD3^+$ cell doses, for example, in aliquots of 5 X 10^4/kg, 1 X 10^5/kg, 5 X10^5/kg, and 1 X 10^6/kg. For patients requiring small doses, donors may give whole blood if ABO compatibility permits the use of whole blood as a source of DLI.

7.5.4 Dose:

Indication for DLI	Persistent mixed chimerism[1] or EBV PTLD or viral infection		Increasing recipient chimerism[2] and/or cytogenetic/molecular relapse		Morphological relapse
Donor type	Slow[3]	Rapid[3]	Slow[3]	Rapid[3]	
Matched unrelated Donor[4]	1 x 10^5/kg	5 x 10^5/kg	5 x 10^5/kg	1 x 10^6/kg	Chemo →1 x 10^6/kg
Haploidentical Donor[5]	1 x 10^4/kg	5 x 10^4/kg	5 x 10^4/kg	1 x 10^5/kg	Chemo → 1 x 10^5/kg

[1] Persistent mixed chimerism requiring treatment will be donor % < 80%.

[2] Increasing mixed chimerism will be one 20 point drop in the percentage of donor cells or two values of greater than 10% over at least 2 weeks. A lower dose (than for persistent mixed chimerism) can be used for increasing recipient chimerism depending on the rate of increase and the clinical scenario.

[3] Disease pace: Slow = Chronic Leukemia and MDS without increased blasts; Rapid = Acute leukemia, lymphoma, and rapidly-progressive EBV PTLD. For diseases not falling into these categories, the choice of using the slow or rapid dose is at physician discretion.

[4] The doses for unrelated donors usually apply to matched unrelated donors. Donors with mismatch at HLA-C that is predicted to not be clinically relevant may also be treated at this dose at physician discretion. Mismatched unrelated donors will be treated at the Haploidentical Donor doses.

[5] The DLI dose for patients with 7/8-matched related donors (mismatch not at DR) can be treated with dose for matched unrelated donors at physician discretion.

7.5.4.1 If < 6 months post-SCT (high risk of GVHD because APCs are still recipient): At physician discretion, a dose that is 50% of that in the table can be given to decrease the risk of GVHD. The $CD3^+$ cell dose can be repeated every1-2 months.

7.5.4.2 If > 6 months post-SCT (APC's are donor so risk of GVHD much less): $CD3^+$ cell dose starts at dose in table above, but may be increased with each dose eg. 1 X 10^5/kg, then 5 X 10^5/kg, then 1 x 10^6/kg, etc.; sequential doses given every1-2 months.

7.5.4.3 Prior to starting DLI in patients that are on immunosuppression for GVHD but <u>without</u> active GVHD at the time, immunosuppression will be discontinued based on indication as follows:

a) Stopped for morphological relapse,

b) Weaned over 1 week for rapidly increasing mixed chimerism and/or cytogenetic/molecular relapse,

c) Weaned over 4 weeks for persistent mixed chimerism or slowly increasing chimerism; for patients with persistent

mixed chimerism, patients will be re-assessed when immunosuppression is stopped prior to giving DLI.

7.5.4.4 For patients with active GVHD, they will remain on their GVHD therapy and DLI will be given as soon as possible.

7.6 Follow-up assessments

7.6.1 Follow-up history and physical evaluations which are standard for all transplant patients occur monthly for 6-12 months, then every 3 months x 1 year then every 6 months x 1 year then yearly until 5 years post-transplant.

7.6.2 Follow-up studies will be performed according to BMT guidelines and include assessment of engraftment, marrow function, immune status, liver and kidney function, pulmonary function tests, and echocardiogram.

7.7 Schedule of immunological and engraftment studies

	Weeks©ƒ				**Months©ƒ**										**Years©**		
	4	8	12		4	5	6	9	12	15	18	21	24		3	4	5
Lymphocyte Phenotyping	X	X	X		X	X	X	X	X	X%	X	X%	X		X	X	X
T cell function*							X	X	X		X		X		X	X	X
Engraftment	X	X	X		X	X	X	X	X		X		X		X	X	X
Research immune recovery testing					Day 120		X		X								

*T cell function studies (mitogens) are optional after they become normal.
% The 15 and 21 month lymphocyte phenotyping are optional.
©If the patient has progression of primary disease, then the above tests are optional at the discretion of the physician.

Assessments can be +/- 7 days for evaluations through Day 120. Assessments can be within one month of target after Day 120 and within three months of target after 24 months.

These are the assessments for study and clinical purposes. In addition, these tests may be performed more often for clinical reasons as well. For example, engraftment studies will usually be performed to monitor for relapse in leukemia patients according to the LCH BMT Post-Transplant Evaluation Guidelines. Patients with non-malignant disease will usually be monitored every 1-2 weeks after engraftment until day +100 depending on the clinical scenario.

7.8 Research samples

7.8.1 NK cell receptor typing

7.8.1.1 NK cell KIR typing will be performed on potential donors in the laboratory of Dr. Wing Leung at St. Jude Children's Research Hospital. The results will be communicated to Dr. Gilman. The results may be used for donor selection for patients with leukemia at Dr. Gilman's discretion.

7.8.1.2 NK cell typing will be optional and dependent on the donor's willingness to participate.

7.8.2 Immune recovery testing at St. Jude

Testing will include analysis of T cell receptor excision circles (TREC) and T cell receptor repertoire (V beta typing). This will be performed at approximately Day 120, 6 months, and 1 year after transplant (see section 7.7). Blood will be sent to Dr. Wing Leung at St. Jude Children's Research Hospital. See Appendix V for details.

7.8.3 Immune recovery testing at Emory University

Testing will include analysis of T and B subsets and T cell activation by flow cytometry. Testing will also include evaluation of CMV and EBV specific T cells by tetramer analysis and cytokine production. This will be performed at approximately Day 120, 6 months, and 1 year after transplant (see section 7.7). Blood will be sent to Dr. Leslie Kean at Emory University. See Appendix V for details.

8.0 CRITERIA FOR TERMINATION

8.1 Conditions for terminating the study

8.1.1 The Principal Investigator may terminate the study for any of the following reasons:

8.1.1.1 Significant toxicities

8.1.1.2 If it becomes clear that the study treatment is less effective than other available treatments.

8.2 Conditions for individual patient termination

8.2.1 The Principal Investigator may terminate the participation of an individual patient for any of the following reasons:

8.2.1.1 Disease progression

8.2.1.2 Need for exclusionary concurrent treatment

8.2.1.3 Withdrawal of informed consent

8.2.1.4 Protocol non-compliance

8.2.1.5 Lost to follow-up

9.0 STATISTICAL CONSIDERATIONS

9.1 Hypotheses

Transplantation of stem cells that have been $CD34^+$ selected and T cell-depleted with the CliniMACS® device will prevent severe (grade III/IV) acute GVHD without the use of prophylactic post-transplant immunosuppression. The incidence of grade III/IV acute GVHD is predicted to be <10%. This has been our experience in 18 reported patients (22) and currently 25 treated recipients of haplocompatible donor transplants.

9.2 Accrual objectives

Originally, the plan was to enroll thirty patients into each of two cohorts (haplocompatible related donor and unrelated donor). The study objective is to determine the ability of $CD34^+$ selection using the CliniMACS® device as the sole GVHD prophylaxis to prevent severe (grade III-IV) acute GVHD by Day +30 after transplant in recipients of alternative donor stem cell transplants. Thirty patients will allow estimating the rate of severe acute GVHD with the 95% confidence interval of the maximum width of 0.25 if the observed rate of severe acute GVHD does not exceed 10%. A very low incidence of severe GVHD is predicted based on a reported incidence of 2% in a large study of adults (17) and a 0% incidence in a large study in children (21) and our experience (22). For example, if 3 severe acute GVHD events are observed in 30 patients, the exact 95% confidence interval is (0.02, 0.27). The accrual goal to the haplocompatible

cohort will be increased to 60. This is necessary for several reasons. First, this study serves to provide patients for the companion study which is evaluating an approach to hasten immune recovery after transplant. The companion study may require another 16-30 patients to complete its objective. Also, adding additional patients will allow us to better address the primary and secondary objectives in subpopulations like those with malignant disease, sickle cell disease, and immunodeficiency. Finally, the accrual of additional patients will allow the determination of acute GVHD with tighter confidence intervals. Because the accrual to the unrelated donor cohort may be lower, the statistical objective for this group can be changed to estimation of severe GVHD incidence as a pilot trial with less tight confidence interval goals than above. Patients who have primary graft failure (never engraft) will not be evaluable. They will be replaced by the enrollment of additional patients. Based on the literature and our experience, graft rejection is expected to occur in < 10% of patients. Patients that are enrolled but who do not receive a transplant will be replaced because they will not be evaluable for study objectives.

It is anticipated that study accrual will fluctuate significantly from quarter to quarter because marked variations in numbers of transplants in common. Also, the accrual to the unrelated donor cohort may be less than expected. If this is the case, then the accrual goal for this cohort will be 10 patients and the statistical objective will be to estimate severe GVHD incidence as a pilot trial for this approach.

9.3 Endpoint definitions

9.3.1 GVHD: Grading of GVHD will be according to Appendix II and III

9.3.2 Engraftment:

9.3.2.1 Primary graft failure (lack of engraftment) will be the lack of recovery of ANC to > 500 by Day +28 after transplant in the absence of immunological graft rejection. This endpoint is intended to monitor graft quality after manipulation.

9.3.2.2 Late (secondary) graft failure

a) Initial evidence for marrow recovery and engraftment with subsequent pancytopenia without another cause (ie. Infection and/or drug therapy) and decrease in donor chimerism by greater than 50% from highest level achieved

b) Decrease in donor chimerism to less than 10%

9.3.3 Immune recovery: Immune recovery will be assessed by the time to CD4>100 and CD4>200.

9.3.4 Severe toxicity: Severe toxicity will include any unexpected Grade 3 (for transplant recipient) toxicity or any non-hematological grade 4-5 toxicity through 1 year post-transplant.

9.3.5 Post-transplant infections: Infections that occur following the initiation of the conditioning regimen.

9.3.6 CMV infection and disease: CMV infection will be reactivation detected by CMV PCR on plasma and CMV disease will be evidence of organ involvement (eg. CMV pneumonia, enteritis).

9.3.7 Post-transplant lymphoproliferative disease (PTLD): PTLD will include patients that have clinical PTLD (for example, detectable EBV viral load and fever and/or adenopathy).

9.3.8 Transplant-related mortality (TRM): TRM will include death due to regimen-related toxicity or GVHD (usually all causes other than disease relapse).

9.3.9 Disease free survival (DFS) and overall survival (OS): Disease-free survival will be survival without relapse, including molecular, cytogenetic, and morphological relapse. DFS and OS at one, two, and 5 years after transplant will be determined from the day of transplant.

9.3.10 Chimerism at Day 100: Chimerism (percentage of donor cells) will be assessed at Day 100 post-transplant in evaluable (without disease progression) patients.

9.3.11 Device (CliniMACS®) performance parameters: The parameters will include the purity of the $CD34^+$ selected stem cell product, yield of $CD34^+$ cells after selection, degree of $CD3^+$ cell depletion after selection, and viability and sterility of the stem cell product after selection.

9.4 Plan of analysis

The primary endpoint of the study is the incidence of severe (grade III/IV) acute GVHD occurring by Day +30 after transplant. In our experience, almost all patients who develop acute GVHD after this type of transplant do so by Day +30. Acute GVHD attributed to the primary graft will not be able to be assessed after Day +30 in patients who are enrolled on the companion prophylactic donor lymphocyte infusion (DLI) protocol. In this situation, there will be no way to know if the GVHD is due to the primary graft or the DLI. It will be more likely to be due to the DLI because a higher dose of T cells will be given with the DLI and the level of circulating ATG will be less after DLI. We anticipate that a few patients will not go on the companion prophylactic study and these patients can be assessed for acute GVHD until Day +100. Although this will not be used for the primary endpoint, it will corroborate the fact that patients do not develop acute GVHD between Day 30 and Day +100. GVHD will be graded per standard criteria as discussed above.

The following variables will be assessed as secondary endpoints:

a. Engraftment: The incidence of primary graft failure and late (secondary) graft failure will be determined.
b. Immune recovery: The time to CD4 count >100 and >200 will be calculated.
c. Severe toxicities: The incidence of severe toxicities will be determined. This will include any unexpected grade 3 (for transplant recipient) toxicity or any non-hematological grade 4-5 toxicity through 1 year post-transplant.
d. Post-transplant infections: Post-transplant infections will be described by incidence and type.
e. CMV infection and disease: The incidence of each will be calculated.
f. Post-transplant lymphoproliferative disease (PTLD): The incidence of PTLD will be calculated.
g. Transplant-related mortality (TRM): The incidence of TRM will be calculated at Day 100 after transplant and long term.
h. Disease-free survival: The method of Craddock et al. (37) will be used to estimate DFS. The definition of DFS will parallel that of leukemia-free survival proposed by Craddock.
i. Overall survival: Overall survival will be estimated using the Kaplan-Meier method (38).
j. Chimerism at Day 100: The percentage of donor cells will be reported for all evaluable (without disease progression) patients.
k. Device (CliniMACS®) performance parameters: The parameters will be summarized for all products that are processed and median and ranges will be determined.

The endpoints will be monitored at least annually. The frequency will depend on the number of patients enrolled. Patients that receive chemotherapy for relapse

of underlying disease will only be followed for overall survival from that the time of chemotherapy.

Additional results that will be monitored include the research studies for immune recovery (TREC, T cell receptor repertoire (V beta typing) T and B subsets and T cell activation by flow cytometry, and CMV and EBV specific T cells by tetramer analysis and cytokine production). This will be performed at approximately Day 120, 6 months, and 1 year after transplant. The analysis of these results will be descriptive.

Stopping rules
The study includes stopping rules based on the incidence of graft failure and on transplant-related mortality (TRM) by Day 100 after transplant.

Stopping rule for primary graft failure: The trial is stopped if there are $\geq b_k$ graft failures out of n resolved patients. Only points where stopping is possible are listed.

Number of Patients, n	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Boundary, b_n	-	2	3	3	3	4	4	4	4	5	5	5	5	5	6	6	6	6	6	6
Number of Patients, n	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Boundary, b_n	7	7	7	7	7	7	8	8	8	8	8	8	9	9	9	9	9	9	10	10
Number of Patients, n	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
Boundary, b_n	10	10	10	10	10	11	11	11	11	11	11	11	12	12	12	12	12	12	13	13

The stopping rule for graft failure yields the probability of stopping the trial of 0.05 if the rate of graft failure is 0.1. The probability of stopping the trial is 0.61 if the graft failure rate is 0.2 and 0.97 if the graft failure rate is 0.3. These probabilities were calculated based on the binomial distribution. The stopping rule was generated as described by Ivanova et al. (39).

Stopping rule for Day 100 TRM: The trial is stopped if there are $\geq b_n$ patients with TRM out of n resolved patients.

Number of Patients, n	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Boundary, b_n	-	-	3	4	4	4	5	5	5	5	6	6	6	6	7	7	7	7	8	8
Number of Patients, n	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Boundary, b_n	8	8	9	9	9	9	10	10	10	10	11	11	11	11	11	12	12	12	12	12
Number of Patients, n	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
Boundary, b_n	13	13	13	13	14	14	14	14	14	15	15	15	15	15	16	16	16	16	16	17

The stopping rule for TRM yields the probability of stopping the trial of 0.05 if the TRM rate is 0.15. The probability of stopping the trial is 0.21 if the TRM rate is 0.2, and 0.80 if the TRM rate is 0.3. These probabilities were calculated based on the binomial distribution. The stopping rule was generated as described by Ivanova et al. (39).

If the study reaches a stopping boundary, the study will be suspended. At this point it may be terminated by the PI or submitted to the DSMC with a description of the failures to date and a rationale for why the study should be continued. Proper use of the stopping rule table will be ensured by the Study Investigator.

9.5 Sample size

Patients will be recruited by the PI and his co-investigator. 30 patients will be enrolled into the unrelated donor cohort and 60 patients into the alternative related donor cohort.

9.6 Estimated duration of study

The study will require 8 years for accrual and up to 5 years for follow-up studies.

9.7 Replacement policy

If a patient becomes unevaluable for engraftment at 4 weeks post-BMT, then an additional patient will be enrolled.

10.0 CRITERIA FOR EVALUATION

10.1 Monitored outcomes

10.1.1 The primary outcome will be severe (grade III/IV) graft-versus-host disease.

10.1.2 Secondary outcomes will be engraftment (ANC>500 and >80% donor cells in blood), survival, disease-free survival, infection, transplant-related toxicity and mortality, grade 3/4 stem cell product infusion-related toxicity, and relapse.

10.2 Toxicity definitions/stopping rules

10.2.1 If any patient develops grade IV acute GVHD by 4 weeks post BMT, the protocol will be halted and the processing re-evaluated before proceeding.

10.2.2 Stopping rules will be used for graft failure.

11.0 DATA SAFETY MONITORING PLAN

11.1 Oversight and monitoring plan

The study will utilize the Data and Safety Monitoring Committee (DSMC) of the Pediatric Blood and Marrow Transplant Consortium (PBMTC). The DSMC will be responsible for safeguarding the interests of participants in this trial. This responsibility will be exercised by providing recommendations for continuation or early termination of the trial, based on assessment of safety. The DSMC may also formulate recommendations related to the selection, recruitment or retention of participants, their management and adherence to protocol-specified regimens, and the procedures for data management and quality control.

The DSMC will be advisory to Andrew Gilman, MD who will serve as sponsor and principal investigator and to his co-investigators. Dr. Gilman and his co-investigators will be responsible for promptly reviewing any recommendations and deciding how to respond.

The DSMC will:

- Review the current protocol
- Review progress of the trial on a semi-annual basis
- Review of all serious adverse events including expected and unexpected events according to PBMTC DSMC guidelines.

If at any time the Investigator stops enrollment or stops the study due to safety issues, the DSMC and IRB will be notified within 24 business hours via e-mail. A formal letter will be submitted within 10 business days and the FDA will be notified.

11.2 Monitoring and reporting guidelines

The PI will conduct continuous review of data and patient safety at weekly Blood and Marrow Transplant meeting where the results of each patient's treatment are discussed. The discussion will be documented by the BMT research team. The discussion may include the number of patients, significant toxicities as described in the protocol, dose adjustments, and observed responses. Semi-annual reports will be submitted to the PBMTC DSMC for review according to PBMTC guidelines. Grade 3 (unexpected for transplant recipient) adverse events, non-hematologic grade 4 adverse events, all grade 5 adverse events and all serious adverse events related to study participation will be submitted to the FDA, IRB and DSMC in accordance with their reporting requirements.

11.3 Review and oversight requirements

11.3.1 Oversight of cell processing

The results of the cell processing at UCSF will be reviewed by the PI. The outcomes and AE's will also be reviewed.

11.3.2 Adverse event definition

An adverse experience is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An adverse experience or event (AE) can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

11.3.3 Adverse event recording

Adverse events will be graded using the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 3.0 found on the following website: http://ctep.cancer.gov/reporting/ctc.html. All non-hematologic grade 3 – 4 adverse events and all grade 5 adverse events will be recorded until 1 year post-transplant. All deaths and other endpoints in Section 9.3 will continue to be recorded through the duration of follow-up. Grading of acute and chronic GVHD will be according to Appendix II and III. Causality will be rated as definitely, probably, possibly, or unlikely related, or unrelated to the $CD34^+$ CliniMACS® collection. **The investigator is responsible for making an assessment of whether or not it is reasonable to suspect a causal relationship between the adverse event and the study treatment.**

11.3.4 Serious adverse events definition

An unexpected adverse event is any adverse drug experience where the specificity or severity of which is not consistent with the current investigator brochure; or, if an investigator brochure is not required or available, the specificity or severity of which is not consistent with the risk information described in the general investigational plan or elsewhere in the current application.

A serious adverse experience (SAE) or serious adverse drug reaction (ADR) is any adverse drug experience occurring at any dose that results in any of the following outcomes:

11.3.4.1 Death

11.3.4.2 Life-threatening (places the subject, in the view of the investigator, at immediate risk of death from the reaction as it occurred)
11.3.4.3 Inpatient hospitalization or prolongs existing hospitalization
11.3.4.4 Persistent or significant disability/incapacity (a substantial disruption of a person's ability to conduct normal life functions)
11.3.4.5 Birth defect/congenital anomaly
11.3.4.6 Any important medical event that may not result in prior listed outcomes but, based upon appropriate medical judgment, may jeopardize the subject, and may require medic al and surgical intervention to prevent one of the prior listed outcomes.

11.3.5 Targeted AE reporting guidelines for this protocol

11.3.5.1 Any evidence of grade III/IV acute or extensive chronic GVHD using protocol appendices II and III
11.3.5.2 Grade 3 (unexpected for transplant recipient), non-hematological grade 4, and all grade 5 adverse events until 1 year post-transplant. See Appendix VI for expected adverse events.
11.3.5.3 Hospitalization
11.3.5.4 Relapse
11.3.5.5 Any problems with CliniMACS device during cell separation or inability to achieve $CD34^+$ cell and $CD3^+$ cell target doses.
11.3.5.6 Failure to engraft or late graft failure.

11.3.6 Adverse event reporting procedures

Reporting will be in accordance with 21 Code of Federal Regulation (CFR) Part 312.32.

Grade 3 (unexpected for transplant recipient) adverse events, non-hematologic grade 4 adverse events, and all grade 5 adverse events will be recorded in the Case Report Forms until 1 year post-transplant. This will include the severity or toxicity grade, the relationship to the study drug, treatment and the outcome of the event.

FDA website for guidance in reporting serious adverse events
http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr+312.32

MedWatch forms and information:
http://www.fda/gov/medwatch/getforms.html

CliniMACS® $CD34^+$ Reagent System:
Fax MedWatch form directly to the Safety Officer, Miltenyi Biotec Inc. (781-782-1920)

Serious Adverse events will be reported on the MedWatch form when it meets the definition of expedited FDA reporting.

Serious Adverse events will be reported to PBMTC DSMC within 10 days of event.

If the SAE is death and determined to be possibly, probably, or definitely related to the investigational drug or any research related procedure, the event must be reported to the DSMC within 24 business hours. The reporting procedure is by personal communication via phone with written documentation of the 1:1 communication or via e-mail with a copy of the e-mail to be placed in the regulatory binder.

11.3.7 Review of adverse event rates

If the study has an increased incidence of grade 3 or grade 4 adverse events associated with the infusion of the CD34+ selected stem cell product above the rate reported in the protocol or investigational brochure, this will be reported to the DSMC at the time of identifying the increased rate.

11.3.8 Semi-annual review of study progress

Principal Investigator is required to submit semi-annual study progress reports to the PBMTC DSMC. The progress reports will describe toxicity and include the rate of grade 3 (unexpected for transplant recipient) adverse events, all non-hematological grade 4 adverse events, all grade 5 adverse events and all serious adverse events through 1 year post-transplant. In addition, the report will include an updated accrual summary, all deaths reported on study, and a brief synopsis of study status.

These semi-annual reports are reviewed by the PBMTC DSMC. These reports are scheduled for May and November as per DSMC procedures. The cut-off date for data will be 2 weeks prior to this date. The reports will be submitted within at least 1 month from the scheduled date.

12.0 ETHICAL ASPECTS

12.1 Regulatory considerations

This study will be reviewed by the Carolinas Healthcare System (CHS) Institutional Review Board. In addition, an Investigational New Drug application will be filed with the FDA. As per FDA regulations (21 CFR 312.33), annual reports will be submitted to the FDA within 60 days of the anniversary date that the IND went into effect.

12.2 Independent Ethics Committees/Institutional Review Board

This protocol and the informed consent will be approved by the CHS IRB. The Principal Investigator is responsible for keeping the IRB advised of the progress of the study and of any changes made in the protocol prior to implementation. The Principal Investigator will also keep the IRB informed of any significant adverse reactions, and any protocol exceptions or deviations. Records of all study review and approval documents must be kept on file by the Principal Investigator and are subject to FDA inspection during or after completion of the study. The IRB will receive notification of the termination of the study.

13.0 DATA FORMS AND SUBMISSION SCHEDULE

Forms will be completed and documented for each patient. The Completion Schedule below is intended as a reasonable guideline only. The exception is the Recipient/Donor eligibility form which should be completed before the recipient receives study treatment. A research chart with completed paper case report forms and supporting documentation of reportable AEs and SAEs for all enrolled patients will be maintained in the BMT Research office. Patient enrollment information and toxicity/reporting information will also be entered by the coordinator into the BMT database. If the patient is enrolled on LCH BMT 09-02 protocol (09-02), then completion of acute and chronic GVHD forms for LCH BMT 09-01 are not required until completion of follow up ends at 2 years for 09-02.

FORMS	Submission Schedule
Enrollment Forms: ▪ **Recipient Eligibility** ▪ **Donor Eligibility** ▪ **Recipient Baseline Information**	Complete recipient eligibility form and baseline form after recipient, parent, or guardian has signed consent and prior to treatment of the recipient. Complete donor eligibility form after donor, parent, or guardian has signed consent and prior to treatment of the donor. Complete baseline information.
Treatment Forms: ▪ **Transplant Data Form** ▪ **CD34+ Cell Processing**	Complete within one month of last dose of conditioning therapy.
Follow Up Forms: ▪ **Engraftment Assessment & Lymphocyte Phenotyping** ▪ **Acute GvHD** ▪ **Chronic GvHD** ▪ **Adverse Events Reporting**	Complete within one month of time points listed in table in Section 7.7).
Toxicity Reporting: ▪ **SAE Forms** ▪ **CHS IRB Form** ▪ **FDA MedWatch**	Complete as required based on reporting criteria for each institution. See sections 11.2 to 11.3.6 for further information.

APPENDICES

APPENDIX I – Alternative conditioning regimens:

1. Chemotherapy Alone Regimen (for patients who have a contraindication to TBI or non-malignant disease (22).
- **1.1.** On day –7, administer melphalan 140mg/m^2 IV over 20-30 minutes x 1 dose.
- **1.2.** On day –6, thiotepa 5mg/kg/dose will be administered intravenously in two doses (each dose over 3-4 hours) for a total dose of 10mg/kg/day.
- **1.3.** On days –6 through –2, fludarabine 40 mg/m^2/d (infuse over 30 minutes) will be administered in the early morning hours on each of the five days (there will be at least 24 hrs between the last dose of fludarabine and the stem cell infusion). Fludarabine dose will be reduced by 20% for creatinine clearance of < 70 ml/min/1.73m^2.
- **1.4.** Rabbit ATG (thymoglobulin) 1.5-2.5 mg/kg on days –5 to day –2 infused over 6 hours. The dose will be at PI discretion. Examples are that patients with non-malignant disease will usually receive the higher dose and patients with solid tumors who have received intensive chemotherapy prior to transplant will usually receive the lower dose.

2. Reduced Intensity Regimen (for patients with decreased organ function) (30,31)
- **2.1.** On day -7 through day -3, fludarabine 30 mg/m^2/d (infused over 30 minutes) will be administered in the early morning hours on each of the five days. Fludarabine dose will be reduced by 20% for creatinine clearance of 30-70 ml/min/1.73m^2.
- **2.2.** On day –3, thiotepa 5mg/kg/dose will be administered intravenously in two doses (each dose over 3-4 hours) for a total dose of 10mg/kg/day.
- **2.3.** On days –2 and –1, melphalan 60 mg/m^2/d (infuse over 20-30 minutes) will be administered in the morning (there will be at least 24 hrs between the last dose of melphalan and the stem cell infusion).
- **2.4.** Rabbit ATG (thymoglobulin) 0.5 mg/kg on day -7 infused over 4 hrs and then 2.5 mg/kg for four daily doses from day –6 to day –3 infused over 6 hours.

3. Alternative Reduced Intensity Regimen (without thiotepa) (32)
- **3.1.** On day –8 and -7, administer busulfan 0.8 mg/kg (1 mg/kg if > 10 kg and < 4 yrs of age) q 6 hrs IV over 2 hrs.
- **3.2.** On days –7 through –3, fludarabine 40 mg/m^2/d (infuse over 30 minutes) will be administered in the morning on each of the five days. Fludarabine dose will be reduced by 20% for creatinine clearance of 30-70 ml/min/1.73m^2.
- **3.3.** On day –2, melphalan 140mg/m^2 IV over 20-30 minutes x 1 dose. Prepare melphalan immediately prior to administration to maintain stability.
- **3.4.** Rabbit ATG (thymoglobulin) 0.5 mg/kg on day -6 infused over 4 hrs and then 2.5 mg/kg for four daily doses from day –5 to day –1 infused over 6 hours.

4. Regimen for chromosome breakage disorders like Fanconi anemia with aplasia (33)
- **4.1.** On day -6 through day -3, cyclophosphamide 10mg/kg/dose will be administered intravenously (each dose to run over 1 hour) on each of the four days.
- **4.2.** On day -6 through day -3, fludarabine 40 mg/m^2/d (infused over 30 minutes) will be administered in the morning on each of the four days. Fludarabine dose will be reduced by 20% for creatinine clearance of < 70 ml/min/1.73m^2.
- **4.3.** Rabbit ATG (thymoglobulin) 0.5 mg/kg on day -6 infused over 4 hrs and then 3.75 mg/kg for four daily doses from day –5 to day –2 infused over 6 hours.

5. Regimen for chromosome breakage disorders like Fanconi anemia with MDS/AML (34)
- **5.1.** On day -7, a 450 cGy single dose of total body irradiation is delivered.
- **5.2.** On day -5 through day -2, cyclophosphamide 10mg/kg/dose will be administered intravenously (each dose to run over 1 hour) on each of the four days.
- **5.3.** On day -6 through day -2, fludarabine 30 mg/m^2/d (infused over 30 minutes) will be administered in the early morning hours on each of the five days (there will be at least

24 hrs between the last dose of fludarabine and the stem cell infusion). Fludarabine dose will be reduced by 20% for creatinine clearance of < 70 ml/min/1.73m^2.

5.4. Rabbit ATG (thymoglobulin) 0.5 mg/kg on day -6 infused over 4 hrs and then 2.5 mg/kg for four daily doses from day –5 to day –2 infused over 6 hours.

6. Alternative Regimen (for patients with immunological disorders with immune activation and those with high risk of autologous marrow recovery including beta-thalassemia major) (40)

6.1. On day –10 and -9, administer cyclophosphamide 50 – 60 mg/kg/dose will be administered intravenously (each dose to run over 1 hour) on each day with adjustments per referenced article.

6.2. On day –8 through -6, administer busulfan daily IV over 2 hrs on days -8 and -7 and q12 on day -6 with adjustments per referenced article.

6.3. On day –5, thiotepa 5mg/kg/dose will be administered intravenously in two doses (each dose over 3-4 hours) for a total dose of 10mg/kg/day.

6.4. On days –8 through –4, fludarabine 40 mg/m^2/d (infuse over 30 minutes) will be administered in the morning on each of the five days. Fludarabine dose will be reduced by 20% for creatinine clearance of 30-70 ml/min/1.73m^2.

6.5. Rabbit ATG (thymoglobulin) 2.5 mg/kg for four daily doses from day –4 to day –1 infused over 6 hours.

6.6. Dose adjustments will be made based on ferritin, hepatomegaly, and age for patients with thalassemia and also for other patients (at physician discretion) per referenced article. Busulfan Css levels will be targeted at 500 – 600 mcg/L.

6.7. Azathioprine and hydroxyurea will be used prior to transplant (as described in the reference) for patients with beta-thalassemia major

APPENDIX II- Acute GVHD staging and grading for children**

ORGAN	STAGE	DESCRIPTION
SKIN	1	Maculopapular rash < 25% of BSA
	2	25 – 50% of BSA
	3	Generalized erythroderma
	4	Desquamation and bullae
LIVER	1	Bilirubin 2 - 3 mg/dL
	2	Bilirubin 3.1 - 6 mg/dL
	3	Bilirubin 6.1 - 15 mg/dL
	4	Bilirubin > 15 mg/dL
GUT	1	Diarrhea > 500 – 1000 ml/day (> 10 mL/kg - 20 mL/kg/day) OR persistent UGI symptoms
	2	Diarrhea > 1000 – 1500 ml/day (> 20mL/kg – 30 mL/kg/day)
	3	Diarrhea >1500 ml/day (>30 mL/kg/day)
	4	Severe abdominal pain or ileus

GRADE	Skin	Liver		Gut
I	1-2	0		0
II	3 and/or	1	and/or	1
III	--	2-3	and/or	2-3
IV	4 and/or	4	and/or	4

** Adapted from Glucksberg and Jacobsohn articles (41,42).

APPENDIX III - Chronic GVHD staging and grading for children**
Chronic GVHD grading will be performed with both a limited/extensive grading system and with the NIH Consensus scoring system.

The limited/extensive grading will use the revised Seattle classification (43) shown below.

Table 2. *Original and Revised Seattle Classification for Limited and Extensive Chronic GVHD*

Original Seattle Classification	Revised Seattle Classification*
Limited	Clinical limited
One or both of:	1. Oral abnormalities consistent with chronic GVHD, a positive skin or lip biopsy, and no other manifestations of chronic GVHD
Localized skin involvement	2. Mild liver test abnormalities (alkaline phosphatase ≤2 × upper limit of normal, AST or ALT ≤3 × upper limit of normal, and total bilirubin ≤1.6) with positive skin or lip biopsy, and no other manifestations of chronic GVHD
Hepatic dysfunction due to chronic GVHD	3. Less than 6 papulosquamous plaques, macular-papular or lichenoid rash involving <20% of BSA, dyspigmentation involving <20% BSA, or erythema involving <50% BSA, positive skin biopsy, and no other manifestations of chronic GVHD
	4. Ocular sicca (Schirmer's test ≤5 mm with no more than minimal ocular symptoms), positive skin or lip biopsy, and no other manifestations of chronic GVHD
	5. Vaginal or vulvar abnormalities with positive biopsy, and no other manifestations of chronic GVHD
Extensive	Clinical extensive
One of:	1. Involvement of 2 or more organs with symptoms or signs of chronic GVHD, with biopsy documentation of chronic GVHD in any organ
Generalized skin involvement	2. Karnofsky or Lansky Clinical Performance scores <60%, ≥15% weight loss, and recurrent infections not due to other causes, with biopsy documentation of chronic GVHD in any organ
Localized skin involvement and/or hepatic dysfunction due to chronic GVHD, plus:	3. Skin involvement more extensive than defined for clinical limited chronic GVHD, confirmed by biopsy
Liver histology showing chronic aggressive hepatitis, bridging necrosis, or cirrhosis, or:	4. Scleroderma or morphea
	5. Onycholysis or onychodystrophy thought to represent chronic GVHD, with documentation of chronic GVHD in any organ
Involvement of eye (Schirmer's test with <5 mm wetting), or:	6. Decreased range of motion in wrist or ankle extension due to fasciitis caused by chronic GVHD
	7. Contractures thought to represent chronic GVHD
Involvement of minor salivary glands or oral mucosa demonstrated on labial biopsy, or:	8. Bronchiolitis obliterans not due to other causes
	9. Positive liver biopsy; or abnormal liver function tests not due to other causes with alkaline phosphatase >2 × upper limit of normal, AST or ALT >3 × upper limit of normal, or total bilirubin >1.6, and documentation of chronic GVHD in any organ
Involvement of any other target organ	10. Positive upper or lower GI biopsy
	11. Fasciitis or serositis thought to represent chronic GVHD and not due to other causes

*Provided by Mary E.D. Flowers and Paul J. Martin, Fred Hutchinson Cancer Research Center.
AST indicates aspartate aminotransferase; ALT, alanine aminotransferase; BSA, body surface area.

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Grading of Chronic GVHD severity by NIH Consensus Guidelines using Organ Scoring Table (44)

Severity	Definition
Mild	Involves on 1 or 2 organs or sites (except the lung; see below), with no clinically significant functional impairment (maximum of score 1 in all affected organs or sites)
Moderate	(1) At least one organ or site with clinically significant but no major disability (maximum score of 2 in any affected organ or site OR (2) 3 or more organs or sites with no clinically significant functional impairment (maximum score of 1 in all affected organs or sites) OR a lung score of 1.
Severe	(1) Major disability caused by chronic GVHD (score of 3 in any affected organ) or site OR a lung score of $\geq$ 2.

NIH Consensus Chronic GVHD Organ Scoring

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

	SCORE 0	SCORE 1	SCORE 2	SCORE 3
LUNGS† FEV1 ☐ DLCO ☐	☐ No symptoms ☐ FEV1 > 80% **OR** LFS=2	☐ Mild symptoms (shortness of breath after climbing one flight of steps) ☐ FEV1 60-79% **OR** LFS 3-5	☐ Moderate symptoms (shortness of breath after walking on flat ground) ☐ FEV1 40-59% **OR** LFS 6-9	☐ Severe symptoms (shortness of breath at rest; requiring 0_2) ☐ FEV1 ≤39% **OR** LFS 10-12
JOINTS AND FASCIA	☐ No symptoms	☐ Mild tightness of arms or legs, normal or mild decreased range of motion (ROM) **AND** not affecting ADL	☐ Tightness of arms or legs **OR** joint contractures, erythema thought due to fasciitis, moderate decrease ROM **AND** mild to moderate limitation of ADL	☐ Contractures **WITH** significant decrease of ROM ***AND*** significant limitation of ADL (unable to tie shoes, button shirts, dress self etc.)
GENITAL TRACT	☐ No symptoms	☐ Symptomatic with mild signs on exam **AND** no effect on coitus and minimal discomfort with gynecologic exam	☐ Symptomatic with moderate signs on exam **AND** with mild dyspareunia or discomfort with gynecologic exam	☐ Symptomatic **WITH** advanced signs (stricture, labial agglutination or severe ulceration) **AND** 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___
M yasthenia Gravis___	Cardiomyopathy___	Eosinophilia > 500µl___
Polymyositis___	Cardiac conduction defects___	Coronary artery involvement___
Platelets <100,000/µl ___	Progressive onset___	

OTHERS: Specify:_____

Organ scoring of chronic GVHD. *AP may be elevated in growing children, and not reflective of liver dysfunction. †Pulmonary scoring should be performed using both the symptom and pulmonary function testing (PFT) scale whenever possible. When discrepancy exists between pulmonary symptom or PFT scores the higher value should be used for final scoring. Scoring using the Lung Function Score (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 [29]. The percent predicted FEV1 and DLCO (adjusted for hematocrit but not alveolar volume) should be converted to a numeric score as follows: > 80% = 1; 70-79% = 2; 60-69% = 3; 50-59% = 4; 40-49% = 5; < 40% = 6. The LFS = FEV1 score + DLCO score, with a possible range of 2-12. GVHD indicates graft versus host disease; ECOG, Eastern Cooperative Oncology Group; KPS, Karnofsky Performance Status; LPS, Lansky Performance Status; BSA, body surface area; ADL, activities of daily living; LFTs, liver function tests; AP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ULN, upper limit of normal.

APPENDIX IV - Device information

Brand: CliniMACS® CD34 Reagent System Information
The CliniMACS® CD34 Reagent System is a medical device that is used *in vitro* to select and enrich specific cell populations. When using the CD34 Reagent, the system selects CD34+ cells from heterogeneous hematological

The CliniMACS CD34 Reagent System is comprised of four primary components:
• CliniMACS CD34 Reagent: a sterile monoclonal antibody reagent specific for CD34+ cells
• CliniMACS plus Instrument: a software controlled instrument that processes the blood sample (cell product)
• CliniMACS Tubing Sets: single-use, sterile, disposable tubing sets with two proprietary cell selection columns (CliniMACS Tubing Set and CliniMACS Tubing Set LS)
• CliniMACS PBS/EDTA Buffer: a sterile, isotonic phosphate-buffered, 1 mM EDTA, saline solution, used as external wash and transport fluid for the *in vitro* preparation of blood cells

University of California, San Francisco CliniMACS® cell sorter Device information:

1) **Serial number: 000288**
2) **Catalogue number: 15101**
3) **Part number: 44085**

Distributor:
Corporate Headquarters
Miltenyi Biotec Inc.
12740 Earhart Avenue
Auburn, CA 95602

Manufacturer:
Mitenyi Biotec GmbH, Clinical Products
Friedrich-Ebert Strasse
Technologiepark H-13
D51429
Bergisch Gladbach, Germany

Sponsor Contact:
Miltenyi Biotec Inc., Suite 305
120 Presidential Way
Woburn, MA 01801
Phone: (781) 782-1910
Fax: (781) 782-1920

APPENDIX V – RESEARCH SAMPLES

Sample for KIR/NK Cell Biology Studies

Blood samples will be obtained for potential donors in order to perform KIR typing. Donor samples for KIR typing will usually be obtained at the time of confirmatory HLA typing of potential donors and after HLA typing results are available for related donors. KIR typing will only be performed upon optional donor consent.

Sample Collection

Two 10 mL ACD yellow-top tubes of peripheral blood will be collected from potential donor and sent to the address below for KIR typing.

Shipping

All samples will be shipped at room temperature by overnight mail to Dr. Wing Leung at SJCRH. No refrigeration is necessary. Blood samples must be protected in shipping containers inside Styrofoam boxes to minimize temperature change because the cells must be tested in a viable condition. Samples sent for KIR typing must be sent via FedEx and must be sent with a specimen transmittal form. Label sample with Patient ID, Protocol ID, and time of sample collection.

Research personnel at LCH will notify Dr. Leung that a donor sample will be sent for KIR typing. For related donors, research personnel will provide the study Donor ID and for unrelated donors, research personnel will provide the study Donor ID, DID, RID and estimated collection date.

All samples for KIR typing should be sent to the address below:

Barbara Rooney
Laboratory of Dr. Wing Leung
St. Jude Children's Research Hospital
262 Danny Thomas Place, Room D5032
Memphis, TN 38105
Phone: (901) 595-4155
Fax: (901) 595-4023
E-mail: wing.leung@stjude.org or Barbara.rooney@stjude.org
Saturday deliveries are permissible; Sunday deliveries are not permissible.
Note: If a Saturday delivery is planned, please notify Dr Leung at the number listed above and clearly mark the package "For Saturday Delivery."

Ship each specimen individually by overnight air freight on the day of its collection. Maximum time from sample collection to shipment should be no greater than 24 hours. Exception: Samples collected on weekends or holidays should be shipped the first working day following collection. Store and ship samples at room temperature. Send fresh.

Samples for Immune Recovery Studies

The St. Jude and Emory samples can be drawn on different days to allow the target amount to be obtained for each lab. The samples for immune recovery studies at St. Jude and Emory University can be sent within 1 week of each time point if the amount of blood taken is limited by patient size. The limit for blood taken in each blood draw is 2 mL/kg (max 60 mL).

Sample for Immune Recovery Studies at St. Jude

Blood samples will be obtained at approximately Day 120, 6 months, and 1 year after transplant as described in Sections 7.7 and 7.8.2.

Sample Collection

Two 10 mL ACD yellow-top tubes of peripheral blood will be collected.

Shipping

All samples will be shipped at room temperature by overnight mail to Dr. Wing Leung at SJCRH. No refrigeration is necessary. Blood samples must be protected in shipping containers inside Styrofoam boxes to minimize temperature change, because the cells must be tested in a viable condition. Samples will be sent via FedEx and must be sent with a specimen transmittal form. Label sample with Patient ID, Protocol ID, and time of sample collection.

All samples for immune recovery studies should be sent to the address below:
Barbara Rooney
Laboratory of Dr Wing Leung
St. Jude Children's Research Hospital
262 Danny Thomas Place, Room D5032
Memphis, TN 38105
Phone: (901) 595-4155
Fax: (901) 595-4023
E-mail: wing.leung@stjude.org or Barbara.rooney@stjude.org
Saturday deliveries are permissible; Sunday deliveries are not permissible.
Note: If a Saturday delivery is planned, please notify Dr Leung at the number listed above and clearly mark the package "For Saturday Delivery."

Ship each specimen individually by overnight air freight on the day of its collection. Maximum time from sample collection to shipment should be no greater than 24 hours. Store and ship samples at room temperature. Send fresh.

Sample for Immune Recovery Studies at Emory University

Blood samples will be obtained at approximately Day 120, 6 months, and 1 year after transplant as described in Sections 7.7 and 7.8.3.

Sample Collection

Peripheral blood will be collected as follows:

Samples will consist of the following:
1. 8 mL into cytochex tubes (can do 4 cc on smaller children if needed).

a. Cyto-Chex BCT is a blood collection tube for the preservation of whole blood samples for immunophenotyping by flow cytometry. Cell morphology and surface markers are maintained in these tubes for up to 7 days allowing for blood to be sent to a central location for processing.
b. Fill a Cyto-Chex tube with 4 ml whole blood
c. Immediately mix the collected tube by gentle inversion 8-10 times.
d. Collected blood should be shipped by priority overnight express to Dr. Kean's laboratory on the same day as collection **at ambient temperature**.

2. 16-32 mL into CPT tubes for viral specific assays as feasible based on patient weight.
   a. Cell Processing Tubes (CPT) are tubes used for the collection of blood for cryopreservation of peripheral blood mononuclear cells (PBMCs) for functional T cell Assays and plasma preservation.
   b. Collected blood should be shipped by priority overnight express to Dr. Kean's laboratory on the same day as collection **at ambient temperature**.

Shipping

All samples will be shipped at room temperature by overnight mail to Dr. Leslie Kean's laboratory at Emory University. No refrigeration is necessary. Blood samples must be protected in shipping containers inside Styrofoam boxes to minimize temperature change, because the cells must be tested in a viable condition. Samples will be sent via FedEx and be sent with a specimen transmittal form. Label sample with Patient ID, Protocol ID, and time of sample collection.

Prior to shipping, send an email to Aneesah Garrett: aneesah.d.polnett@emory.edu notifying her of an impending shipment. Include the Fed Ex tracking number in the email. Aneesah's phone #: 404-727-4738

Copy Jennifer Cheeseman (jcheese@emory.edu) on the email to Aneesah.

Samples are to be shipped to the Dr. Leslie Kean's Laboratory at:

Emory Transplant Center Biorepository
101 Woodruff Circle, #5014-WMB
Emory University
Atlanta, GA 30322

Ship each specimen individually by overnight air freight on the day of its collection. Maximum time from sample collection to shipment should be no greater than 24 hours. Store and ship samples at room temperature. Send fresh.

Appendix VI –Grade 3 Adverse Events Expected for Transplant Recipient

Cardiac
Hypertension
Constitutional Symptoms
Fatigue
Weight gain
Gastrointestinal
Anorexia
Colitis
Diarrhea
Dysphagia
Enteritis
Esophagitis
Gastritis
Ileus, GI
Mucositis/ stomatitis (clinical exam)
Mucositis/ stomatitis (functional)
Nausea
Typhlitis
Vomiting
Growth and Development
Growth velocity
Hemorrhage/ Bleeding
Petechiae/ purpura
Infection
Colitis, infectious
Febrile Neutropenia
Infection
Opportunistic infection
Metabolic/ Laboratory
ALT
AST
GGT
Hypomagnesemia
Hypophosphatemia
Hypokalemia
Hyponatremia
Musculoskeletal/ Soft Tissue
Muscle weakness
Osteonecrosis (avascular necrosis)
Neurology
Mood Alteration (agitation, anxiety, depression)
Pain
Sexual/ Reproductive function
Irregular menses

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