

Phase I/II Study of Donor Lymphocyte Infusion with Methotrexate GVHD Prophylaxis to Hasten Immune Reconstitution after CD34<sup>+</sup> Cell-Selected Transplant

**[Companion study to A Phase II Study Using the CliniMACS® Device for CD34<sup>+</sup> Cell Selection and T Cell Depletion for Graft-versus-Host Disease Prophylaxis in Alternative Donor Stem Cell Transplant Recipients]**

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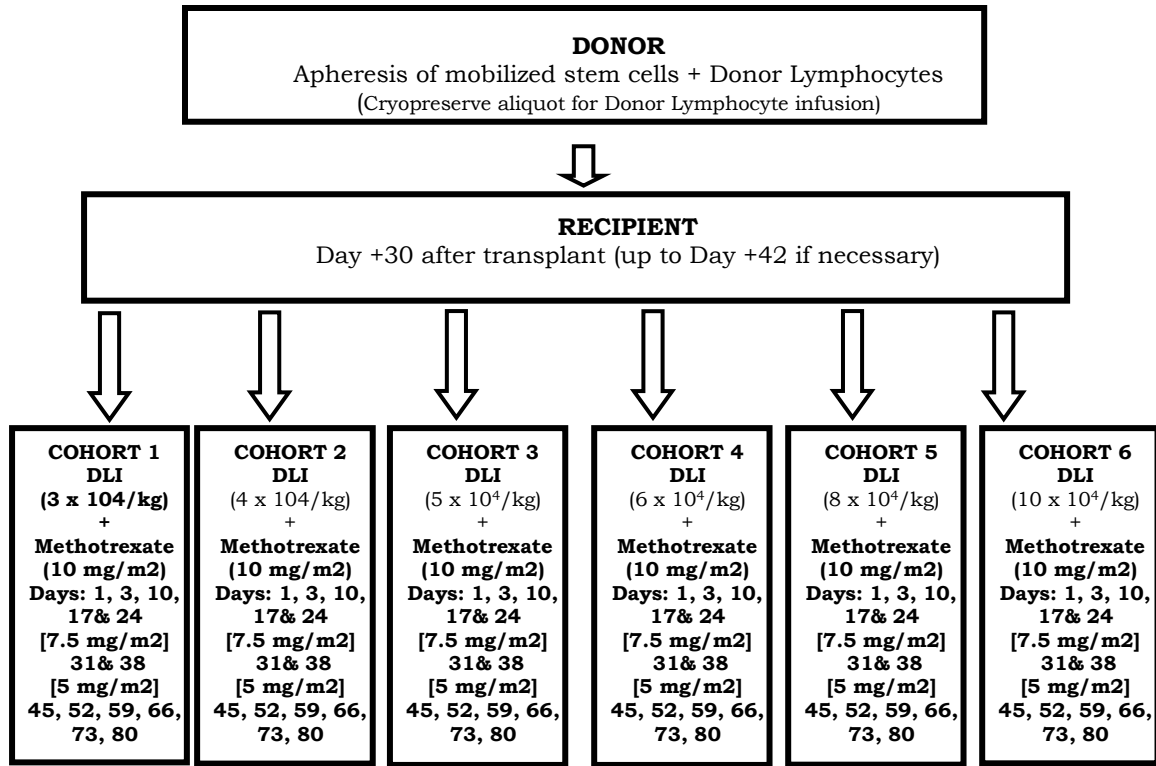
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**SHORT ABSTRACT**

A previous clinical trial has suggested that giving donor T cells after a mismatched T cell-depleted stem cell transplant resulted in recovery of T cells in the patient. However, this approach is associated with a risk of graft-versus-host disease (GVHD). The purpose of this study is to determine whether giving a donor lymphocyte infusion (DLI) with methotrexate GVHD prophylaxis can accelerate immune recovery in recipients of T cell-depleted stem cell transplants without causing significant GVHD. At day 30 (up to Day +42 if necessary) after transplant, patients will receive DLI. The patients will be monitored for immune recovery as measured by CD4 count. They will also be monitored for graft versus host disease (GVHD). The dose of DLI will be escalated to determine a dose at which immune recovery is achieved without significant GVHD. The starting dose will be  $3 \times 10^4$  T cells/kg and the highest dose will be  $10 \times 10^4$ /kg. In addition, all patients will receive methotrexate after the DLI to prevent GVHD. All patients will be followed for 2 years after transplant to determine outcomes.

**SCHEMA**



**LIST OF ABBREVIATIONS**

AE	Adverse Event
ALL	Acute Lymphocytic Leukemia
AML	Acute Myelocytic Leukemia
ANC	Absolute Neutrophil Count
ASCO	American Society for Clinical Oncology
BMT	Bone Marrow Transplant
BSA	Body Surface Area
CIBMTR	Center for International Blood and Marrow Transplant Research
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
FDA	Food and Drug Administration
FHCRC	Fred Hutchinson Cancer Research Center
GCP	Good Clinical Practice
G-CSF	human Granulocyte-Colony Stimulating Factor
GMP	Good Manufacturing Practice
GVHD	Graft Versus Host Disease
HLA	Human Leukocyte Antigen
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
MTX	Methotrexate
PBSC	Peripheral Blood Stem Cells
PCR	Polymerase Chain Reaction
PI	Principal Investigator
PTLD	Post-transplant Lymphoproliferative Disorder
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 assess the ability of a donor lymphocyte infusion given with methotrexate GVHD prophylaxis to accelerate immune recovery following transplantation of a CD34<sup>+</sup>selected, T cell-depleted stem cell product.

### **1.2 Secondary objectives**

- 1.2.1** To determine the optimum dose of DLI that will accelerate immune recovery without causing significant GVHD
- 1.2.2** To determine the incidence and severity of GVHD after this treatment
- 1.2.3** To determine the incidence of infection and EBV-related post-transplant lymphoproliferative disease (PTLD) after this treatment

## **2.0 BACKGROUND AND RATIONALE**

### **2.1 Introduction**

This study will be a companion study to a study which uses the CliniMACS® device for CD34<sup>+</sup> (hematopoietic stem cell) cell selection and T cell depletion to prevent severe (grade III-IV), life-threatening acute graft-versus-host disease (GVHD) after alternative donor stem cell transplant. The purpose of this study is to investigate post-transplant immunotherapy to hasten immune recovery and decrease post-transplant infections.

Blood stem cell and bone marrow transplants (SCT) can cure patients with leukemia, lymphoma, myelodysplastic syndrome, and bone marrow failure. Only 15-25% of patients will have histocompatible (HLA-identical) family member donors. For patients without a matched family donor, alternative donors can include mismatched or mismatched volunteer unrelated adult donors or cord blood, or mismatched family members. A major complication of unmanipulated, unrelated adult donor or cord blood is severe (grade III-IV), life-threatening acute GVHD, which occurs in 25-30% of patients (1;2). Mismatched family member donor transplant is associated with a very high risk of severe acute GVHD (3). GVHD results in rash, diarrhea, and jaundice. GVHD is caused by T cells (or T lymphocytes). GVHD is amplified by inflammatory proteins (cytokines) that are released as a result of high-dose chemotherapy and radiation therapy given just prior to the transplant (4). Removal of the T cells from unrelated and mismatched related SCT can prevent severe GVHD. Older studies reported that T cell depletion is associated with a high incidence of graft rejection and leukemic relapse (5). More recent experience shows that newer techniques for T cell depletion and for transplantation have overcome these challenges (6-10). However, the low number of T cells given with the SCT results in minimal passive transfer of immunity and delayed immune recovery. This is associated with high risk of morbidity and mortality due to infections and remains as a challenge (6;11-13).

We are participating in a study sponsored by University of California – San Francisco (UCSF # 01151) that is close to completing accrual. The study uses the CliniMACS® device for CD34<sup>+</sup> cell selection and T cell depletion to prevent graft-versus-host disease (GVHD) after mismatched family member donor transplant. The study uses the conditioning regimen (radiation and chemotherapy) established by Martelli and colleagues in Perugia, Italy (14). The study attempts to improve immune recovery and decrease post-transplant



infections by the following: (1) giving a large dose of CD34<sup>+</sup> cells, (2) reducing the dose of anti-thymocyte globulin (ATG) given to the patient before transplant to decrease the number of donor T cells that are killed after transplant, (3) giving a fixed small dose of T cells with the stem cell transplant. Immune recovery is judged by achievement of > 100 CD4<sup>+</sup> T cells/uL of blood by 100 days after transplant. The study also allowed a donor T cell infusion of 3 x 10<sup>4</sup> T cells/kg to be given for patients who have serious viral infections or those who do not have a CD4<sup>+</sup> T cells/uL by 100 days after transplant. The study has enrolled 17 of the planned 21 patient accrual. Sixty-five percent of patients remain alive with a median follow-up of 2 years (range 1 month – 4 years). Although overall survival is very good, immune recovery has not been accelerated in most patients and post-transplant infections have caused significant morbidity. Only 3/17 (18%) of patients achieved > 100 CD4<sup>+</sup> T cells/uL by 100 days after transplant and 3/4 of these patients either had received donor T cell infusion for a 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). We have therefore developed this study to build on the success of the UCSF study, and add a new approach to hasten immune recovery and decrease post-transplant infections.

Because the overall survival has been very good and because there has been no severe acute GVHD, we will extend this approach to patients with unrelated donors as well.

## **2.2 Experience with donor T lymphocyte infusions (DLI) after T cell-depleted transplants**

As part of a retrospective review with UCSF, we recently reported giving low dose DLI (3-6 x 10<sup>4</sup>/kg) to 16 recipients of mismatched related donor SCT (15). The DLI given were from the CD34-negative fraction of the G-CSF mobilized blood stem cells that were cryopreserved at the time of SCT. Ten of these patients were transplanted for malignancies or aplastic anemia, and 6 were transplanted for immunodeficiency. DLI were planned to be given to hasten immune recovery in patients that had a CD4<sup>+</sup> T cell count < 100/mcl at 100 days after SCT for malignancy or at 4 weeks after SCT for immunodeficiency, or for the treatment of infection. DLI were given at a median 12 weeks (range 4-34 wks) after SCT. The median time to have a CD4<sup>+</sup> T cell count > 100/mcl was 1 month (range 0.5-5.5 months) after DLI. Four patients never achieved this response. Grade I-II GVHD was seen in 19% of patients and no severe GVHD occurred. Of the 15 patients that survived more than 100 days, 20% developed chronic GVHD. Six patients died of pre-existing or new infections, including 2 in the context of treatment for chronic GVHD. These results suggest that DLI given at a median of 12 weeks after a mismatched SCT are reasonably well tolerated and result in a recovery of T cells. However, this approach doesn't address the marked risk of infection during the first few months after SCT.

Lewalle et al. reported a dose-finding study of G-CSF mobilized mismatched related donor DLI in 11 adults (16). Nine patients given 1 x 10<sup>4</sup> CD3<sup>+</sup> cells/kg starting one month after SCT and 4 developed Grade I to Grade III GVHD. Two patients were given a dose of 3 x 10<sup>4</sup> CD3<sup>+</sup> cells/kg and both had Grade II GVHD. Both eventually died from complications of chronic GVHD. In comparison to our experience, this data suggests that either early (ie. 1 month) after SCT DLI and/or adult recipients is associated with an increased risk of GVHD.

Handgrettinger et al. reported the use of DLI in recipients of mismatched related donor SCT (17). The DLI were given from freshly collected blood (not from the G-CSF mobilized stem cell collection). Eleven patients were given doses from  $2.5 \times 10^4$  to  $10 \times 10^4$  CD3<sup>+</sup>/kg around 1 month after transplant. The DLI could be repeated at monthly intervals. Six patients developed acute GVHD – grade I in 2 patients (dose  $2.5 \times 10^4$  for one and  $5 \times 10^4 \times 2$  for one), grade II in 3, patients and grade 4 in 1 patient. Immune recovery was faster than usually reported for this type of transplant, with the median time to reach CD3<sup>+</sup> cells > 100/mcl being 72 days after transplant. This study also suggests that DLI soon after SCT is associated with an increased risk of acute GVHD.

### **2.3 Other approaches to hasten immune reconstitution**

One approach to hasten immune recovery involves the administration of donor T lymphocytes that are not alloreactive (don't recognize the transplant recipient's tissues as different) and therefore not capable of causing GVHD. This approach involves the stimulation of donor blood with recipient blood and then the subsequent removal of activated (alloreactive) T cells. The basis for this approach is that after removal of alloreactive T cells, the T cells that remain are not alloreactive but can provide protection against infectious agents. The removal of the activated (alloreactive) T cells has been done using immunomagnetic selection (in a similar fashion to how CD34<sup>+</sup> cells are obtained with the CliniMACS® device) with antibodies against CD25 or CD69, which are activation antigens on T cells (18). A similar approach uses an antibody against CD25 that has been linked to a toxin to kill the activated cells (19). The approaches for depletion of alloreactive T cells are technically cumbersome, require GMP facilities for cellular therapy, and have a potential risk of transmitting infection to the recipient as a result of the *ex vivo* manipulation. It is unlikely that such approaches will ever be widely available.

An approach that would provide killing of alloreactive T cells *in vivo* would be feasible at a larger number of centers and would reduce costs. Such an approach has been used for mismatched related donor transplantation. Donor stem cells (not T cell depleted) were used and high-dose cyclophosphamide given 3-4 days after transplantation to delete alloreactive T cells (20). Patients were also given cyclosporine and mycophenolate mofetil to prevent GVHD after transplant. The incidence of grade III-IV acute GVHD was 6%. However, the incidence of relapse was high and survival was only 36%. The study was conducted in adults with advanced hematological malignancies, preventing a comparison with our experience.

### **2.4 Rationale for planned approach**

This study will use a donor T lymphocyte (CD3<sup>+</sup> cells) infusion (DLI) to help immune recovery. As discussed above, T cells cause GVHD. The likelihood of GVHD depends on the T cell dose given, when they are given, and if ATG is given around the time of T cell infusion. GVHD prophylaxis will be given with the donor T cell infusion to prevent severe GVHD. Our hypothesis is that the use of GVHD prophylaxis with methotrexate will prevent GVHD by killing T cells responding to alloantigens in the recipient while sparing a sufficient number of viral-specific T cells to provide some immunity and protection from infection.

#### **2.4.1 GVHD prophylaxis**

Murine models suggested that a delayed DLI separated from the transplant would prevent GVHD (21). However, the delay in humans probably needs to be 3 to 6 months and this leaves patients at high risk for infection. Early (ie. 1 month after transplant) DLI carries a significant

risk of severe acute GVHD when given without GVHD prophylaxis (14;22).

This study will explore an approach that combines aspects of the approaches described above. Methotrexate, which is an anti-proliferative chemotherapy drug like cyclophosphamide, will be used. Methotrexate has been used for the prevention of GVHD for many years (23). This approach is also supported by laboratory data that showed that trimetrexate (a folate antagonist similar to methotrexate) can delete alloreactive T cells. Remaining lymphocytes were able to respond to cells from a third party and to candidal antigens (24).

#### **2.4.2 Rituximab**

Following a comprehensive review of study data in September 2014, it was noted that a significant number of subjects began to have B cell recovery as early as 8 weeks after transplant. This recovery may be due to B cells in the donor lymphocyte infusion (DLI) and may have contributed to disease, such as post-transplant lymphoproliferative disease (PTLD) and autoimmune disease, in some patients. We believe that we may be able to prevent these complications by giving rituximab with the DLI. The risk of giving rituximab will be slower B cell reconstitution but we think that the benefit of preventing disease due to B cells will outweigh this risk. A lower dose of rituximab than used with transplant was chosen because we are only trying to eliminate the B cells in the DLI and not those in the patient. The dose that we will use has recently been reported in the setting of T alpha beta depletion where B cells are given at the time of transplant (31)

#### **2.4.3 Timing of T cell infusion**

T cells can be given with the stem cells at the time of transplant. As noted above, this has been done with T replete alternative donor transplants using post-transplant cyclophosphamide. Most approaches utilize very T cell-depleted transplants (12). The UCSF study used a very T cell-depleted transplant, but added back a fixed dose ( $3 \times 10^4$  CD3<sup>+</sup> cells/kg) of T cells to the transplant. Immune recovery was not accelerated, perhaps due to the ATG that had been given prior to transplant. Residual ATG in the patient may have killed these T cells.

We will delay the DLI until 30 days after the SCT. Reasons for the delay are: residual ATG in the patient should be less at that time point to decrease the chance of T cells being killed; delaying the DLI will allow engraftment and healing from the transplant conditioning regimen-related toxicity. Methotrexate given soon after the conditioning regimen adds to oral and GI mucositis and slows recovery of blood counts. Patients receiving CD34<sup>+</sup> selected transplants with a large stem cell dose typically have very robust engraftment at one month after transplant and should be able to tolerate the methotrexate without a significant decrease in blood counts.

#### **2.4.4 Cell dose**

Our treatment plan will be to target the T cell dose at transplant to  $< 1 \times 10^4$  CD3<sup>+</sup> cells/kg recipient. A dose of  $< 1 \times 10^4$  CD3<sup>+</sup> cells/kg should minimize the risk of GVHD from the transplant (12). We will then give DLI with methotrexate GVHD prophylaxis in an attempt to hasten immune reconstitution while preventing significant GVHD from these cells.

We have chosen 6 DLI (CD3<sup>+</sup> or T cell) doses to evaluate. We are attempting to balance the chance of benefit from the DLI - faster immune recovery and fewer infections - with the risk of severe GVHD. Our experience in children with viral infections after this type of transplant is that they have benefited from anti-viral responses against cytomegalovirus, Epstein-Barr Virus, and adenovirus following DLI doses of  $3 \times 10^4$ /kg. Viral-specific T cells are present at a level of 1 per  $1 \times 10^4$  cells to 1 per  $10^5$  T cells CMV and EBV, respectively (25;26). A DLI dose of  $3 \times 10^4$ /kg to an average size patient weighing 30 kg will provide  $9 \times 10^5$  T cells. This should provide adequate viral-specific clones that can be expanded *in vivo* to prevent viral infections.

We have seen GVHD in patients with a dose of  $3 \times 10^4$  cells/kg. In the haploidentical donor setting, others have reported a T cell dose of  $> 1-5 \times 10^4$ /kg as the threshold for causing GVHD when no GVHD prophylaxis is used. The range of doses probably reflects different variables like time post-transplant, number of T cells in the PBSC graft, and conditioning regimen (16;17). Therefore, there will not be a cohort of patients receiving DLI for immune reconstitution without methotrexate. Our hypothesis is that the methotrexate prophylaxis will prevent GVHD and allow these doses of T cells to be given. If there is not acceleration of immune recovery and severe GVHD is not seen with a dose of  $3 \times 10^4$  cells/kg, the dose of DLI will be escalated to a maximum of  $1 \times 10^5$  cells/kg. We will not exceed this dose because we feel that the risk of severe GVHD outweighs the potential benefit at higher doses.

### 3.0 STUDY DESIGN AND ELIGIBILITY CRITERIA

#### 3.1 Study design

Patients will receive a donor lymphocyte infusion (DLI) on Day +30 (up to Day +42 if necessary) after transplant. The DLI dose will be escalated for each cohort as described below. Rituximab 200 mg/m<sup>2</sup> IV will be given on the day of DLI infusion prior to the infusion if possible. It can be given +/- 1 day if necessary for logistical reasons. After the DLI, patients will be given IV methotrexate as GVHD prophylaxis. The methotrexate dose will be as follows:

Days 1, 3, 10, 17, 24: 10 mg/m<sup>2</sup>

Days 31, 38: 7.5 mg/m<sup>2</sup>

Days 45, 52, 59, 66, 73, 80: 5mg/m<sup>2</sup>

	Day 0	Day 1	Day 3	Day 10	Day 17	Day 24	Day 31	Day 38	Day 45	Day 52	Day 59	Day 66	Day 73	Day 80
<b>DLI</b>	<b>X</b>													
<b>MTX</b>		<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>

Days 0 denotes day of donor lymphocyte infusion.

#### 3.2 Patient inclusion criteria

**3.2.1** Patients treated on the study “A Phase II Study Using the CliniMACS® Device for CD34<sup>+</sup> Cell Selection and T Cell Depletion for Graft-versus-Host Disease Prophylaxis in Alternative Donor Stem Cell Transplant Recipients”.

**3.2.2** All patients or legal guardians must have agreed to participate by signing an informed consent document.

### **3.3 Patient exclusion criteria**

- 3.3.1** Active GVHD at the time when DLI are due
- 3.3.2** History of acute GVHD > grade I prior to DLI
- 3.3.3** Disease due to viral infection (eg. CMV) when DLI are due (asymptomatic viral replication or viral shedding is not a contraindication)
- 3.3.4** Uncontrolled bacterial or fungal infection
- 3.3.5** O<sub>2</sub> saturation by pulse oximetry < 95%
- 3.3.6** Bilirubin > 3mg/dL or ALT > 5 x upper limit of normal
- 3.3.7** Creatinine > 3x baseline (baseline is creatinine at admission for transplant)
- 3.3.8** ANC (WBC x % neutrophils + bands) < 500/uI
- 3.3.9** Significant effusions (eg. pleural or pericardial) or ascites
- 3.3.10** EBV-related PTLD
- 3.3.11** Persistent or increasing mixed chimerism requiring therapeutic DLI as defined on the LCH 09-01 protocol

### **3.4 Patient recruitment**

All patients who have a transplant on the companion study using the CliniMACS® device (referenced above) will be evaluated for eligibility. Patients who meet the eligibility criteria will be offered the opportunity to participate in this study.

### **3.5 Patient registration**

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

### **3.6 Donor selection criteria**

The DLI donor will be the same as the donor for the stem cell transplant.

## **4.0 INVESTIGATIONAL TREATMENT PLAN**

### **4.1 Treatment dosage and administration**

Patients will receive a donor lymphocyte infusion on Day +30 after transplant (may be given up to day +42 if necessary). The DLI dose for the first cohort will be  $3 \times 10^4$  cells/kg and will be obtained from cells cryopreserved from the G-CSF-mobilized PBSC at the time of the transplant. The dose administered will be based on the cryopreserved dose. Dose escalation will continue as described in the Statistical section (8.0).

#### **4.1.1 Donor lymphocyte infusion (DLI)**

DLI will be cryopreserved at the time of the CD34<sup>+</sup> selection from donors. The DLI will be prepared from the “negative” fraction of the stem cell harvest. DLI will be cryopreserved in bags containing  $3-10 \times 10^4$  CD3<sup>+</sup> cells/kg of recipient weight.

DLI will be thawed and infused according to the LCH BMT procedures for thawing and infusion of cryopreserved hematopoietic progenitor cells. When a dose is needed that is less than a dose that was frozen, an aliquot of a volume proportional to the desired dose may be drawn into a syringe.

#### **4.1.2 Rituximab**

Rituximab 200 mg/m<sup>2</sup> IV will be given on the day of DLI infusion prior to the infusion if possible. It can be given +/- 1 day if necessary for logistical reasons.

**4.1.3 Patients will receive methotrexate IV as follows:**

Day 1 (approximately 24 hrs after DLI infusion): methotrexate 10 mg/m<sup>2</sup>

Day 3: methotrexate 10 mg/m<sup>2</sup>

Day 10: methotrexate 10 mg/m<sup>2</sup>

Day 17: methotrexate 10 mg/m<sup>2</sup>

Day 24: methotrexate 10 mg/m<sup>2</sup>

Day 31: methotrexate 7.5 mg/m<sup>2</sup>

Day 38: methotrexate 7.5 mg/m<sup>2</sup>

Day 45: methotrexate 5 mg/m<sup>2</sup>

Day 52: methotrexate 5 mg/m<sup>2</sup>

Day 59: methotrexate 5 mg/m<sup>2</sup>

Day 66: methotrexate 5 mg/m<sup>2</sup>

Day 73: methotrexate 5 mg/m<sup>2</sup>

Day 80: methotrexate 5 mg/m<sup>2</sup>

The dose should be given on these days whenever possible, especially for doses through Day +24. Doses after day 3 dose may be given +/- 2 days if necessary.

**Concurrent use of other myelosuppressive drugs should be minimized to avoid neutropenia.**

**4.1.4 There will be a dose escalation of DLI as follows:**

Cohort 1: DLI 3 x 10<sup>4</sup>/kg

Cohort 2: DLI 4 x 10<sup>4</sup>/kg

Cohort 3: DLI 5 x 10<sup>4</sup>/kg

Cohort 4: DLI 6 x 10<sup>4</sup>/kg

Cohort 5: DLI 8 x 10<sup>4</sup>/kg

Cohort 6: DLI 10 x 10<sup>4</sup>/kg

The dose escalation will occur separately for Cohorts A (mismatched related donors) and B (unrelated donors) as discussed in the statistical section.

Details of the dose escalation are provided in the Statistical section (8.0).

The DLI dose will be calculated using the weight used to calculate the CD34+ cell dose for PBSC transplant. At investigator discretion, the dose may be adjusted in the following situations:

(1) the dose may be reduced to a dose based on current weight at time of DLI if the patient has lost more than 10% body weight from the weight used to calculate the CD34+ dose

(2) 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.

**4.2 Duration of therapy**

Patients will be followed for study outcomes for 2 years after transplant.

## 5.0 DOSE MODIFICATIONS AND TOXICITIES

### 5.1 Methotrexate

#### 5.1.1 Availability

Methotrexate is commercially available in a number of dosage forms:

- Solution for Injection: 2.5 mg/ml (2ml vial), or 25 mg/ml (2ml, 4ml, 8ml, and 10ml vials)
- Powder for Reconstitution/Injection: 20 mg, 100 mg, 1000 mg
- Oral Tablet: 2.5 mg

#### 5.1.2 Storage and stability

Store tablets and intact vials at room temperature and protect from light. Dilute powder with D5W or NS to a concentration of  $\leq 25$  mg/ml (for 20 mg and 50 mg vials) or 50 mg/ml (for the 1g vial size). Further dilutions are stable for 24 hours at room temperature. Reconstituted solutions with a preservative may be stored under refrigeration for up to 3 months and for up to 4 weeks at room temperature.

#### 5.1.3 Preparation

The 1 g vial may be diluted in 100 mL of saline or D5W.

#### 5.1.4 Administration

Methotrexate, in this protocol, will be administered as a slow push IV. No leucovorin rescue is required at the doses used in this protocol.

#### 5.1.5 Toxicity

Most common adverse effects are alopecia, rash, diarrhea, nausea/vomiting, and mucositis.

Most serious adverse effects are skin ulceration, hyperuricemia, myelosuppression (usually with higher doses), LFT abnormalities, liver failure, hepatic cirrhosis/fibrosis/necrosis, acute renal failure, and interstitial pneumonitis.

### 5.2 Rituximab

#### 5.2.1 Availability

Rituximab is commercially available as a solution for injection:

- 10mg/ml (10ml and 50ml vials)

#### 5.2.2 Storage and stability

Vials are stable at 2°C-8°C (36°F-46°F) protected from direct sunlight. Do not freeze or shake. Rituximab solutions for infusion may be stored at 2°C-8°C (36°F-46°F) for 24 hours and at room temperature for an additional 24 hours.

#### 5.2.3 Preparation

Dilute to a final concentration of 1 mg/ml in an infusion bag containing either 0.9% Sodium Chloride, USP, or 5% Dextrose in Water, USP.

#### 5.2.4 Administration

Infuse at an initial rate of (0.5 mg/kg/hour; Maximum of 50 mg/hour) for the first hour. If no hypersensitivity or infusion related events

increase rate by (0.5 mg/kg/hour; Maximum 50 mg increase every 30 minutes) every 30 minutes to a maximum rate of 3x maintenance IV fluids (Max 400mg/hr).

### 5.2.5 Toxicity

Most 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.

### 5.3 Dose Modifications

Patients that develop neutropenia (ANC < 1000/ul) during the three months after DLI will be given G-CSF approximately 5 mcg/kg IV/SQ as needed. G-CSF will not be given within 24 hrs of a dose of methotrexate. The investigator may decide to not give G-CSF depending on the clinical situation. The reason should be documented.

If a patient has an ANC < 500/ul when methotrexate is due, the methotrexate will be delayed until the ANC is > 500/ul.

ANC is the absolute number of neutrophils + bands (% neutrophils + % bands X WBC).

#### 5.3.1 The dose of methotrexate will be adjusted as follows:

Bilirubin (mg/dl)	%dose	Creatinine mg/dl	%dose
< 3.0	100	=< 2x baseline	100
3.1 - 6.0	50	> 2x baseline	50
> 6.0	HOLD	> 3x baseline	HOLD

If creatinine level is > 2x baseline, then leucovorin (10 mg/m<sup>2</sup> IV or PO q6h for 4 doses) will be started 24 hours after the Methotrexate has been given. The baseline creatinine is the creatinine at the time of transplant admission.

**5.3.2** Hold methotrexate dose in the presence of significant effusions or ascites.

**5.3.3** Hold methotrexate for serum ALT > 20x upper limit of normal (ULN).

**5.3.4** When a dose of methotrexate is held, it should be given as soon as the reason for holding has resolved (for example, ALT < 20 X ULN). The investigator may choose to continue to hold it depending on the clinical scenario. The reason should be documented.

**5.3.5** A methotrexate dose may be held if required by the patient's condition (eg. sepsis). Methotrexate may continue to be held for ongoing serious infection such as significant viral infection. The reason should be documented and should also be discussed with the Principal Investigator.



## 6.0 SCHEDULE OF ASSESSMENTS

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. 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.

### 6.1 Time and events table

Assessment	Pre-study	Prior to MTX*	Follow up <sup>§</sup>							Off Study
			Day 56	Day 100	Day 120	6 Mo	9 Mo	12 Mo	24 Mo	
Informed Consent	<b>X</b>									
History and PE	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>
Performance Status	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>
O2 saturation	<b>X</b>									
Bilirubin, ALT, Cr	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>
CBC, diff	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>
Lymphocyte markers (CD3, CD4, CD8, CD19, CD16/56)	<b>X</b>		<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	

\*MTX = methotrexate on days 1, 3, 10, 17, 24, 31, 38, 45, 52, 59, 66, 73, and 80 after DLI; The assessments are preferably performed within 2 days prior to methotrexate, but history, PE, and performance status may be up to 5 days prior to methotrexate.

§Follow up is days/months after transplant. Assessments can be +/- 7 days for evaluations at Day 56, 100 and 120. The evaluation at Day 120 should be within 2 days of Day 120 if possible. Assessments can be within one month of target after Day 120.

©If the patient has progression of primary disease or receives therapeutic DLI (eg. For the treatment of viral infection or relapse), chemotherapy, or a PBSC infusion then the above follow-up tests are optional at discretion of the physician.

### 6.2 Patient pre-study assessments

The patients will have a full history and physical examination before participating in this study. In addition, the assessments listed in section 6.1 will be completed for all recipients.

### 6.3 Treatment assessments

The assessments listed in section 6.1 will be followed during the treatment portion of the protocol. In addition, the recipients will be routinely evaluated for GVHD and other toxicities.

#### **6.4 Follow up assessments**

Recipients will be followed for survival and development of GVHD up to 2 years after transplant for efficacy of the protocol. After 2 years, the recipients will be followed as standard procedure for transplants.

### **7.0 CRITERIA FOR TERMINATION**

#### **7.1 Conditions for terminating the study**

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

- 7.1.1** Significant toxicities
- 7.1.2** If it becomes clear that the study treatment is less effective than other available treatments.

#### **7.2 Conditions for individual patient termination**

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

##### **7.2.1 Extraordinary medical circumstances**

If at any time the constraints of this protocol are detrimental to the patient's health, the patient will be removed from protocol therapy. In this event:

- Principal Investigator will be notified.
- Document the reason(s) for withdrawal (Case Report Form).
- Follow the patient for survival.
- CHS IRB will be notified as part of the annual renewal unless the reason for removal is unexpected for the patient's condition, transplant, and/or therapy, in which case the IRB will be notified promptly.

##### **7.2.2 Voluntary withdrawal**

The patient may at any time withdraw from protocol therapy. The steps outlined above will be followed.

##### **7.2.3 Excessive toxicity**

Excessive toxicity will be determined by the Principal Investigator and steps outlined above will be followed.

##### **7.2.4 Investigator's judgment**

The Principal Investigator or attending physician may deem that it is in the best interest of the patient to be removed from protocol. The steps outlined above will be followed. The patients will be included in the intent-to-treat analysis.

### **8.0 STATISTICAL CONSIDERATIONS**

#### **8.1 Study design**

The goal of the study is to determine a DLI dose that (1) results in a biological endpoint of CD4<sup>+</sup> count > 100 at Day +120 after transplant and (2) avoids dose-limiting toxicity defined as unacceptable GVHD. This will be accomplished by a dose escalation of DLI to a dose that results in a high percentage of patients with a CD4<sup>+</sup> count > 100. Dose escalation will also be limited by the occurrence of dose-limiting toxicity, which will be unacceptable GVHD. Unacceptable GVHD will be defined as grade IV acute GVHD or an excessive rate of grade II/III acute GVHD as defined in Section 8.4. Clinical success for a patient in this trial is to have successful immune recovery (CD4<sup>+</sup> > 100 at Day +120) without experiencing any grade IV by Day +180.

The study can be amended during its progress by adapting the methotrexate dosing if GVHD occurs and CD4<sup>+</sup> recovery in the absence of GVHD doesn't. This may include a change in the methotrexate dose or prolongation of methotrexate to more closely mimic long course methotrexate (15 mg/m<sup>2</sup> on Day +1 followed by 10 mg/m<sup>2</sup> on Days +3, 6, 11 and then weekly until Day +102). After a change in therapy, future patients will be considered as new cohorts even at the same DLI dose.

## 8.2 Sample size

The study could enroll up to 46 patients (6 at each dose level plus up to 10 additional patients at the estimated target dose) for each cohort (see below). It is unlikely that 6 patients will be enrolled at each dose level. It is more likely that enrollment will approximate the original estimate of 28 patients.

## 8.3 Data management procedures/process

Proper use of the study design will be ensured by the Study Investigator informing the study statistician A. Ivanova (aivanova@bios.unc.edu or 919-843-8086) whenever a grade IV acute GVHD (following DLI) occurs (except for unevaluable patients as defined below in section 8.4) or a new patient is ready for assignment.

## 8.4 Data analysis

The *target* dose in this study is defined as the dose where:

- 1) The probability of grade II and III GVHD is at most 33% and the probability of grade III GVHD is at most 17%;
- 2) No grade IV GVHD events are observed;
- 3) Probability of successful immune recovery (CD4<sup>+</sup> > 100) by Day +120 is at least 66%.

The design for the dose-finding portion of the study is described below in subsection *Dose finding design*. The following treatments with DLI will be considered:

- Cohort 1: DLI 3 x 10<sup>4</sup>/kg
- Cohort 2: DLI 4 x 10<sup>4</sup>/kg
- Cohort 3: DLI 5 x 10<sup>4</sup>/kg
- Cohort 4: DLI 6 x 10<sup>4</sup>/kg
- Cohort 5: DLI 8 x 10<sup>4</sup>/kg
- Cohort 6: DLI 10 x 10<sup>4</sup>/kg

Patients will be evaluated for acute GVHD due to prophylactic DLI between the day of prophylactic DLI infusion and Day +180 after transplant. Patients that have not had grade II, III, or IV acute GVHD following prophylactic DLI but receive therapeutic DLI (eg. For the treatment of viral infection or relapse), chemotherapy, or a PBSC infusion prior to Day +180 will be considered unevaluable because these therapies may cause or prevent GVHD and affect the

determination of this endpoint. Patients that receive third party anti-viral cells within 14 days of Day +180 may be considered evaluable at the discretion of the Principal Investigator. The decision is based upon the fact that different cells may have a different risk of GVHD. In general, the risk of GVHD due to these cells will be very low. Patients will be replaced by enrollment of additional patients.

There will be two cohorts of patients that will be analyzed separately:

COHORT A: Recipients of transplant from mismatched related donors

COHORT B: Recipients of transplant from unrelated donors

#### **8.4.1 Dose finding design**

All patients who meet the eligibility criteria will be offered the opportunity to participate in this study.

The study design consists of two phases, a dose-escalation phase and dose confirmation phase. If at any point one grade IV acute GVHD event is observed at a specific dose, no more patients will be assigned to this or higher dose levels unless the methotrexate dosing is modified. In the dose-escalation phase, patients will be enrolled in groups of 3. The first group of 3 patients will be enrolled at the lowest of six dose levels.

- If none of the initial three patients at a dose level experiences grade II/III GVHD and  $< 2/3$  have a CD4<sup>+</sup> count  $> 100$  at Day +120, the next three patients will be assigned to the next higher dose level.
- If 1 out of 3 patients has a grade III GVHD event or 1 – 2 out of 3 patients have a grade II GVHD event and/or  $\geq 2/3$  have a CD4<sup>+</sup> count  $> 100$  at Day +120, the dose is repeated for the next group of patients.
  - If  $\leq 2/6$  grade II/III GVHD events and  $\leq 1/6$  grade III GVHD events occur and  $< 4/6$  patients have a CD4<sup>+</sup> count  $> 100$  at Day +120, the dose is increased for the next group of patients.
  - If  $\leq 2/6$  grade II/III GVHD events and  $\leq 1/6$  grade III GVHD events are observed and  $\geq 4/6$  patients have a CD4<sup>+</sup> count  $> 100$  at Day +120, this dose is selected for the dose confirmation phase.
- If all 3 patients have grade II GVHD events or 2 grade III GVHD events are observed, 3 more patients are assigned to the next lower dose level, unless there are already 6 patients at that dose level.

Because of the relatively long time for a patient to be evaluable and the excellent early clinical outcomes on the study:

- While the initial 3 patients at a dose level are being followed, additional patients may be enrolled at the same dose level if none of the initial 3 have grade II/III GVHD within 30 days of DLI.
- While patients in the second group of 3 at a dose level are being followed, additional patients may be enrolled at the same dose level if: (a) 0-1 of the initial 3 patients had grade II GVHD and none had grade III GVHD, and (b) none

- of the second group of 3 have grade II/III GVHD within 30 days of DLI.
- When patient 6 is being followed, additional patients may be treated at the next higher dose level if (a) none of the first 3 patients had grade II/III GVHD and < 2 of the first five evaluable patients have grade II/III GVHD, (b) patient 6 did not have grade II/III GVHD within 30 days of DLI, (c) and < 3/5 patients have CD4+ count >100 at Day +120. Patient 6 will not be replaced if the patient becomes not evaluable.
  - If the above criteria are not met, then the additional patients will be enrolled at a previous, lower dose level.
  - When the patients being followed are at the initial dose ( $3 \times 10^4/\text{kg}$ ), the additional patients will be treated at this dose.

The dose-escalation phase is continued until the maximum dose of DLI is reached or until escalation is stopped for toxicity or efficacy.

After the dose-escalation phase is completed and preliminary estimate of the target dose is obtained, up to 10 more patients will be assigned to the estimated target dose in each cohort (cohorts A and B).

The confirmatory stage will be stopped and the dose re-evaluated if one of the following occurs:

- The lower bound of the 50% confidence interval for the rate of grade II and III GVHD is above 33%
- The lower bound of the 50% confidence interval for the rate of grade III GVHD is above 17%;
- Grade IV GVHD event is observed;
- The upper bound of the 50% confidence interval for the rate of successful immune recovery ( $\text{CD4}^+ > 100$ ) by Day +120 is lower than 66%. If patients are having clinical benefit despite not achieving the target efficacy ( $\text{CD4}^+ > 100$  at Day +120) but are not having unacceptable rates of GVHD, then up to 10 more patients can still be entered.

### **8.5 Estimated duration of study**

The study will require 5 years for accrual and up to 2 years for follow-up studies.

## **9.0 CRITERIA FOR EVALUATION**

### **9.1 Monitored outcomes**

**9.1.1** The outcomes will be CD4 count at day +120 post-transplant, GVHD, infections, and EBV-related PTLD.

### **9.2 Toxicity definitions**

**9.2.1** Toxicity will be graded according to the CTCAE v3.0.

## **10.0 DATA SAFETY MONITORING PLAN**

### **10.1 Oversight and monitoring plan**

The study will utilize the Data and Safety and Monitoring Committee (DSMC) of the Pediatric Blood and Marrow Transplant Consortium (PBMTTC). 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 all serious adverse events including expected and unexpected events according to PBMTTC DSMC guidelines.

### **10.2 Monitoring and reporting guidelines**

The PI will conduct continuous review of data and patient safety at the weekly Blood and Marrow Transplant meeting where the results of each patient's treatment are discussed. The discussion will be documented in the minutes. The discussion may include for each dose level, the number of patients, significant toxicities as described in the protocol, dose adjustments, and observed responses. Semi-annual reports will be submitted to the PBMTTC DSMC for review according to PBMTTC guidelines. Unexpected grade 3 adverse events (AE), all grade 4-5 adverse events and all serious adverse events (SAE) related to study participation will be submitted to the FDA, IRB and DSMC.

### **10.3 Review and oversight requirements**

#### **10.3.1 Adverse events 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.

#### **10.3.2 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> for grading the severity of adverse events. All grade 3-5 adverse events will be recorded until 1 year post-transplant and all other endpoints in Section 9.1 will continue to be recorded through the duration of follow-up. Grading of acute and chronic GVHD will be according to Appendix I and II. Causality of AE will be rated as definitely, probably, possibly, unlikely related, or unrelated to the DLI. **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.**

### **10.3.3 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:

- 10.3.3.1 Death
- 10.3.3.2 A life-threatening adverse drug experience
- 10.3.3.3 Inpatient hospitalization or prolongation of existing hospitalization.
- 10.3.3.4 Persistent or significant disability/incapacity (a substantial disruption of a person's ability to conduct normal life functions).
- 10.3.3.5 Birth defect/ congenital anomaly
- 10.3.3.6 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 medical or surgical intervention to prevent one of the prior listed outcomes.

### **10.3.4 Reporting procedures**

Reporting of serious adverse events will be in accordance with 21 CFR 312.32 and 21 CFR 314.80. If required, an NCI Adverse Drug Reaction (ADR) form will be completed and copies kept in the regulatory binder and submitted to the IRB.

For all adverse events reported or observed, the information will be recorded in the Case Report Forms for that patient. This will include a full description of the event, its severity or toxicity grade, the relationship to the study drug, and the treatment, outcome and sequelae of the event.

FDA website for guidance in reporting serious adverse events <http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSe arch.cfm?fr=312.32>

MedWatch forms and information:

Change this to FDA Adverse Event Reporting System (AERS)  
<http://www.fda.gov/medwatch/getforms.html>

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, or his designee within 24 business hours. The reporting procedure is by personal communication via phone or in person with written documentation of the 1:1 communication via e-mail with a copy of the e-mail placed in the regulatory binder.

**10.3.5 Review of adverse event rates**

Each semi-annual report will indicate if the AE incidence is considered to be higher than expected by the PI. 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.

**10.3.6 Semi-annual review of study progress**

Principal Investigator is required to submit semi-annual study progress reports to determine whether accrual projections are being met, to summarize unexpected grade 3 adverse events, all grade 4 and 5 adverse events, SAE reports, and the rate of unexpected grade 3 and all grade 4-5 AE's. In addition, progress on recruitment and subjects known response to the investigational therapy should be reported.

These semi-annual reports are reviewed by the PBMTDC 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.

**11.0 ETHICAL ASPECTS****11.1 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 for the companion protocol using the CliniMACS® device. 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.

**11.2 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.

**12.0 DATA FORMS AND SUBMISSION SCHEDULE****12.1 Study monitoring**

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 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 a locked cabinet in the BMT Research office. Patient enrollment information and toxicity/reporting information will also be entered by the coordinator into the BMT Research database.

Forms	Completion Schedule
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<b>Enrollment Forms:</b> <ul style="list-style-type: none"> <li>▪ <b>Recipient Eligibility</b></li> <li>▪ <b>Recipient Baseline Information</b></li> </ul>	Complete after recipient, parent, or guardian has signed consent and prior to treatment of the recipient
<b>Treatment Form</b>	Complete within one month after last dose of methotrexate
<b>Treatment Forms:</b> <ul style="list-style-type: none"> <li>▪ <b>Immune Recovery Form</b></li> <li>▪ <b>GVHD</b></li> <li>▪ <b>AE form</b></li> </ul>	Complete within one month of the time points listed in table 6.1.
<b>Toxicity Reporting:</b> <ul style="list-style-type: none"> <li>▪ <b>SAE Form</b></li> <li>▪ <b>CHS IRB Form</b></li> <li>▪ <b>FDA Medwatch Form</b></li> </ul>	Complete as required based on reporting criteria for each institution.

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

<b>ORGAN</b>	<b>STAGE</b>	<b>DESCRIPTION</b>	
<b>SKIN</b>	1	Maculopapular rash < 25% of BSA	
	2	25 – 50% of BSA	
	3	Generalized erythroderma	
	4	Desquamation and bullae	
<b>LIVER</b>	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	
<b>GUT</b>	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	
<b>GRADE</b>	<b>Skin</b>	<b>Liver</b>	<b>Gut</b>
<b>I</b>	1-2	0	0
<b>II</b>	3 and/or	1 and/or	1
<b>III</b>	--	2-3 and/or	2-3
<b>IV</b>	4 and/or	4 and/or	4

\*\* Adapted from Glucksberg and Jacobsohn articles (27;28).

**APPENDIX II - 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 (29) shown below.**

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

Original Seattle Classification	Revised Seattle Classification*
<b>Limited</b>	<b>Clinical limited</b>
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 $\leq 2 \times$ upper limit of normal, AST or ALT $\leq 3 \times$ upper limit of normal, and total bilirubin $\leq 1.6$ ) 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 $\leq 5$ 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
<b>Extensive</b>	<b>Clinical extensive</b>
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 $<60\%$ , $\geq 15\%$ 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
Involvement of eye (Schirmer's test with $<5$ mm wetting), or:	5. Onycholysis or onychodystrophy thought to represent chronic GVHD, with documentation of chronic GVHD in any organ
Involvement of minor salivary glands or oral mucosa demonstrated on labial biopsy, or:	6. Decreased range of motion in wrist or ankle extension due to fasciitis caused by chronic GVHD
Involvement of any other target organ	7. Contractures thought to represent chronic GVHD
	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 $>2 \times$ upper limit of normal, AST or ALT $>3 \times$ upper limit of normal, or total bilirubin $>1.6$ , and documentation of chronic GVHD in any 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.

**Grading of Chronic GVHD severity by NIH Consensus Guidelines using Organ Scoring Table (30)**

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

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

**REFERENCES**

- (1) **Balduzzi A, Gooley T, Anasetti C, Sanders J, Martin P, Petersdorf E et al. Unrelated donor marrow transplantation in children. Blood 1995; 86:3247-3256.**
- (2) **Wagner JE, Barker JN, Defor TE, Baker KS, Blazar BR, Eide C et al. Transplantation of unrelated donor umbilical cord blood in 102 patients with malignant and nonmalignant diseases: influence of CD34 cell dose and HLA disparity on treatment-related mortality and survival. Blood 2002; 100(5):1611-1618.**
- (3) **Beatty PG, Clift RA, Mickelson EM, Nisperos BB, Flournoy N, Martin PJ et al. Marrow transplantation from related donors other than HLA-identical siblings. N Engl J Med 1985; 313(13):765-771.**
- (4) **Ferrara JL, Deeg HJ. Graft-versus-host disease. N Engl J Med 1991; 324(10):667-674.**
- (5) **Rowe JM, Lazarus HM. Genetically haploidentical stem cell transplantation for acute leukemia. Bone Marrow Transplant 2001; 27(7):669-676.**
- (6) **Sleight B, Cowan M, Horn B, Jaroscak J, McGuirk J, Gilman A. Selected haplocompatible donor stem cell transplantation in children. Blood 2006; 108:5417a.**
- (7) **Aversa F, Tabilio A, Terenzi A, Velardi A, Falzetti F, Giannoni C et al. Successful engraftment of T-cell-depleted haploidentical "three-loci" incompatible transplants in leukemia patients by addition of recombinant human granulocyte colony-stimulating factor-mobilized peripheral blood progenitor cells to bone marrow inoculum. Blood 1994; 84(11):3948-3955.**
- (8) **Aversa F, Tabilio A, Velardi A, Cunningham I, Terenzi A, Falzetti F et al. Treatment of high-risk acute leukemia with T-cell-depleted stem cells from related donors with one fully mismatched HLA haplotype. N Engl J Med 1998; 339(17):1186-1193.**
- (9) **Lang P, Handgretinger R, Niethammer D, Schlegel PG, Schumm M, Greil J et al. Transplantation of highly purified CD34+ progenitor cells from unrelated donors in pediatric leukemia. Blood 2003; 101(4):1630-1636.**
- (10) **Lang P, Klingebiel T, Bader P, Greil J, Schumm M, Schlegel PG et al. Transplantation of highly purified peripheral-blood CD34+ progenitor cells from related and unrelated donors in children with nonmalignant diseases. Bone Marrow Transplant 2004; 33(1):25-32.**
- (11) **Eyrich M, Lang P, Lal S, Bader P, Handgretinger R, Klingebiel T et al. A prospective analysis of the pattern of immune reconstitution in a paediatric cohort following transplantation of positively selected human leucocyte antigen-disparate haematopoietic stem cells from parental donors. Br J Haematol 2001; 114(2):422-432.**
- (12) **Aversa F, Terenzi A, Tabilio A, Falzetti F, Carotti A, Ballanti S et al. Full haplotype-mismatched hematopoietic stem-cell transplantation: a phase II**

- study in patients with acute leukemia at high risk of relapse. J Clin Oncol 2005; 23(15):3447-3454.**
- (13) **Waller EK, Giver CR, Rosenthal H, Somani J, Langston AA, Lonial S et al. Facilitating T-cell immune reconstitution after haploidentical transplantation in adults. Blood Cells Mol Dis 2004; 33(3):233-237.**
- (14) **Lee CK, Magalhaes-Silverman M, Hohl RJ, Hayashi M, Buatti J, Wen BC et al. Donor T-lymphocyte infusion for unrelated allogeneic bone marrow transplantation with CD3+ T-cell-depleted graft. Bone Marrow Transplant 2003; 31(2):121-128.**
- (15) **Dvorak C, Gilman A, Horn B, Jaroscak J, Dunn E, Baxter-Lowe L et al. Clinical and Immunologic Outcomes Following Haplocompatible Donor Lymphocyte Infusions. Bone Marrow Transplant 2009; In Press.**
- (16) **Lewalle P, Triffet A, Delforge A, Crombez P, Selleslag D, De MH et al. Donor lymphocyte infusions in adult haploidentical transplant: a dose finding study. Bone Marrow Transplant 2003; 31(1):39-44.**
- (17) **Handgretinger R, Klingebiel T, Lang P, Schumm M, Neu S, Geiselhart A et al. Megadose transplantation of purified peripheral blood CD34(+) progenitor cells from HLA-mismatched parental donors in children. Bone Marrow Transplant 2001; 27(8):777-783.**
- (18) **Fehse B, Frerk O, Goldmann M, Bulduk M, Zander AR. Efficient depletion of alloreactive donor T lymphocytes based on expression of two activation-induced antigens (CD25 and CD69). Br J Haematol 2000; 109(3):644-651.**
- (19) **Amrolia PJ, Mucioli-Casadei G, Huls H, Heslop HE, Schindler J, Veys P et al. Add-back of allodepleted donor T cells to improve immune reconstitution after haplo-identical stem cell transplantation. Cytotherapy 2005; 7(2):116-125.**
- (20) **Luznik L, O'Donnell PV, Symons HJ, Chen AR, Leffell MS, Zahurak M et al. HLA-haploidentical bone marrow transplantation for hematologic malignancies using nonmyeloablative conditioning and high-dose, posttransplantation cyclophosphamide. Biol Blood Marrow Transplant 2008; 14(6):641-650.**
- (21) **Johnson BD, Truitt RL. Delayed infusion of immunocompetent donor cells after bone marrow transplantation breaks graft-host tolerance allows for persistent antileukemic reactivity without severe graft-versus-host disease. Blood 1995; 85(11):3302-3312.**
- (22) **Goerner M, Weber-Nordt R, Hoepfner S, Benner A, Luft T, Ho AD. Addition of a low fixed number of CD3+ cells to CD34-enriched allografts: effects on engraftment, graft-versus-host disease, and survival after related and unrelated peripheral stem cell transplantation. J Hematother Stem Cell Res 2003; 12(3):309-320.**
- (23) **Ramsay NK, Kersey JH, Robison LL, McGlave PB, Woods WG, Krivit W et al. A randomized study of the prevention of acute graft-versus-host disease. N Engl J Med 1982; 306(7):392-397.**



- (24) Szabolcs P, Park KD, Marti L, Deoliveria D, Lee YA, Colvin MO et al. Superior depletion of alloreactive T cells from peripheral blood stem cell and umbilical cord blood grafts by the combined use of trimetrexate and interleukin-2 immunotoxin. *Biol Blood Marrow Transplant* 2004; 10(11):772-783.
- (25) Hebart H, Deginik S, Stevanovic S, Grigoleit U, Dobler A, Baur M et al. Sensitive detection of human cytomegalovirus peptide-specific cytotoxic T-lymphocyte responses by interferon-gamma-enzyme-linked immunospot assay and flow cytometry in healthy individuals and in patients after allogeneic stem cell transplantation. *Blood* 2002; 99(10):3830-3837.
- (26) Lucas KG, Burton RL, Zimmerman SE, Wang J, Cornetta KG, Robertson KA et al. Semiquantitative Epstein-Barr virus (EBV) polymerase chain reaction for the determination of patients at risk for EBV-induced lymphoproliferative disease after stem cell transplantation. *Blood* 1998; 91(10):3654-3661.
- (27) Jacobsohn DA. Acute graft-versus-host disease in children. *Bone Marrow Transplant* 2008; 41(2):215-221.
- (28) Glucksberg H, Storb R, Fefer A, Buckner CD, Neiman PE, Clift RA et al. Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched sibling donors. *Transplantation* 1974; 18(4):295-304.
- (29) Lee SJ, Vogelsang G, Flowers ME. Chronic graft-versus-host disease. *Biol Blood Marrow Transplant* 2003; 9(4):215-233.
- (30) Filipovich AH, Weisdorf D, Pavletic S, Socie G, Wingard JR, Lee SJ et al. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report. *Biol Blood Marrow Transplant* 2005; 11(12):945-956.
- (31) Bertaina A, Merli P, Rutella S et al. HLA-haploidentical stem cell transplantation after removal of  $\alpha\beta^+$  T and B cells in children with nonmalignant disorders. *Blood* 2014, 124(5) 822-826.