A STUDY OF HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT) IN IMMUNE FUNCTION DISORDERS USING A REDUCED-INTENSITY PREPARATORY REGIME (The HIT Study)

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1.0 STUDY OBJECTIVES

This study hypothesizes that a reduced intensity immunosuppressive preparative regimen will establish engraftment of donor hematopoietic cells with acceptable early and delayed toxicity in patients with immune function disorders. A regimen that maximizes host immune suppression is expected to reduce graft rejection and optimize donor cell engraftment. We will test this hypothesis in immunological diseases that are benefited by transplantation and for whom myeloablation is not necessary to eradicate malignant disease. Host immunosuppression will be achieved with alemtuzumab, fludarabine, melphalan, and low dose thiotepa. All agents have been previously tested in the stem cell transplant setting and are known to provide the desired immunosuppression. Matched or minimally mismatched marrow, peripheral blood, or cord blood products (related and unrelated) will be accepted as stem cell sources. A standard regimen of cyclosporine (CsA) or tacrolimus and short course methotrexate will be administered for the prevention of graft-versus-host disease (GVHD). Steroids will be added for patients receiving unrelated donor transplants. An alternative regimen of cyclosporine or tacrolimus plus mycophenolate mofetil (MMF) is permitted per institution preference for cord blood transplants.

1.1 Primary Objectives

1. To determine if treatment with alemtuzumab, fludarabine, melphalan, and low dose thiotepa followed by hematopoietic stem cell transplantation (HSCT) using bone marrow (BM), peripheral blood (PB), or umbilical cord stem cells (UCB) will result in donor engraftment in patients with immune function disorders.

2. To determine major transplant-related toxicities (CTCAE version 4 grades 4 and 5) during the first year after HSCT in patients with immune function disorders treated with the above-described reduced intensity preparatory regimen.

1.2 Secondary Objectives

1. To determine the time to neutrophil engraftment (defined as ANC > 500/dL for the first of three consecutive days) and platelet engraftment (defined as platelet count > 50,000/dL for the first of seven days without platelet transfusions) in patients with immune function disorders treated with the above-described reduced intensity preparatory regimen followed by HSCT.

2. To determine the incidence of acute GVHD in these patients.

3. To determine the incidence of chronic GVHD in these patients.

4. To determine engraftment within the first two years after HSCT in these patients.

5. To determine infectious complications (> grade 3) during the first 2 years after HSCT.

6. To assess immune reconstitution for 2 years in these patients.

7. To assess overall survival (OS) and disease-free survival (DFS) for 2 years post–transplant in these patients.

8. To determine grade 4 non-hematologic toxicity for 2 years after HSCT.

2.0 BACKGROUND AND RATIONALE

2.1 Allogeneic Stem Cell Transplantation in Immunologic Disorders

Primary immune deficiencies or immune dysfunction/dysregulation disorders encompass a broad group of diseases that affect a single immune cell lineage (lymphoid or myeloid). As a whole these disorders result in inadequate clearance of infections and/or control of immune responses. As a result, patients with these disorders have recurrent infections with significant morbidities, end organ damage and failure, and an increased risk of early mortality. Currently, the only known cure for these patients is hematopoietic stem cell transplant (HSCT). Recent studies have demonstrated high disease-free (DFS) and overall survival (OS) rates following transplant for diseases such as severe combined immune deficiency (SCID), Wiskott-Aldrich Syndrome (WAS), and chronic granulomatous disease (CGD) [1-8].

Stem cell transplantation in patients with immune disorders is associated with several unique attributes compared to HSCT for malignancies [9]. First, the most profound immune deficiencies do not require any conditioning as there is no immunological resistance to engraftment of donor cells (i.e., no host-versus-graft response). Second, the immune defect may be corrected by stable partial reconstitution of normal immune cells. In other words, full donor chimerism of the affected cell type or other hematopoietic components is not necessary. Third, there is no potential benefit of graft-versus-host disease (GVHD) in contrast to patients with malignancies in which a graft-versus-tumor effect is advantageous. Fourth, due to the immune defects, patients often present to transplant with concurrent infections or significant organ toxicities from previous infections and treatments. For instance, organ function (i.e., lung or liver) may be compromised secondary to granulomas or scarring from recurrent infections. Collectively, these unique aspects of transplant for immune defects support utilization of a transplant regimen with reduced organ toxicities and minimal GVHD and the acceptance of partial engraftment, if necessary, to attain these reduced toxicities. We propose a reduced intensity preparative regimen that will achieve engraftment with less toxicity.

2.2 Toxicities of Transplantation and Myeloablative Conditioning Regimen

The use of allogeneic transplantation for non-malignant disorders is limited by toxicities associated with this procedure [10, 11]. The limiting factors are primarily transplant related mortality (TRM), acute and chronic GVHD, infections, and early and/or late toxicities of individual agents and the combination chemotherapy/radiotherapy used for conditioning [12, 13]. Graft rejection can be an additional complication due to host immune competence, which can result in host-versus-graft immune responses, depending on the disorder transplanted.

The incidence of acute GVHD (within the first 100 days post transplant) is 30-50% in allogeneic transplantation [14]. The incidence of chronic GVHD is 30% for related transplants and considerably higher in unrelated donor transplantation [15]. GVHD rates vary with age, conditioning therapy, degree of HLA-mismatching between donor and recipient, stem cell source, prior transfusions, GVHD prophylaxis, and infections [16].

An ideal transplant for a non-malignant disorder should allow stable and adequate engraftment of donor cells, have low TRM, and absent or minimal GVHD. Allogeneic bone marrow transplant is traditionally performed following administration of myeloablative therapy to ablate recipient marrow and allow donor marrow to engraft, and in most cases is targeted at hematologic malignancies. The most commonly used conditioning regimens include total body irradiation

(TBI) or high-dose busulfan [17, 18]. These conditioning regimens have two purposes: 1) eliminate malignant cells and recipient marrow, and 2) allow engraftment of donor cells.

Myeloablative regimens are associated with significant morbidity and mortality. In addition to profound myelosuppression, organ toxicities include: gastrointestinal, such as mucositis, diarrhea, nausea, vomiting, and persistent anorexia; hemorrhagic cystitis; cardiac dysfunction; cutaneous toxicity including erythema, desquamation, and hyperpigmentation; irreversible gonadal dysfunction with infertility and premature menopause; seizures; renal failure; acute and chronic pulmonary dysfunction; and hepatic injury including veno-occlusive disease [19, 20]. The long-term side effects of TBI and busulfan in children also include impaired growth, hormone deficiencies, developmental/cognitive impairment, and a higher life-long risk of second cancers [19, 20].

Following myeloablative HSCT, transplant-related causes of death include regimen-related toxicity, GVHD, or infection. In a recent summary of allogeneic transplants reported to the IBMTR, actuarial treatment related mortality ranged from 3% (0-16%) in patients with chronic myelogenous leukemia in stable phase (good risk) to 12% (1-23%) in patients with acute myeloid leukemia (poor risk) [21]. Hence, it is necessary to find a preparative regimen that will provide engraftment of donor cells with minimal treatment-related complications and a low rate of graft rejection, to make this a safer procedure for treatment of non-malignant disorders.

2.3 Reduced Intensity Conditioning: Rationale and Clinical Experience

Myeloablative conditioning therapy for allogeneic HSCT rapidly and completely eliminates recipient hematopoietic cells and allows engraftment of donor cells. Ablation of recipient bone marrow cells is desirable in transplantation for malignant disorders of the bone marrow in order to eradicate residual malignancy. However, it also renders the recipient susceptible to toxicities of the agents used. Complete ablation of the recipient bone marrow is not necessary for non-malignant disorders and, thus donor-recipient chimerism is an acceptable goal. Stable mixed chimerism is adequate for disease control in many non-malignant disorders [22].

An increased risk of infection following HSCT prior to immune reconstitution is a significant contributor to post-transplant morbidity and mortality. The high risk for serious infections is due in part to the prolonged neutropenia and myelosuppression, a consequence of the intensity of the conditioning regimen and regimen-related tissue damage such as mucositis or skin injury, and central vein catheters. Altering the intensity of the preparative regimen is expected to reduce the interval of absolute neutropenia by 6-8 days while continuing to provide adequate immunosuppression to support donor cell engraftment [23-26]. The shorter neutropenic period can decrease the risk of fungal infections, common in patients with prolonged neutropenia. This shortened window of neutropenia is particularly relevant to patients with immune deficiencies. The absence of normal immune function prior to transplant increases the risk of infection in the peri-transplant period prior to donor derived immune reconstitution.

Several studies support that myeloablation is unnecessary for donor engraftment and host immunosuppression of lymphocyte function is adequate to facilitate this process [23-26]. This approach has the following advantages: 1) a reduction in regimen-related toxicity and 2) a shorter period of neutropenia that should translate into reduced infectious complications. In combination, these attributes of reduced intensity conditioning could reduce hospitalization times, decrease the cost of transplantation, and permit transplantation in patients with disease-related end organ dysfunction, which would prohibit high dose myeloablative regimens [27].

Past experience with reduced intensity transplants (RIT) and non-ablative conditioning regimens

is positive, with low rates of early regimen-related toxicity, minimal myelosuppression, and early hematopoietic engraftment [28-31]. These regimens are based on host immunosuppression provided by fludarabine or low dose TBI, with additional agents such as ATG, melphalan and busulfan. The results are summarized in the table below.

Reference	N	Prep Regimen	GVHD Proph y	Mucositis	Duration ANC<500	Duratio n Plt<20K	AGVHD Grade II-IV	CGVH D	Donor chimerism at day 100	TR M
Nagler, et al. 2001[32]	16	FLU 180 BU 8 ATG 40	CsA	3-mild	17 days	17.5 days	3 Gr II 2 Gr III 1 Gr IV	0	15 1 mixed	2
Slavin, et al 1998[30]	48	Flu 180 BU & ATG	CsA	None	Mild	NA	8	nil	40	1
McSweeney, et al. 1998[33]	8	TBI 200 CsA MMF	CsA MMF	None			1	0	5	0
Spitzer, et al. 1998[34]	15	CY150-200 ATG/CsA Thymic irradiation		None	16	17	8	0	NA	0
Giralt, et al 1998[35]	29	FLU/2CDA MEL 180	CsA/Tacro MTX/St.		14	21	5		21	
Kottaridis, et al 2000[36]	44	CAM 100 FLU 150 MEL 140	CAM CsA MTX		13	13	2	1	18 full 13 mixed	4
Childs, et al 1999[31]	15	FLU 125 CY 120	CsA	None	11	8	6 Gr II 3 Gr III	4 mild	13 graft failure 1	0

FLU = Fludarabine; CY = Cyclophosphamide; CsA = Cyclosporine; BU = Busulfan; ATC = Anti-thymocyte glboulin; MEL = Melphalan; CAM = Campath-1H; Tacro = tacrolimus; MTX = Methotrexate; St = Steroids; TRM = Treatment Related Mortality; AGVHD = Acute GHVD; CGVHD = Chronic GHVD; MMF = mycophenolate mofetil

2.4 Reduced-Intensity Conditioning in Immune Disorders

Our success with RIT using alemtuzumab, fludarabine, and melphalan has been previously reported in several non-malignant disorders [23-26]. However, a barrier to the use of very low intensity conditioning regimens for stem cell transplant in immune deficiencies has been graft rejection. Many of these immunological disease states are associated with hyperactive inflammatory or dysregulated immune responses that prevent engraftment or favor reconstitution of the native immune system. In our own institutional experience of HSCT following alemtuzumab, fludarabine, and melphalan, 39% of patients with immune deficiencies required additional intervention (i.e., immune suppression withdrawal or donor lymphocyte infusion) to attain stable donor engraftment. Full donor engraftment was achieved in 39% whereas 54% achieved stable mixed chimerism with the above interventions. Importantly, with these additional steps, 7% of patients with immune deficiencies had graft rejection. In contrast, other benign hematologic disorders, such as bone marrow failure disorders, readily achieved > 90% donor engraftment with the same preparative regimen without further intervention (unpublished data).

Additionally, donor engraftment was compromised when umbilical cord blood was used as the stem cell source, regardless of underlying disease [23]. The increased graft rejection in immune deficiencies and with cord blood products is likely secondary to robust immune or inflammatory

responses in the host. To overcome these obstacles, thiotepa is added to the preparative regimen to increase the immune suppression. The rationale for the use of thiotepa is adopted from previously reported successful trials of transplants following the addition of hydroxyurea and thiotepa in thalassemia transplants [37, 38]. A national protocol (the URTH trial) for reduced intensity unrelated donor transplant for thalassemia used modified reduced intensity conditioning (a combination of alemtuzumab, melphalan, fludarabine, and thiotepa) similar to that proposed in this study. Though too early to report long-term outcomes, the study completed enrollment of both marrow and cord blood transplants and did not meet stopping rules for mortality or graft rejection. In this study, thiotepa has been included to reduce graft rejection in similar fashion.

2.5 Goals of Conditioning Regimen Proposed in this Trial

In non-malignant disorders, the goal of transplant conditioning is to achieve low transplant-related mortality with successful engraftment from the best available stem cell source. As previously discussed, we propose to reduce the incidence of graft failure by achieving host T cell depletion with alemtuzumab (monoclonal humanized antibody against CD52 antigen), fludarabine, and low level myelosuppression with melphalan and thiotepa. This reduced intensity regimen is designed to offset the morbidity associated with a myeloablative regimen. Alemtuzumab is administered on Days -14, -13, and -12 to reduce the level of the antibody in recipients at the time of donor stem cell infusion, followed by fludarabine, melphalan, and thiotepa in the immediate pre-transplant period. Thiotepa is included in the preparative regimen to enhance immune suppression (see Section 2.4 above).

The adjusted timing of alemtuzumab administration is designed to achieve host immune suppression to facilitate engraftment but simultaneously provide protection against GVHD by low level donor T cell depletion. GVHD is another major barrier to transplant especially in non-malignant disorders. The incidence of GVHD is based on stem cell source and is higher with bone marrow or peripheral blood than with umbilical cord blood. Other factors that influence GVHD are the level of HLA matching, concurrent infection, and the development of an inflammatory state, which is often initiated by the conditioning agents used. The use of immunosuppressive agents with a prolonged half-life, such as alemtuzumab, administered in the peri-transplant can achieve donor T cell depletion and significantly reduce the incidence of GVHD [39, 40]. Alemtuzumab has been given on transplant Days -8 to -4. Given that the half-life of this antibody is 42-57 hours, antibody persisted in recipients' serum through the early post-transplant period. This proximal alemtuzumab treatment may explain the very low frequency of acute GVHD in these patients. Only 7% of patients developed GVHD and no cases of Grade III-IV acute GVHD were observed [39]. However, this approach also has several disadvantages including increased graft rejection due to donor T cell depletion and delayed immune reconstitution resulting in late infection related morbidity [39]. In contrast, administration of alemtuzumab 3 weeks in advance of transplant preferentially suppresses the host immune system but can lead to an increased risk of GVHD [23-26]. More recently, alemtuzumab administration in between these two times, i.e., at 14 days pre-transplant, has allowed for a lower GVHD risk with less pronounced effects on immune reconstitution [41]. We have adopted this newer intermediary approach of alemtuzumab administration for this protocol to support engraftment while simultaneously reducing the incidence of GVHD.

2.6 Stem Cell Source

The stem cell source, HLA-matching, and early availability are key features to a successful transplant in immune function disorders that present with early serious complications such as infections and cytopenias. Though an unaffected HLA matched family donor is ideal, this is a

viable option for < 25% of recipients due to the lack of HLA-matched family members. For patients without a family donor, the ability to quickly identify HLA-compatible unrelated donors (URD) permits HSCT and cure before complications compromise outcome.

Unrelated marrow donors matched by serology at 6 antigens (A, B, and DRB1) can be identified in the majority of Caucasian recipients. However, the likelihood of identifying a matched URD is lower for other ethnicities due to the decreased representation of non-whites in the donor pool. Based on outcome analyses, acceptable bone marrow donors especially for non-malignant disorders should be matched at 8/8 loci (HLA A, B, C, and DRB1) or allele mismatched at a single, preferably Class I, locus as determined by high resolution typing [42].

Umbilical cord blood (UCB) is rich in hematopoietic progenitors and is available from established UCB cryopreservation banks. By utilizing available marrow and cord sources of stem cells, it should be possible to identify suitable donors for patients within a reasonable time frame in the majority of children with immune disorders [43]. UCB transplants have lower rates of GVHD and this allows a higher degree of mismatching at HLA loci, thus increasing the chances of finding potential donors [44, 45]. TRM and engraftment is acceptable with UCB grafts that are matched at 5-6 of 6 HLA loci (A, B, and DRB1) [46]. The limiting factor for the use of UCB is currently the CD34+ cell dose or the total nucleated cell count (TNC). Engraftment is impaired or delayed at a CD34+ cell dose less than 5.0 x 10^5/kg recipient weight or a TNC less than 5 x 10^7/kg making the recipient susceptible to graft rejection or delayed engraftment and life threatening infections/mortality [16]. The number of cord blood cells for UCBT in non-malignant disorders including immune disorders will need to remain high. Thus, single cords with high cell numbers are acceptable donor products. Double cord products have been shown to afford good engraftment and have similar outcomes to single UCB products provided they are suitably matched to the recipient [47]. Based on this data, this trial will allow the use of stringently matched double cord products [48, 49].

Peripheral blood stem cells are acceptable as a stem cell source for transplant but have the disadvantage of an increased risk of chronic GVHD. Since non-malignant disorders have no benefit from GVHD, we will restrict the use of PBSC to related donor transplants only as in that situation, the risk of GVHD is low [50].

An algorithm for donor selection is incorporated into this trial [51].

3.0 ELIGIBILITY CRITERIA

Patients must be enrolled prior to starting protocol therapy (i.e. Alemtuzumab test dose).
All eligibility criteria must be performed within 60 days prior to beginning protocol therapy unless otherwise listed below.
If the patient's lab values are outside the limits of eligibility or their clinical status declines post enrollment but prior to starting protocol therapy, the patient's continuation on study must be discussed with the study chair and the patient may be removed from study.

3.1 Recipient Inclusion Criteria

1. Diagnosis of immune deficiency disorder that is benefited by conditioning followed by bone marrow transplantation. This includes, but is not limited to:

- Severe combined immunodeficiency (SCID)
- Chronic granulomatous disease (CGD)
- X-linked aggamaglobulinemia (XLA)
- Wiskott-Aldrich Syndrome (WAS)
- Hyper-IgM Syndrome
- DiGeorge Syndrome
- Chédiak-Higashi syndrome
- Common Variable Immune Deficiency (CVID)

OR

Diagnosis of immune regulatory disorder that is benefited by conditioning followed by transplant. This includes, but is not limited to:
- Hemophagocytic lymphohistiocytosis (HLH)
- IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked) syndrome
- Autoimmune lymphoproliferative syndrome (ALPS)
- X-linked lymphoproliferative syndrome (XLPS)

2. No more than 28 years of age.

3. Karnofsky/Lansky performance status $\geq$ 40 (Appendix C).

4. DLCO (corrected for hemoglobin) or pulmonary function tests $\geq$ 40% of expected values if performed.
   If PFTs are not performed: O_2 sats $\geq$ 88% at the time of enrollment or chest X ray that indicates no evidence of active infection or a condition that markedly alters lung function as determined by study chair and local institutional PI. In the absence of PFTs, the final eligibility determination will be the responsibility of the study chair and institutional PI.

5. LVEF $\geq$ 40% OR LVSF (left ventricular shortening fraction) $\geq$ 26%.

6. Serum creatinine $\leq$ 2 x upper limit of normal for age.

7. Liver enzymes $\leq$ 5 x upper limit of normal.

8. Negative pregnancy test within 2 weeks prior to the start of conditioning for females of childbearing potential.

9. Female and male patients (along with their female partners) must agree to use two forms of acceptable contraception, including one barrier method, during participation in the study and for 6 months following the last dose of conditioning therapy.

10. Patient must have a suitably matched donor (see section 3.4 for Donor Choice Algorithm).

11. Written informed consent and assent must be obtained. Consents must be provided by either the recipient or the legal guardian for the recipient.

12. **For patients receiving cord blood units, please note: Per FDA guidelines all patients receiving an unlicensed Umbilical Cord Blood unit must also be enrolled on the NMDP Protocol 10-CBA and follow the protocol specific reporting requirements.**

3.2 Recipient Exclusion Criteria

1. Known diagnosis of HIV I/II.

2. Pregnant or breastfeeding.

3. Uncontrolled invasive fungal or bacterial infections capable of tissue/organ seeding cultured from blood or tissue within 1 month prior to starting alemtuzumab. Infections that developed prior to this time period should have received adequate treatment per institution guidelines and should be well controlled or eradicated. Ideally, all infections will be cleared prior to initiation of alemtuzumab. **If any new or on-going infections are present at the time of enrollment and/or at the time of transplant, the study chair must be notified to discuss eligibility and infectious treatment plan prior to initiating therapy.**

4. Uncontrolled viral infections within a week prior to starting alemtuzumab. **All patients should be tested for CMV, EBV and adenovirus infections (by blood PCR) and respiratory viral infections (by NP swab) within 7 days prior to starting conditioning.**

3.3 Donor/Stem Cell Product Eligibility Criteria

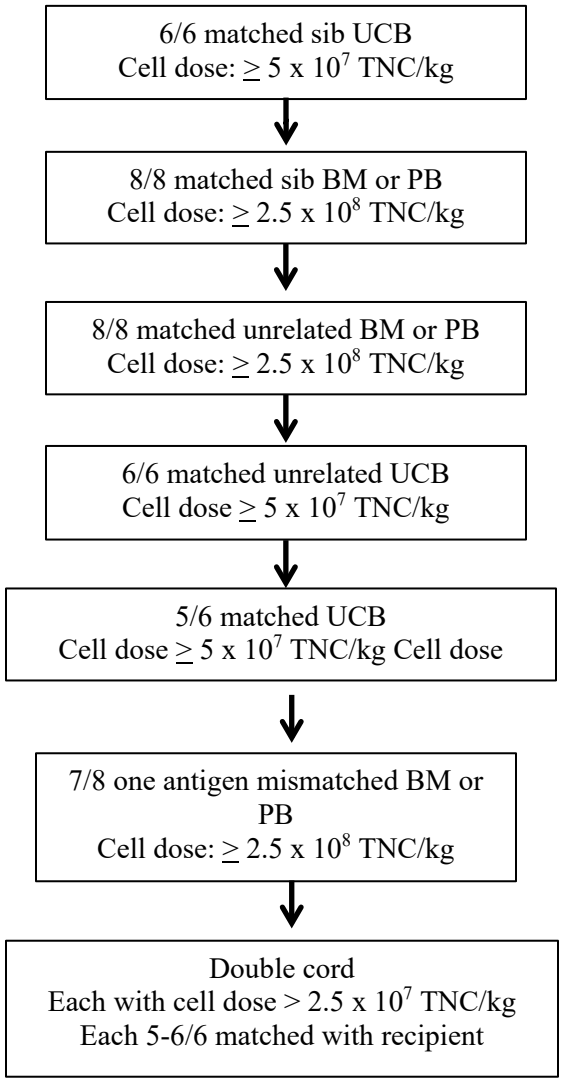
1. Donor stem cells should be HLA typed by high resolution typing if unrelated bone marrow. Low resolution typing is acceptable for family donors. Bone marrow or peripheral blood typing should include A, B, C, and DRB1 loci and should be matched at 7-8/8 loci (see algorithm - section 4.5). Cord blood should have intermediate resolution molecular typing at class I A and B loci and high resolution typing at the DRB1 locus. Cord products should be matched at 5-6/6 loci (A, B, and DRB1) with the recipient.

2. Donor must be in good health based on review of symptoms, results of physical examination, and routine testing per standards of good medical care.

3. Female donors of childbearing age must have a negative pregnancy test and must not be lactating.

4. Hematopoietic stem cell product should be targeted to meet the recommended cell doses listed below.
   If fewer cells than recommended are available, transplant may proceed as planned per the treating physician's decision. It is recommended that the treating physician contact the NMDP to inquire about obtaining additional stem cells. As it is not always possible or feasible to acquire or infuse additional cells, the procurement and decision to infuse the additional cells will be at the discretion of the treating physician and after discussion with the study chair.

3.4 Donor Choice Algorithm



4.0 REGISTRATION

Patients will be registered prior to conditioning by emailing the eligibility data form along with required supporting documentation to Thomas Pfeiffer at pthomas@wustl.edu (preferably at least 2 days prior to beginning conditioning therapy). Therapy should not be initiated without confirmation of enrollment. For questions regarding registration, please contact either Thomas Pfeiffer at pthomas@wustl.edu.

5.0 TREATMENT PLAN

The hypothesis of the current proposal is that an immunosuppressive reduced-intensity conditioning regimen will result in sustained engraftment of donor stem cells irrespective of the source. A brief duration of neutropenia is expected, and stem cell infusion will be followed by daily doses of G-CSF to recipients commencing on Day +7.

The conditioning for patients will be as follows:

- Alemtuzumab IV over 6 hours on 3 successive days. The infusions should be given on Days -14, -13, and -12 or, if not possible, on any 3 consecutive days between Day -16 and Day -11. The first dose of alemtuzumab will be 10 mg, the second dose will be 15 mg, and the third dose will be 20 mg (or, for patients $\leq$ 10 kg, dose will remain 10 mg for all 3 days). At least 6 hours and no more than 7 days prior to the first 10 mg dose of alemtuzumab, patients will receive a test dose of 3 mg IV over 2 hours. It is recommended that alemtuzumab be infused on weekdays whenever possible.
    - The Alemtuzumab may be administered subcutaneously instead of IV according to the treating physician preference, though the IV administration route is the preferred for this study.
- Fludarabine 30 mg/m^2 IV over 30 minutes on Days -8 to -4 (or, for patients $\leq$ 10 kg, dose will be 1 mg/kg)
- Thiotepa 4 mg/kg IV over 2 hours on Day -4 twice, 12 hours apart (total dose is 8 mg/kg)
- Melphalan 140 mg/m^2 IV over 30 minutes on Day -3 (or, for patients $\leq$ 10 kg, dose will be 4.7 mg/kg)

Treatment Schema for Patients > 10 Kg

Day	Treatment
$\geq$6 hours and $\leq$7 days prior to the 1st dose of Alemtuzumab	Alemtuzumab test dose 3 mg IV or SQ
-14 -13 -12 (or 3 consecutive days between Days -16 and -11)	Alemtuzumab 10 mg IV or SQ Alemtuzumab 15 mg IV or SQ Alemtuzumab 20 mg IV or SQ
-8	Fludarabine 30mg/m^2 IV
-7	Fludarabine 30mg/m^2 IV
-6	Fludarabine 30mg/m^2 IV
-5	Fludarabine 30mg/m^2 IV
-4	Fludarabine 30mg/m^2 IV Thiotepa 4 mg/kg IV Q 12 hours (total 8mg/kg)
-3	Melphalan 140 mg/m^2 IV
-2	Rest
-1	Rest
0	Stem cell infusion
+7	G-CSF 5 µg/kg/day continue until neutrophil engraftment

Treatment Schema for Patients ≤ 10 Kg

Day	Treatment
Between -23 and -15*≥6 hours and ≤7 days prior to the 1st dose of Alemtuzumab	Alemtuzumab test dose 3 mg IV or SQ
-14 to -12 (or 3 consecutive days between Days -16 and -11)	Alemtuzumab 10 mg IV or SQ each day
-8	Fludarabine 1mg/kg IV
-7	Fludarabine 1mg/kg IV
-6	Fludarabine 1mg/kg IV
-5	Fludarabine 1mg/kg IV
-4	Fludarabine 1mg/kg IV Thiotepa 4 mg/kg IV Q 12 hours (total 8mg/kg)
-3	Melphalan 4.7 mg/kg IV
-2	Rest
-1	Rest
0	Stem cell infusion
+7	G-CSF 5 µg/kg/day continue until neutrophil engraftment

Prior to beginning treatment with fludarabine, patient will undergo placement of a double lumen Hickman or similar intravenous catheter.

Collection of allogeneic bone marrow or blood stem cells will be performed either through the National Marrow Donor Program (NMDP) or by participating institution using standard collection procedures. HSCT infusion will occur at least 30 hours after the dose of melphalan. If necessary, an additional day 0 may be added to accommodate delays in stem cell product arrival or preparation.

G-CSF at 5 mcg/kg/day will be administered beginning Day +7 to facilitate engraftment. It should be administered until ANC is > 1500/dl for 3 consecutive days.

5.1 Premedication Administration

Premedication should be given 30 minutes prior to each infusion of alemtuzumab and should follow institutional standard of care. Recommended premedications include: diphenhydramine 1 mg/kg IV or PO (maximum recommended dose 50 mg), acetaminophen 10-15mg/kg PO, and hydrocortisone 2mg/kg IV (maximum recommended dose 100 mg). Patients **should** continue to receive the same medications IV or PO every 4-6 hours until at least 48 hours after the last dose of alemtuzumab. Additional medications which can be given to treat infusion-related symptoms include meperidine 1mg/kg IV q 2-4 hours PRN (rigors) and additional doses of hydrocortisone. Vital signs should be taken ever 15-30 minutes during each infusion of alemtuzumab.

If infusion reaction is noted during alemtuzumab infusions despite pre-medication, the study PI should be contacted regarding intervention and continuation (at pthomas@wustl.edu anytime or 314-454-6018 on weekdays).

5.2 GVHD Prophylaxis

GVHD prophylaxis will be methotrexate with cyclosporine or tacrolimus. Steroids will be used for unrelated donor transplants. For cord blood transplants, centers have the option of choosing an alternative GVHD prophylaxis regimen of mycophenolate mofetil and cyclosporine or tacrolimus. Patients will receive one of two regimens described in the table below. Regimen 1 is the preferred regimen for this study; however, cord blood recipients may receive Regimen 2.

GVHD Prophylaxis Regimens

Day	Regimen 1	Regimen 2
-3	Tacrolimus or cyclosporine dosed to maintain appropriate levels. Given through Day 100 then slowly tapered.	Tacrolimus or cyclosporine dosed to maintain appropriate levels. Given through Day 100 then slowly tapered. Mycophenolate mofetil (MMF) 1 gram IV q 8 hours for children > 50 kg or 15 mg/kg IV q 8 hours for children < 50 kg MMF is given through Day +45 or 7 days after engraftment, whichever is later.
0	Stem cell infusion	Stem cell infusion
+1	Methotrexate 7.5 mg/m^2 IV	
+3	Methotrexate 7.5 mg/m^2 IV	
+6	Methotrexate 7.5 mg/m^2 IV	
+7	**For unrelated donors only:** Methylprednisolone 1.0 mg/kg/day IV in divided doses twice daily OR Prednisone 1.2 mg/kg/day PO in divided doses twice daily Continued through Day +28	

5.2.1 GVHD Prophylaxis Regimen 1

This is the preferred regimen for all recipients, and is the only one permitted for bone marrow or peripheral blood recipients.

Patients will receive tacrolimus or cyclosporine followed by methotrexate. Tacrolimus administration will commence on Day -3, and doses will be adjusted to maintain a level of 8-15 ng/mL. Tacrolimus can be administered by intermittent infusion over 6 hours twice daily or by continuous infusion to maintain therapeutic levels per institutional guidelines. Patient can be changed to oral tacrolimus when tolerated or per institutional guidelines.

Alternatively, cyclosporine may be administered beginning on Day -3 and doses will be adjusted to maintain a level of 250-500 ng/mL. Cyclosporine can be administered by continuous or intermittent infusion per institutional guidelines. Patient can be changed to oral cyclosporine when tolerated or per institutional guidelines.

Patients will be continued on tacrolimus or cyclosporine through Day 100. After that time, tacrolimus or cyclosporine should be slowly weaned as tolerated per institutional guidelines (recommended tapering of approximately 5-10% per week).

Methotrexate will be given at a dosage of 7.5 mg/m^2 IV on Day 1, 3 and 6. There are no dose adjustments of methotrexate for patients under 10 kg. The Day 1 dose of methotrexate should not be administered until 24 hours following completion of the marrow infusion. Leucovorin rescue may be used per institutional practice guidelines.

Methotrexate dose will be adjusted as follows:

	MTX dose		
	Full	**50%**	**Hold dose**
Direct bilirubin	< 2.0 mg/dL	2.0-4.0 mg/dL	> 4.0 mg/dL
AST or ALT	< 399 U/L	400-600 U/L	> 600 U/L
Serum creatinine	-	-	> 2.5 mg/dL

Finally, methylprednisolone/prednisone will be administered to patients who received stem cells from unrelated donors only. Starting on Day 7, methylprednisolone 1.0 mg/kg/day IV in two divided doses daily or prednisone 1.2 mg/kg/day PO in two divided doses daily will be administered and continued until Day 28. In the absence of GVHD, prednisone will be tapered per institutional guidelines (recommended taper of 20% per week).

5.2.2 GVHD Prophylaxis Regimen 2

This regimen may be used instead of regimen 1 for cord blood recipients only.

Tacrolimus or cyclosporine will be administered as described in Section 5.2.1.

Mycophenolate mofetil (MMF) will be given at a dose of 1 gram IV q 8 hours if patient weighs ≥ 50 kg or 15 mg/kg IV q 8 hours if patient weighs < 50 kg beginning the morning of Day -3. (If renal failure occurs, or GFR is < 25 mL/min, do not exceed dose of 1 gram q 8 hours.) No dose adjustment is necessary for liver disease. MMF should be given IV until patient can tolerate oral medications and has a normal gastro-intestinal transit time. MMF will be given orally at a dose of 1 gram PO q 8 hours if patient weighs > 50 kg or 15 mg/kg PO q 8 hours if patient weighs < 50 kg. Tablets or suspension may be used to achieve calculated doses.

Stop MMF at Day + 45 *or* 7 days after engraftment, whichever day is later, if no acute GVHD. If no donor cell engraftment, MMF continues per institutional standards.

If the patient has active acute GVHD requiring systemic therapy, MMF may be stopped 7 days after control of GVHD (e.g., resolution of skin rash, vomiting, and diarrhea) according to institutional guidelines.

5.3 Supportive Care Guidelines

5.3.1 Thiotepa Skin Toxicity

Frequent baths (every 6 hours during the day) is recommended during and for 24 hours after thiotepa, or per institutional standard practices, to minimize potential for skin toxicity.

5.3.2 Fever with Presumed or Confirmed infection

Due to infection risks with immune suppression, recipients will be managed in the inpatient setting until WBC engraftment. Patients with fever (temperature of 38.5° C or above) with ANC < 500/dl should receive IV antibiotics per treating institution preference. For fever not responding in 3-5 days to antibacterial agents, or for evidence of fungal infection, voriconazole is recommended at full therapeutic doses or as per institutional guidelines.

5.3.3 Engraftment Syndrome

Engraftment syndrome is a clinical diagnosis. The most frequently reported manifestations are transient fever, rash, and respiratory symptoms not attributable to infection or GVHD. The pathophysiology is multifactorial mediated by cellular, complement, and cytokine components. Diagnostic criteria include fever (temperature >38.5° C) without an identifiable infectious cause within 4 days of the start of neutrophil recovery and an erythematous rash not attributable to GVHD or medications or capillary leak (weight gain, edema, ascites, effusions) or respiratory symptoms not attributable to IPS. Mild symptoms may not require therapy due to the self-limiting nature of this syndrome. For progressive symptoms, methylprednisolone at 2 mg/kg/day is recommended for 5 days or as per institutional guidelines. If recurrent or prolonged, investigation for GVHD is recommended.

5.3.4 Hypertension

Nifedipine administration is recommended for control of hypertension. Hypertension should be strictly controlled to prevent CNS toxicity. Blood pressure should be monitored closely and both systolic and diastolic hypertension should be treated promptly to maintain blood pressure at the patient's pre-transplant baseline ± 20% starting from the time of beginning conditioning with alemtuzumab. Dosing in children is 1 mg/kg/day in three divided doses (10 mg maximum per dose) for the prevention or treatment of hypertension with calcineurin inhibitors or steroids. Nifedipine may be substituted with amlodipine for long-term control of hypertension at a dose of 0.1 mg/kg/day once or twice daily as needed (maximum 5 mg/dose). Monitor for side effects of the drug such a peripheral edema.

5.3.5 Transfusion Guidelines

Platelets are required to be maintained > 30,000/dl after stem cell infusion until platelet engraftment.

All patients should receive irradiated, leukoreduced blood products.

5.3.6 HSV

Prophylactic acyclovir is recommended for all patients, particularly those who are seropositive for HSV, starting 24 hours after last dose of alemtuzumab and continuing until 6 months, or until immunosuppression is discontinued, whichever is later. The recommended dose is 750 mg/m^2 orally or IV divided three times daily.

5.3.7 CMV - Surveillance Cultures

Starting after alemtuzumab infusion, recipients should be tested weekly for CMV reactivation until Day +100. Subsequently, all patients should be tested at least twice monthly until day 180 and then every 2 weeks if 1) previously abnormal and not resolved or 2) as indicated while on immune suppression. Treatment should be commenced pre-emptively if the CMV test is positive, with continued monitoring for response. If CMV reactivation occurs prior to engraftment, consider therapy with foscarnet instead of ganciclovir to prevent marrow suppression. **This treatment guideline should be rigidly enforced due to the risk of CMV disease following the highly immunosuppressive conditioning.**

5.3.8 Pneumocystis Infection Prophylaxis

Prophylaxis for pneumocystis infection is **required** after hematologic recovery and will be continued until immunosuppression is discontinued or for a minimum period of 1 year post-transplant. Suggested prophylaxis includes trimethorpim/sulfamethoxazole, pentamidine, dapsone, atavaquone, or other agent per institutional standards.

5.3.9 Fungal Prophylaxis

Prophylaxis against invasive fungi such as aspergillus is **required.** We recommend using itraconazole and following levels for appropriate dosing. However, other agents can be substituted based on institution preference. Itraconazole prophylaxis 5 mg/kg/day is recommended and should be started 24 hours after last dose of alemtuzumab and continued until Day 180 or when definitive therapy is commenced with an antifungal agent of choice for a suspected or proven fungal infection. Following completion of antifungal therapy, fungal prophylaxis will be commenced again until Day 180 or when corticosteroids are discontinued whichever is later. It is recommended (not required) that itraconazole levels be monitored and the dose be adjusted to achieve therapeutic target of > 1 mcg/ml for the sum of itraconazole and hydroxyitraconazole levels. **The prophylaxis approach is mandated due to the very immunosuppressive nature of the conditioning regimen, and the high risk of invasive fungal infections, such as the *Aspergillus* species.**

5.3.10 EBV

Starting after alemtuzumab infusion, recipients should have EBV PCR tested weekly until Day +100. Subsequently, all patients should be tested at least twice monthly until day 180 and then every 2 weeks if 1) previously abnormal and not resolved or 2) as indicated while on immune suppression. In the event of persistent EBV viremia or signs/symptoms consistent with EBV-related PTLD (adenopathy, fever, etc.), therapy with rituximab (dosing per institutional guidelines) is recommended.

5.3.11 Adenovirus

Starting after alemtuzumab infusion, recipients should have adenovirus PCR tested weekly until Day +100. Subsequently, all patients should be tested at least twice monthly until day 180 and then every 2 weeks if 1) previously abnormal and not resolved or 2) as indicated while on immune suppression. Additionally, testing for adenovirus infection by PCR is recommended in the event of symptoms suspicious for infection such as diarrhea, hepatic dysfunction, or respiratory symptoms. If an active systemic infection is diagnosed, therapy should be instituted with cidofovir or other active agents per institution guidelines.

5.3.12 Other Infectious Prophylaxis

All patients will be at risk for bacterial infections secondary to immunodeficiency or chronic neutropenia. Prophylaxis with ciprofloxacin (10 mg/kg/dose given twice daily) should be started 24 hours after last dose of alemtuzumab and continued until Day +180. This can be substituted with other antibiotics as necessary during the transplant period.

Intravenous gammaglobulin (IVIG) infusions are strongly recommended due to underlying immune deficiencies of these patients and the highly immunosuppressive nature of the conditioning regimen. IVIG should be given at 400 mg/kg monthly beginning after start of conditioning and continued until Day 100 or more frequently as needed during acute infections.

5.4 Women of Childbearing Potential

Women of childbearing potential (defined as women with regular menses, women with amenorrhea, women with irregular cycles, women using a contraceptive method that precludes withdrawal bleeding, and women who have had a tubal ligation) are required to have a negative serum pregnancy test within 14 days prior to the first dose of alemtuzumab.

Female and male patients (along with their female partners) are required to use two forms of acceptable contraception, including one barrier method, during participation in the study and for 6 months following the last dose of conditioning therapy.

If a patient is suspected to be pregnant, study therapy should be immediately discontinued. In addition a positive urine test must be confirmed by a serum pregnancy test. If it is confirmed that the patient is not pregnant, the patient may resume dosing.

If a female patient or female partner of a male patient becomes pregnant during therapy or within 6 months after the last dose of conditioning therapy, the investigator must be notified in order to facilitate outcome follow-up.

5.5 Duration of Therapy

If at any time the constraints of this protocol are considered to be detrimental to the patient's health and/or the patient no longer wishes to continue protocol therapy, the protocol therapy should be discontinued and the reason(s) for discontinuation documented in the case report forms.

In the absence of treatment delays due to adverse events, treatment may continue as described above or until one of the following criteria applies:

- Documented and confirmed disease progression
- Death
- Adverse event(s) that, in the judgment of the investigator, may cause severe or permanent harm or which rule out continuation of study therapy
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator
- Suspected pregnancy
- Serious noncompliance with the study protocol
- Lost to follow-up
- Patient withdraws consent
- Investigator removes the patient from study
- Investigator decides to close the study

Patients who prematurely discontinue treatment for any reason will be followed as indicated in the study calendar.

5.6 Duration of Follow-up

Patients will be followed every 3 months for 2 years or until death, whichever occurs first. Patients removed from study for unacceptable adverse events will be followed until resolution or stabilization of the adverse event.

6.0 REGULATORY AND REPORTING REQUIREMENTS

6.1 Adverse Events (AEs)

Definition: any unfavorable medical occurrence in a human subject including any abnormal sign, symptom, or disease.

Grading: the descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for all toxicity reporting. A copy of the CTCAE version 4.0 can be downloaded from the CTEP website.

Attribution (relatedness), Expectedness, and Seriousness: the definitions for the terms listed that should be used are those provided by the Department of Health and Human Services' Office

for Human Research Protections (OHRP). A copy of this guidance can be found on OHRP's website: http://www.hhs.gov/ohrp/policy/advevntguid.html

6.2 Unanticipated Problems

Definition:

- unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;
- related or possibly related to participation in the research (in this guidance document, possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

6.3 Noncompliance

Definition: failure to follow any applicable regulation or institutional policies that govern human subjects research or failure to follow the determinations of the IRB. Noncompliance may occur due to lack of knowledge or due to deliberate choice to ignore regulations, institutional policies, or determinations of the IRB.

6.4 Serious Noncompliance

Definition: noncompliance that materially increases risks, that results in substantial harm to subjects or others, or that materially compromises the rights or welfare of participants.

6.5 Protocol Exceptions

Definition: A planned deviation from the approved protocol that are under the research team's control. Exceptions apply only to a single participant or a singular situation.

Pre-approval of all protocol exceptions must be obtained prior to the event.

6.6 Reporting to the Human Research Protection Office (HRPO) and the Quality Assurance and Safety Monitoring Committee (QASMC) at Washington University

The study PI is required to promptly notify the Washington University IRB of the following events:

- Any unanticipated problems involving risks to participants or others which occur at WU, any BJH or SLCH institution, or that impacts participants or the conduct of the study.
- Noncompliance with federal regulations or the requirements or determinations of the IRB.
- Receipt of new information that may impact the willingness of participants to participate or continue participation in the research study.

These events must be reported to the Washington University IRB within **10 working days** of the occurrence of the event or notification to the PI of the event. The death of a research participant that qualifies as a reportable event should be reported within **1 working day** of the occurrence of the event or notification to the PI of the event.
These events will be reported to the Washington University IRB by the coordinating center.

6.7 Reportable Adverse Events

6.7.1 Expedited Reporting of Serious Adverse Events and Other Events to the Coordinating Center

- Patient death (regardless of cause) must be reported to the coordinating center within 24 hours of knowledge of the event.
- Adverse events that are both unexpected and reasonably related must be reported to the coordinating center within 24 hours of knowledge of the event.
- Noncompliance and serious noncompliance as described in Sections 6.3 and 6.4 must be reported to the coordinating center within 24 hours of knowledge of the event.
- Graft failure (as defined in Section 7.0) must be reported to the coordinating center within 15 days of occurrence.

6.7.2 Routine Adverse Event Reporting

The following adverse events are to be reported on the case report forms during the reporting period in which they occurred at the timeframes specified in Section 11.0.

- All grade 4 or 5 toxicities (excluding hematologic toxicities)
- All microbiologically documented infections
- All symptoms of GVHD with $\geq$ grade 3 toxicity

7.0 ENGRAFTMENT AND GRAFT FAILURE

Neutrophil engraftment will be defined as the first of 3 days on which the absolute neutrophil count (ANC) rises to $\geq$ 500 cells/dl occurring after the post-preparative regimen neutrophil nadir.

Platelet engraftment will be defined as the **first** day on which the platelet count rises to $\geq$ 50,000/dl over a 7 day interval without transfusion support, and occurring after the post-preparative regimen platelet count nadir.

Two forms of graft failure will be recognized, early and late.

Early graft failure will be defined as the absence of donor engraftment (<10% donor chimerism either in all lineages tested or in the lineage of interest based on the disorder) and will be determined by molecular assessment (STR or VNTR analysis) and/or cytogenetic studies on peripheral blood or bone marrow on approximately Day +30 (BM or PB graft) or approximately Day +42 (UCB graft).

Late graft failure will be defined as the loss of donor chimerism (<10% donor) either in all lineages tested or in the lineage of interest based on the disorder after initial engraftment detected by molecular or cytogenetic analysis of peripheral blood or bone marrow within 2 years post-transplant.

8.0 PHARMACEUTICAL INFORMATION

8.1 Alemtuzumab

8.1.1 Alemtuzumab Description

Alemtuzumab (Campath) is a recombinant DNA-derived humanized monoclonal antibody directed against the 21-28 kD cell surface glycoprotein, CD52.

8.1.2 Clinical Pharmacology

Alemtuzumab binds to CD52, an antigen present on the surface of B and T lymphocytes, a majority of monocytes, macrophages, NK cells, and a subpopulation of granulocytes. A proportion of bone marrow cells, including some CD34+ cells, express variable levels of CD52. The proposed mechanism of action is antibody-dependent cellular-mediated lysis following cell surface binding of alemtuzumab to the leukemic cells.

8.1.3 Pharmacokinetics and Drug Metabolism

Alemtuzumab pharmacokinetics were characterized in a study of 30 previously treated B-CLL patients in whom alemtuzumab was administered at the recommended dose and schedule. Alemtuzumab pharmacokinetics displayed nonlinear elimination kinetics. After the last dose, the mean volume of distribution at steady-state was 0.18 L/kg. Systemic clearance decreased with repeated administration due to decreased receptor-mediated clearance (i.e., loss of CD52 receptors in the periphery). Mean half-life was 11 hours after the first dose. The pharmacokinetics of alemtuzumab in pediatric patients have not been studied.

8.1.4 Supplier

Alemtuzumab is commercially available. The drug can be obtained from Sanofi Pharmaceuticals – see supplemental forms.

8.1.5 Dosage Form and Preparation

Alemtuzumab (Campath-1H; Sanofi Pharmaceuticals) is available by request as a purified preparation diluted in phosphate buffered saline (PBS) with 0.05 mm EDTA. Tween 80 is added to a concentration of 0.01%. The final product is a clear, colorless isotonic solution free from visible particulate matter. The ampoules contain 30 mg of antibody in 3 mL of sterile PBS at a concentration of 10 mg/mL. Infusion of alemtuzumab will be diluted in 100cc of 0.9% NS and administered over approximately 6 hours each day for 3 consecutive days (total=3 doses). The test dose of alemtuzumab should be diluted in at least 30 cc of 0.9% NS and administered over approximately 2 hours.

8.1.6 Storage and Stability

Alemtuzumab should be stored protected from light, in a refrigerator at between 2 and 8ºC (36 to 46ºF).

8.1.7 Administration

Alemtuzumab will be infused intravenously as follows:

- Test dose is 3 mg over 2 hours
- First dose is 10 mg over 6 hours
- Second dose is 15 mg over 6 hours (if patient is ≤ 10 kg, dose remains 10 mg)
- Third dose is 20 mg over 6 hours (if patients is ≤ 10 kg, dose remains 10 mg)
- If the patient has significant reaction to the infusion (i.e., hypotension or dyspnea that requires intervention), then the infusion time can be lengthened to 8 hours. Infusion time can be extended up to a maximum of 24 hours. Once reconstituted the alemtuzumab is only stable for 8 hours. If infusion needs to be lengthened beyond 8 hours, then the dose will need to be split and reconstituted accordingly.

8.1.8 Special Handling Instructions

Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration. If particulate matter is present or the solution is discolored, the vial should not be used. DO NOT SHAKE VIAL.

8.1.9 Expected Adverse Events

Administration can cause fevers, rigors, nausea, vomiting, hypotension, fatigue, rash, urticaria, dyspnea, headache, cough, pruritus, diarrhea, pain, anorexia, increased sweating, sepsis, myalgia, asthenia, herpes simplex, hypertension, pharyngitis, abdominal pain, back pain, dizziness, anemia, infections, neutropenia and thrombocytopenia.

8.2 Fludarabine

8.2.1 Fludarabine Description

Chemical name: 9H-Purin-6-amine, 2-fluoro-9-(5-0-phosphono-β-D-arabino-furanosyl) (2-fluoro-ara-AMP).

Molecular formula: $C_{10}H_{13}FN_5O_7P$.

Molecular weight: 365.2.

8.2.2 Clinical Pharmacology

Fludarabine phosphate is rapidly dephosphorylated to 2-fluoro-ara-A and then phosphorylated intracellularly by deoxycitidine kinase to the active triphosphate, 2-fluoro-ara-ATP. This metabolite appears to act by inhibiting DNA polymerase alpha, ribonucleotide reductase and DNA primase, thus inhibiting DNA synthesis. The mechanism of action of this antimetabolite is not completely characterized and may be multifaceted.

8.2.3 Pharmacokinetics and Drug Metabolism

Limited pharmacokinetic data for fludarabine in a pediatric population are available from a published study of children with refractory acute leukemias or solid tumors. When

fludarabine was administered as a loading dose over 10 minutes immediately followed by a 5-day continuous infusion, steady-state conditions were reached early.

8.2.4 Supplier(s)

Fludarabine is commercially available.

8.2.5 Dosage Form and Preparation

Fludarabine is available in 50 mg vials and should be reconstituted by adding 2 mL of sterile water for injection, USP. The resulting solution will contain 25 mg/mL of fludarabine phosphate. This will be further diluted in 0.9% sodium chloride to a concentration not exceeding 10 mg/mL and infused intravenously over approximately 30 minutes.

8.2.6 Storage and Stability

Store under refrigeration between 2-8ºC.

8.2.7 Administration

Fludarabine will be infused intravenously over 30 minutes at a dose of 30 mg/m2 on Days -8 to -4 (or at 1 mg/kg for patients $\leq$ 10 kg).

8.2.8 Special Handling Instructions

The use of latex gloves and safety glasses is recommended to avoid exposure in case of breakage of the vial or other accidental spillage.

8.2.9 Expected Adverse Events

The administration of fludarabine, thiotepa, and melphalan is expected to produce a period of pancytopenia with ANC < 500/dl, hemoglobin < 7-8 gms/dl and platelet < 20,000/dl. Transfusion support is anticipated during this period. In addition, nearly all of those treated will develop fever and approximately 30% will develop infection during the period of neutropenia. Pancytopenic complications may be life-threatening or fatal. Additionally, administration of fludarabine can cause hemolytic anemia, neutropenia or thrombocytopenia, low blood counts secondary to bone marrow suppression, nausea, vomiting, diarrhea, stomatitis, skin rash, pneumonitis, fatigue, blurred vision, decreased immunity and rarely encephalopathy (in very high doses).

8.3 Thiotepa

8.3.1 Thiotepa Description

Chemical name: Tris(1-azidinyl)phosphine sulfide.

Molecular formula: $C_6H_{12}N_3PS$

Molecular weight: 189.22.

8.3.2 Clinical Pharmacology

Thiotepa is a cytotoxic agent of the polyfunctional type, related chemically and pharmacologically to nitrogen mustard. The radiomimetic action of thiotepa is believed to occur through the release of ethylenimine radicals which, like irradiation, disrupt the bonds of DNA. One of the principal bond disruptions is initiated by the alkylation of guanine at the N-7 position, which severs the linkage between the purine base and the sugar and liberates alkylated guanines.

8.3.3 Supplier(s)

Thiotepa is commercially available.

8.3.4 Dosage Form and Preparation

Thiotepa is available as a powder and is reconstituted as a 15 mg solution for injection which is further diluted for infusion over 2-3 hours. Reconstitute Thiotepa with 1.5 mL sterile water for injection to a concentration of 10 mg/mL. Solution is hypotonic and must be diluted further with normal saline prior to use. A solution of 1-5 mg/m is stable at room temperature for up to 24 hrs. Stability decreases significantly at concentrations of less than 0.5 mg/mL should be used immediately.

8.3.5 Storage and Stability

Store in refrigerator (2-8ºC). Protect from light at all times.

8.3.6 Administration

Intravenous injection of 4 mg/kg over 2 hours twice on Day -4 (12 hours apart). Thiotepa can cause skin toxicity (contact dermatitis) and discoloration. Patient must be bathed approximately every 6 hours per day during and for 24 hours after drug is administered, and bed linens changed frequently.

8.3.7 Expected Adverse Events

The administration of fludarabine, thiotepa, and melphalan is expected to produce a period of pancytopenia with ANC < 500/dl, hemoglobin < 7-8 g/dl and platelet < 20,000/dl. Transfusion support is anticipated during this period. In addition, nearly all of those treated will develop fever and approximately 30% will develop infection during the period of neutropenia. Pancytopenic complications may be life-threatening or fatal. Administration of thiotepa can cause nausea, vomiting, anorexia, anemia, neutropenia, thrombocytopenia, mouth sores, inflammation of the passage between the throat and stomach, absence of sperm or stopped monthly periods and infertility (with high doses), pain at the injection site, dizziness, headaches, confusion, drowsiness, increased liver enzymes, increased bilirubin, darkening of the skin, hives, rash, and sudden high fever.

8.4 Melphalan

8.4.1 Melphalan Description

Melphalan is a phenylalanine derivative of nitrogen mustard. Melphalan is a bifunctional

alkylating agent that is active against selected human neoplastic diseases.

Chemical name: 4-[bis(2-chloroethyl)amino]-*L*-phenylalanine

Molecular formula: $C_{13}H_{18}Cl_2N_2O_2$

Molecular weight: 305.20

8.4.2 Clinical Pharmacology

Melaphalan is an alkylating agent of the bischloroethylamine type. As a result, its cytotoxicity appears to be related to the extent of its interstrand cross-linking with DNA, probably by binding at the N^7 position of guanine. Like other bifunctional alkylating agents, it is active against both resting and rapidly dividing tumor cells.

8.4.3 Pharmacokinetics and Drug Metabolism

Following injection, drug plasma concentrations declined rapidly in a biexponential manner with distribution phase and terminal elimination phase half-lives of approximately 10 and 75 minutes, respectively.

Melphalan is eliminated from plasma primarily by chemical hydrolysis to monohydroxymelphalan and dihydroxymelphalan. Aside from this hydrolysis products, no other melphalan metabolites have been observed in humans.

8.4.4 Supplier(s)

Melphalan is commercially available.

8.4.5 Dosage Form and Preparation

Melphalan (Alkeran) is available as dry powder and will be reconstituted to 5 mg/mL with the diluent supplied by the manufacturer. This reconstituted solution will be immediately diluted in 0.9% sodium chloride to a concentration of 0.45 mg/mL. Freshly reconstituted and diluted drug will be infused over approximately 30 minutes. **The time between reconstitution and administration of melphalan should be kept to a minimum because reconstituted and diluted solutions of melphalan are unstable.** Administration should be completed within approximately 60 minutes of reconstitution.

8.4.6 Storage and Stability

Store in a controlled room temperature 15-30ºC and protect from light. Do NOT refrigerate the reconstituted product.

8.4.7 Administration

Melphalan will be administered at a dose of 140 mg/m^2 IV over 30 minutes on Day -3. For patients $\leq$ 10 kg, dose will be 4.7 mg/kg.

8.4.8 Expected Adverse Events

The administration of fludarabine, thiotepa, and melphalan is expected to produce a period of pancytopenia with ANC < 500/dl, hemoglobin < 7-8 gms/dl and platelet < 20,000/dl. Transfusion support is anticipated during this period. In addition, nearly all of those treated will develop fever and approximately 30% will develop infection during the period of neutropenia. Pancytopenic complications may be life-threatening or fatal. Administration of melphalan can cause decreased blood counts, seizures, nausea, vomiting, diarrhea, oral ulcers, decreased immunity, sterility, interstitial pneumonitis, lung fibrosis, allergic reactions and rarely seizures (with very high doses).

9.0 IMMUNOLOGIC STUDIES

On Day +30, Day +100, Day +180, 1 year, and 2 years post-transplant, record the following immune reconstitution results:

1. Lymphocyte subpopulations including absolute lymphocyte count, CD3, CD4, CD8, CD19, and CD16+56
2. T cell receptor excision circles (TREC) and recent thymic emigrants (CD45RA and CD45RO testing on CD4 cells)
3. Lymphocyte proliferation (stimulation) to PHA (phytohemagglutinin). If available, proliferation to PWM (pokeweed mitogen), candida and tetanus should also be tested.
4. Quantitative immunoglobulins G, A, and M
5. Tetanus toxoid antibody titer

These studies are collected on peripheral blood and can be assayed at the enrolling institution or appropriate referral lab. Quantity of blood needed and processing should be performed according to the assessing laboratory's guidelines.

10.0 STUDY PARAMETERS[1]

	Baseline	**Btwn Days -23 and -14**	**Days -14 thru -12**	**Days -8 thru -5**	**Day -4**	**Day -3**	**Day 0**	**Day +1**	**Day +3**	**Day +6**	**Day +7**	**Day +30**	**Day +100**	**Day +180**	**1 Yr**	**18 Mo**	**2 Yr**
Physical exam, incl. ht, wt, and PS	X											X	X	X	X	X	X
CBC with diff	X							X[10]				X[10]	X[10]	X[10]	X[10]	X[10]	X[10]
CMP[2]	X							X[10]				X[10]	X[10]	X[10]	X[10]	X[10]	X[10]
Echocardiogram	X																
PFTs[3]	X																
HIV test	X																
Infectious disease eval. [4]	X[16]	Please see section 5.0 for CMV, EBV, and adenovirus monitoring post transplant. CMV, EBV, adenovirus PCR should be done weekly until Day 100 then twice monthly until day 180[4]															
Pregnancy test[5]	X																
Alemtuzumab		X[11]	X														
Fludarabine				X	X												
Thiotepa					X												
Melphalan						X											
SCT							X										
G-CSF											X[12]						
Tacrolimus or cyclosporine						X ---------- X[13]											
Methotrexate								X	X	X							
Prednisone[14]											X ---- X						
MMF[15]						X ---------- X[15]											
Chimerism (Engraftment)[6]												X[9]	X	X	X	X	X

Immune reconstitution[7]													X	X	X		X
TREC and thymic emigrants[17]													X	X	X		X
Lymphocyte stimulation[8]													X	X	X		X
Tetanus antibody titer													X	X	X		X
Adverse events	X ---------- X																

[1] Baseline evaluations to be obtained within 60 days prior to starting protocol therapy unless otherwise noted. Subsequent evaluations should be performed within 2 weeks (pre or post) of the target date. 1 year, 18 month, and 2 year evaluations should be performed within 30 days (pre or post) of the target date

[2] Blood chemistries must include serum creatinine, AST, and ALT.

[3] If PFTs are not performed: O_2 sats > 88% at the time of enrollment or chest X ray that indicates no evidence of active infection or a condition that markedly alters lung function as determined by the study chair and local institutional PI.

[4] CMV, EBV, Adenovirus PCR. To be obtained weekly beginning after completion of alemtuzumab and continued until Day +100, and then should be obtained twice monthly until day 180 post-transplant and then every 2 weeks if 1) previously abnormal and not resolved or 2) as indicated while on immune suppression. NP swab to be obtained prior to starting campath and then as clinically indicated.

[5] For females of childbearing potential, must be performed within 2 weeks prior to beginning conditioning.

[6] Chimerism (engraftment) will be measured by molecular methods (STR or cytogenetics) on bone marrow or peripheral blood. Fractionated chimerism for the lymphoid and myeloid fractions is preferred but total chimerism is acceptable.

[7] Immune reconstitution (absolute lymphocyte numbers) by flow cytometry for lymphocyte subpopulations (CD3, CD4, CD8, CD19, & CD16+56 cell subsets) and immunoglobulin levels (G, A, M).

[8] Lymphocyte proliferation (stimulation) to PHA (phytohemagglutinin). If available, proliferation to PWM (pokeweed mitogen), candida and tetanus should also be tested.

[9] May be performed between Days 28 and 42.

[10] At each clinic visit: Weekly through Day +100, then monthly until Day +180, then every 2 months until 1 year.

[11] Test dose- must be given at least 6 hours and no more than 7 days prior to the first 10 mg dose of Alemtuzumab

[12] Continues until neutrophil engraftment

[13] Through Day 100 and then slowly tapered

[14] Unrelated donors only, through Day 28

[15] This option is for cord blood recipients only, and is in lieu of methotrexate. Should be given through Day 45 or 7 days after engraftment, whichever is later.

[16] Within 7 days prior to starting conditioning.

[17] T cell receptor excision circles (TREC) and recent thymic emigrants (CD45RA and CD45RO testing on CD4 cells)

11.0 DATA SUBMISSION SCHEDULE

Case Report Form	Time Point
Signed informed consent form Eligibility Data Form	Prior to enrollment
Infection Data Form	At least 48 hours prior to anticipate start of alemtuzumab test dose
Transplant Data Form	Within 14 days of transplant
Day 100 Data Form	Within 14 days of Day 100 visit
Day 180 Data Form	Within 30 days of Day 180 visit
Day 365 Data Form	Within 30 days of Day 365 visit
1.5 Year Data Form	Within 30 days of 1.5 Year visit
2 Year Data Form	Within 30 days of 2 Year visit

12.0 STATISTICAL CONSIDERATIONS

12.1 Overview

This is a phase II study that assesses the frequency of engraftment (for 1 year) and toxicities and treatment related morbidity and mortality (TRM) (Grade IV and V) (for 1 year) as primary endpoints after matched and mismatched related and unrelated donor stem cell transplantation following a reduced intensity conditioning regimen. Overall survival, disease-free survival, acute and chronic GVHD, Grade IV non-hematologic toxicities, $\geq$ Grade III infectious complications, time to neutrophil and platelet engraftment, 2-year engraftment rate, infectious complications and immune reconstitution will be described as secondary endpoints. Estimates of toxicity, incidence of acute GVHD, TRM, and engraftment from all stem cell source cohorts will be described in aggregate.

12.2 Accrual and Monitoring

The study population will consist of 40 recipients. It is estimated that three years of accrual will be necessary to enroll the targeted sample size. Accrual will be reported by gender and age.

12.3 Sample Size Justification

This trial will be considered successful if the 1-year event-free survival (EFS) after hematopoietic cell transplantation is ≥75%. If the true EFS is 75%, a sample size of 20 patients will provide 90% probability of observing ≥65% (=13/20) EFS, 79% probability of observing ≥70% (=14/20) EFS, and 62% probability of observing ≥75% (=15/20) EFS. Phrased another way, this means that if the true percent of EFS is 75%, then there is only a 10% chance of observing <65% EFS, another 28% chance of observing between 65-75% EFS, and a 62% chance of observing ≥75% EFS. On the other hand, if the true EFS is really 80%, then there is an 80% chance of observing ≥75% EFS, and a 91% chance of observing ≥70% EFS. . Additionally, if the observed proportion of EFS is 0.75, the standard error for the estimate of EFS will be 0.097, yielding a 95% confidence interval of width 0.38. Although this interval is wide, it will allow the study to assess the safety of this transplant regimen in a small population of children.

12.4 Study Duration

Patients will be evaluated for two years post-transplant. Follow-up data will be requested for a total of 5 years post-transplant, but the investigator or participant may opt not to participate in this long-term follow-up.

12.5 Primary Endpoint

The primary objective is to assess engraftment and therapy-associated toxicities for 1 year post-transplant. Rates of engraftment will be reported as percentage of patients with donor engraftment > 20% at 1 year post-transplant.

12.6 Analysis of Primary Endpoints

As this is a single arm study, no formal statistical inference will be made. One- and 2-year engraftment rates, total grade IV-V toxicity rates, rates of acute and chronic GVHD, 3-year infectious complications rate, 2-year immune reconstitution rate, and grade 4 non-hematologic toxicity rate will be summarized as proportions with 95% confidence intervals. Transplant-related mortality will be summarized in the same fashion separately from total grade IV-V toxicity rate. Days to neutrophil engraftment and days to platelet engraftment, overall survival and disease free survival will be summarized with means and interquartile ranges calculated by Kaplan-Meier models. Overall survival is defined as time from transplant to death or last follow-up. Time to neutrophil or platelet engraftment is defined as time from transplant to one of the engraftment criteria described above in section 7.0, death or last follow-up. Time to acute GVHD (Appendix A) is defined as time from transplant to first onset of GVHD at any site, death or last follow-up. Time to chronic GVHD is defined as time from transplant to first onset of chronic GVHD (Appendix B) at any site, death or last follow-up.

12.7 Analysis of Secondary Endpoints

Median days to neutrophil and platelet engraftment as well as overall survival and disease-free survival will be reported using time to event models. Incidence of acute GVHD, chronic GVHD and infectious complications will be reported as percentages. Immune reconstitution will be reported as median values with standard error at individual time points and will be assessed in a time to event model to evaluate return to normal immune function.

12.8 Stopping Guidelines

The possibility of stopping the trial or amending the study will be considered for 100 day treatment-related mortality ≥ 20%, graft rejection and/or disease recurrence ≥ 20%, or grade IV acute GVHD ≥ 20%. For each of these three events, the study will be suspended for review if the number of cases reaches 4 in the first 4 patients, or 5 in the first 7 patients, or 6 in the first 10 patients, or 7 in the first 12 patients, or 8 in the first 15 patients, or 9 in the first 19 patients. The probability of early stopping without cause for each event taken separately is 0.0167. The probability of early stopping is 0.12 if the true rate is 0.3 and 0.38 if the true rate is 0.40. The maximum number of occurrences of any one of these events is 9.

12.9 Safety Monitoring

The study chair will be notified immediately of any deaths occurring among patients on the study and the relation of the death to study treatment evaluated. Additional reviews will be requested whenever necessary. In the event of an unexpected grade 4 SAE during the first 100 days post-transplantation attributed to conditioning regimen or death deemed related to the transplant process within one year of transplantation, the Principal Investigator will be notified. Toxicities will be reported in timely fashion by sub-investigators and will be monitored by the Principal Investigator. Graft failure (see Section 7.0) should be reported within 15 days of occurrence. If at any time 2 of 3 successive patients fail to engraft (defined as <10% donor cells in all lineages tested) or experience grade 4 toxicity, patient accrual will be held pending review.

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Appendix A: Grading of Acute GVHD

Clinical Stage of acute GVHD according to Organ System

Stage	**Skin**	**Liver**	**Intestine** (based on 3 day mean stool output)
1	Rash on < 25% of skin	Bilirubin 2-3 mg/dl	Diarrhea > 500 ml/day Patients ≤ 12 years: < 10-19.9 ml/kg/day Unexplained nausea, vomiting or anorexia with biopsy proven upper GI GVHD
2	Rash on 25-50% of skin	Bilirubin 3-6 mg/dl	Diarrhea > 1000 ml/day Patients ≤ 12 years: 20-30 ml/kg/day
3	Rash on > 50% of skin	Bilirubin 6-15 mg/dl	Diarrhea > 1500 ml/day Patients ≤ 12 years: > 30 ml/kg/day
4	Generalized erythroderma with bullous formation	Bilirubin >15 mg/dl	Severe abdominal pain ± ileus or (regardless of stool volume)

CIBMTR GVHD Grade

Grade	Skin	GI	Liver
I	Stage 1-2	None	None
II	Stage 3 or	Stage 1 or	Stage 1
III	_	Stage 2-3 or	Stage 2-4
IV	Stage 4 or	Stage 4	

Percent Body Surfaces

Body Area	**Percent**	**Total Percentage**
Each Arm	9%	18%
Each Leg	18%	36%
Chest & Abdomen	18%	18%
Back	18%	18%
Head	9%	9%
Pubis	1%	1%

Appendix B: Classification of Chronic Graft-Versus-Host Disease (GVHD)

Limited Chronic GVHD

1. Localized skin involvement resembling localized scleroderma with or without
2. Liver involvement
3. No other organ involvement

Extensive Chronic GVHD

1. Generalized skin involvement and/or liver dysfunction
2. Localized skin involvement and/or hepatic dysfunction due to chronic GVHD
3. Liver histology showing chronic aggressive hepatitis, bridging necrosis, or cirrhosis
4. Involvement of the eye
5. Involvement of the salivary glands or oral mucous membranes
6. Involvement of any other target organ.

Appendix C: Performance Status Scales

Lansky Performance Status Scale (< 16 yrs)

Percentage	
100	Fully active
90	Minor restriction in physically strenuous play
80	Restricted in strenuous play, tires more easily, otherwise active
70	Both greater restrictions of, and less time spent in active play
60	Ambulatory up to 50% of time, limited active play with assistance/supervision
50	Considerable assistance required for any active play; fully able to engage in quiet play
40	Able to initiate quiet activities
30	Needs considerable assistance for quiet activities
20	Limited to very passive activity initiated by others (e.g. TV)
10	Completely disabled, not even passive play

Karnofsky Performance Status Scale (≥ 16 yrs)

Percentage	
100	Normal, no complaints
90	Able to carry on normal activity, minor signs or symptoms of disease
80	Normal activity with effort; some signs or symptoms of disease
70	Cares for self; unable to carry on normal activity or do active work
60	Requires occasional assistance, but is able to care for most of his/her needs
50	Requires considerable assistance and frequent medical care
40	Disabled; requires special care and assistance
30	Severely disabled, hospitalization indicated. Death not imminent
20	Very sick, hospitalization necessary, active supportive treatment necessary
10	Moribund, fatal processes, progressing rapidly
0	Dead

Reference

Karnofsky DA: Meaningful clinical classification of therapeutic responses to anti-cancer drugs. Editorial: Clin Pharmacol Ther 2: 709-712, 1961.

Appendix D: Expedited Report Form

(see section 6.7.1 for reporting requirements)

A Study of Hematopoietic Stem Cell Transplantation (HSCT) in Immune Function Disorders Using a Reduced-Intensity Preparatory Regime (The HIT Study)

<table>
<tr><td colspan="4">1. Patient Information</td></tr>
<tr><td>Patient ID:</td><td>Age:</td><td>Sex:</td><td>Weight:</td></tr>
<tr><td colspan="4">2. Adverse Event Assessment</td></tr>
<tr><td colspan="2">Date of Event:</td><td colspan="2">Event Type:
☐ Death
☐ Serious Adverse Event
☐ Graft failure
☐ Noncompliance (protocol error)
☐ Protocol exception
☐ Other: _____</td></tr>
<tr><td>Type of event</td><td colspan="3">☐ Expected ☐ Unexpected</td></tr>
<tr><td colspan="4">Description of events:</td></tr>
<tr><td>Action Taken:</td><td colspan="3"></td></tr>
<tr><td>Protocol Treatment at time of event</td><td colspan="3"></td></tr>
<tr><td>Relevant tests/ Lab data (include dates)</td><td colspan="3"></td></tr>
<tr><td>Other relevant history (including pre-existing conditions and condomitant meds)</td><td colspan="3"></td></tr>
<tr><td>Probable or presumed cause of event</td><td colspan="3"></td></tr>
<tr><td>Relationship to study</td><td colspan="3">☐ Definite ☐ Probable ☐ Possible ☐ Unlikely ☐ Unrelated</td></tr>
<tr><td>Has this event been reported to your institution’s IRB?</td><td colspan="3">☐ Yes ☐ No</td></tr>
</table>

Signature of PI: _____ Date: _____

Institution: _____

Protocol Chair Review:

Action to be taken:

☐ None, continue protocol approval

☐ Protocol suspended pending additional review

☐ Protocol closed to accrual

_____ _____

Signature of Protocol Chair Date