Phase II Clinical Trial of the Use of Post-Transplant Cyclophosphamide for Graft Versus Host Disease (GvHD) Prophylaxis Following Matched Unrelated Donor (MUD)

Hematopoietic Stem Cell Transplant (HSCT)

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Bone Marrow Transplantation and Cellular Therapy Program

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1 BACKGROUND

1.1 Allogeneic Stem Cell Transplantation in Hematologic Malignancies

Allogeneic hematopoietic stem cell transplantation (allo HCT) has become widely used as a possible curative option for many hematologic malignancies.[1, 2] Although the HLA matching techniques have improved significantly over the last several years, including DNA typing, the incidence of graft versus host disease (GVHD) of unrelated donor (MUD) transplant, particularly, mismatched unrelated donor (MMUD) transplant is still higher then matched related donor transplant.[3-5] The high incidence of GVHD is associated with lower survival.[4] Efforts have been made to improve the result of MUD and MMUD transplantation; however, none of these efforts have been very successful.

Anti-thymocyte globulin (ATG) has been used for many years for prevention of GVHD[6, 7], but a systematic review showed it is not associated with significant reduction in non-relapse mortality.[8] The benefit of reduced incidence of GVHD is unfortunately offset by increased risk of infections[9] and increased risk of non-relapse mortality (NRM). Thus the use of ATG for GVHD prophylaxis has become controversial.[10] The main prophylactic regimens for prevention of GVHD after allo HCT are based on pharmacologic agents such as calcineurin inhibitors and methotrexate.[11] These agents have narrow therapeutic window and are often associated with adverse effects such as severe mucositis with methotrexate and renal impairment with calcineurin inhibitors.[11]

Alemtuzumab (Campath-1H) has also been introduced for GVHD treatment[12] and prophylaxis[13, 14], but because of its significant immune suppression, the patient becomes more susceptible to infections. Also, because of the significant T-cell suppression effect which may continue for months after the stem cell transplant, the patients may gradually lose the donor chimeric component which may predispose them to relapse. Thus, the use alemtuzumab for GVHD prophylaxis has become controversial[15].

For these reasons, the optimal regimen for prevention of GVHD in MUD and MMUD transplantation has yet to be identified.

1.2 Post-Transplant Cytoxan as a GVHD Prophylaxis for Haploidentical Transplant

Haploidentical HCT has traditionally been done as a T-cell depleted transplant to avoid the high risk of GVHD by the alloreactive donor T-cells.[16-18] However, risk of engraftment failure and poor immune reconstitution remained barriers for significant survival improvement.[18]

Johns Hopkins group has used post-transplant Cyclophosphamide (Cytoxan) with T-cell replete haploidentical HCT to prevent GVHD.[19, 20] They developed this method based on animal experiments. Post-transplant Cytoxan was shown to be effective in preventing GVHD in haploidentical HCT. These favorable data has been reproduced.[21, 22] The benefit of post- transplant Cytoxan has also been shown with the use of non-myeloablative regimens with haploidentical HCT.[20, 23]

1.3 Post-Transplant Cytoxan as a GVHD Prophylaxis for Matched Transplant

Post-transplant Cytoxan (after myeloablative By/Cy preparative regimen) has also been tested before as a sole GVHD prophylaxis regimen after T-cell replete allogeneic bone marrow transplant (not PBSC) with matched related and MUD.[24] The 100-day and 2-year non-relapse mortality (NRM) in this study were 9% and 17% respectively.[24]

We hypothesize that post-transplant Cytoxan at a lower dose (than that used in haploidentical HCT) is effective in preventing GVHD in the setting of MUD and MMUD after myeloablative transplant. Because the HLA disparity in these 2 settings is not as much as in haploidentical transplant, we will use the post-transplant Cytoxan, 50 mg/kg for one dose, instead of the two doses in the original haploidentical transplant regimens.

2 OBJECTIVES AND ENDPOINTS

2.1 Primary Objective

The primary objective is to determine the incidence of grade II-IV acute GVHD following allo HCT using post-transplant cyclophosphamide for patients with human leukocyte antigen (HLA) matched unrelated (MUD) donors.

2.2 Secondary Objectives

- To determine disease-free survival (DFS) and overall survival (OS) following allo HCT using post-transplant cyclophosphamide for patients with HLA matched unrelated (MUD) donors.

- To assess the safety of post-transplant cyclophosphamide for MUD transplantation.
- To evaluate disease recurrence and time to recurrence in patients receiving post-transplant cyclophosphamide compared to historical control without post-transplant cyclophosphamide.
- To determine the time of onset, severity, responsiveness to treatment, organs involved of acute and chronic GVHD.
- Observation of Immune Reconstitution over time.

2.3 Primary Endpoint

The primary study endpoint is grade II-IV acute GVHD.

2.4 Secondary Endpoints

- OS at one-year post-transplant.
- DFS at one-year post-transplant.
- Regimen related toxicity within the first 100 days post-transplant.
- Time to neutrophil and platelet engraftment. Neutrophil engraftment is defined as the first of 3 consecutive days with an absolute neutrophil count (ANC) > 500/μL. Platelet engraftment is defined as the first of 3 consecutive days with a platelet count > 20,000/μL without platelet transfusion support for 7 days.
- Relapse rate at 2 years post-transplant.

3 PATIENTS ELIGIBILITY CRITERIA

3.1 Inclusion Criteria

1) Disease Criteria: patients must meet diagnostic criteria of acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), chronic myeloid leukemia (CML), chronic lymphocytic leukemia (CLL), non-Hodgkin lymphoma (NHL), Hodgkin lymphoma (HL), myelodysplastic syndrome (MDS), myelofibrosis, or severe aplastic anemia. Patients will be allowed on study if they are deemed eligible for allo HCT regardless of remission status.

2) Age Criteria: 19 to 65 years in age.

3) Organ Function Criteria: All organ function testing should be done within 28 days of study registration.

4) Cardiac: Left ventricular ejection fraction (LVEF) ≥ 50% by MUGA (Multi Gated Acquisition) scan or echocardiogram.

5) Pulmonary: FEV1 (Forced expiratory volume in 1 second) and FVC (Forced vital capacity) ≥ 50% predicted, DLCO (diffusing capacity of the lung for carbon monoxide) (corrected for hemoglobin) ≥ 50% of predicted.

6) Renal: The estimated creatinine clearance (CrCl) must be equal or greater than 60 mL/min/1.73 m2 as calculated by the Cockcroft-Gault Formula:

   CrCl=(140-age) x weight(kg) x 0.85 (if female)/72 x serum creatinine (mg/dL)

7) Hepatic:
   - Serum bilirubin ≤1.5 × upper limit of normal (ULN)
   - Aspartate transaminase (AST)/alanine transaminase (ALT) ≤2.5 × ULN
   - Alkaline phosphatase ≤ 2.5 × ULN

8) Performance status: Karnofsky ≥ 70%.,

9) Patient must be informed of the investigational nature of this study in accordance with institutional and federal guidelines and have the ability to provide written informed consent prior to initiation of any study-related procedures, and ability, in the opinion of the principal investigator, to comply with all the requirements of the study.

10) Patient has a suitable and willing HLA-8/8 matched unrelated donor identified.

3.2 Exclusion Criteria

1) Patient is NOT a candidate for myeloablative regimen.
2) Non-compliant to medications.
3) No appropriate caregivers identified.
4) HIV1 (Human Immunodeficiency Virus-1) or HIV2 positive
5) Uncontrolled medical or psychiatric disorders.
6) Uncontrolled infections, defined as positive blood cultures within 72 hours of study entry, or evidence of progressive infection by imaging studies such as chest CT scan within 14 days of registration.
7) Active central nervous system (CNS) leukemia.
8) Preceding allogeneic HSCT.
9) Pregnancy or Breastfeeding.
10) Prior autologous or allogeneic stem cell transplant.

4 STUDY TREATMENT

We are going to use this method for various diagnoses so this GVHD prophylaxis regimen will be included in the following preparative regimens.

4.1 Preparative Regimens

1) **Fludarabine / Busulfan** regimen is a modified Fludarabine plus Busulfan preparative regimen that will be used for myeloid malignancy and severe aplastic anemia. In the myeloablative regimen of fludarabine plus busulfan, the target AUC (area under the concentration curve) of busulfan is typically 20 - 24,000 μM·min. Since we are adding post-transplant cytoxan to this regimen, we will use a busulfan target AUC of 20,000. Patients will receive MESNA and hydration for prophylaxis of hemorrhagic cystitis per standard practice. Seizure prophylaxis will be administered per standard practice while on busulfan.

2) **Total body irradiation (TBI) / Cytoxan** regimen will be used for ALL or aggressive NHL or HL in young patients (patients </= 40 years old) with no major comorbidities. The standard myeloablative regimen for those patients is TBI 1,200 cGy and Cytoxan 60 mg/kg x 2 days. In our study, TBI dose will be the same, but the 2 doses of pre-transplant Cytoxan will be 35 mg/kg, in addition to post-transplant Cytoxan of 50 mg/kg on Day +3. Thus the total dose of Cytoxan is unchanged in this regimen. Patient will receive MESNA and hydration for prophylaxis of hemorrhagic cystitis per standard practice.

3) **TBI / Fludarabine** regimen will be used for ALL or aggressive NHL or HL in patients who are older than 40 years or at any age with major comorbidities that portends high NRM with fully myeloablative Cy/TBI. These patients will receive Fludarabine 40 mg/m^2 x 4 doses plus TBI 1,000 cGy instead of usual 1,200 cGy. Post-transplant Cytoxan of 50 mg/kg on Day +3 will be given. Patient will receive MESNA and hydration for prophylaxis of hemorrhagic cystitis per the transplant physician's clinical practice standard.

4) **Fludarabine/Melphalan** regimen may be used for NHL or HL patients. These patients will receive Fludarabine 40 mg/m^2 x 4 doses plus melphalan 140 mg/m^2 x one dose. Post-transplant Cytoxan of 50 mg/kg on Day +3 will be given. Patient will receive MESNA and hydration for prophylaxis of hemorrhagic cystitis per the transplant physicians clinical practice standard.

4.2 Study Treatment Schedule for Myeloid Diseases

Flu/Bu - Preparative Regimen #1

Day -6 (+/- 2 days) Admit to Hospital

Day -5 (+/- 2 days) Busalfan 3.2mg/kg and Fludarabine 40 mg/m^2 IV

Day -4 (+/- 2 days) Busulfan- 3.2mg/kg, Fludarabine 40 mg/m^2

IV Day -3(+/- 2 days) Busulfan PK directed dosing IV, Fludarabine 40 mg/m^2

IV Day -2 (+/- 2 days) Busulfan PK directed dosing IV, Fludarabine 40 mg/m^2

Day -1 (+/- 2 days) Rest Day(s)

Day 0 Transplant

Day +3 Cyclophosphamide 50mg/kg IV

4.3 Study Treatment Schedule for ALL or high-risk NHL patients ≤ 40 years old

TBI / Cy – Preparative Regimen #2

Day -6 (+/- 2 days) TBI 200cGy/ fraction (2 fractions)

Day -5 (+/- 2 days) TBI 200cGy/ fraction (2 fractions)

Day -4 (+/- 2 days) TBI 200cGy/Fraction (2 fractions)

Day -3 (+/- 2 days) Cyclophosphamide 35 mg/kg IV

Day -2 (+/- 2 days) Cyclophosphamide 35 mg/kg IV

Day -1 (+/- 2 days) Rest day(s)

Day 0 Transplant

Day +3 Cyclophosphamide 50mg/kg IV

4.4 Study Treatment Schedule for ALL or high-risk NHL patients ≥ 40 years old

Flu / TBI – Preparative Regimen #3

Day -7 (+/- 2 days) Fludarabine 40 mg/m^2 IV

Day -6 (+/- 2 days) Fludarabine 40 mg/m^2 IV

Day -5 (+/- 2 days) Fludarabine 40 mg/m^2 IV

Day -4 (+/- 2 days) Fludarabine 40 mg/m^2 IV

Day -3 (+/- 2 days) TBI 200cGy/fraction (2 fractions)

Day -2 (+/- 2 days) TBI 200cGy/fraction (2 fractions)

Day -1 (+/- 2 days) TBI 200cGy/fraction (1 fraction)

Day 0 Transplant

Day +3 Cyclophosphamide 50mg/kg IV

4.5 Study Treatment Schedule for Lymphoma patients

Flu/Mel – Preparative Regimen #4

Day -5 (+/- 2 days) Fludarabine 40 mg/m^2 IV

Day -4 (+/- 2 days) Fludarabine 40 mg/m^2 IV

Day -3 (+/- 2 days) Fludarabine 40 mg/m^2 IV

Day -2 (+/- 2 days) Fludarabine 40 mg/m^2 IV

Day -1 (+/- 2 days) Melphalan 140 mg/m^2 IV

Day 0 Transplant

Day +3 Cyclophosphamide 50mg/kg IV

Rituximab may be added to this regimen (at the physician's discretion) on days -6, +1, and/or +8 for lymphoma not in CR at the time of transplant.

5 MEDICATIONS

5.1 Cyclophosphamide

Cyclophosphamide (35mg/kg) IV will be administered in preparative regimen #2 as noted above.

Cyclophosphamide (50mg/kg) IV will be administered at day +3 as GVHD prophylaxis in all 3 treatment plans.

Cyclophosphamide will be infused over 2 hours.

Cyclophosphamide will be dosed based on actual body weight unless patient is greater than 40% above IBW in which case we will use adjusted body weight.

Ideal Body Weight is calculated as follows:

Males: IBW= 50 + {[(Ht in cm x 0.39) – 60] x 2.3}

Females: IBW= 45.5 + {[(Ht in cm x 0.39) – 60] x 2.3}

Adjusted ideal body weight is calculated as follows:

Ideal Weight + (Actual Weight-Ideal Weight) x 0.4

If actual weight is lower than ideal weight, we will use actual weight.

Mesna will be administered as an adjuvant for cyclophosphamide metabolites. Dosing will follow standard practice.

5.2 Fludarabine

Fludarabine (40 mg/m2/day) IV will be administered in preparative regimens #1 and #3 as noted above.

Fludarabine will be dosed based on actual body weight unless patient is greater than 40% above IBW in which case we will use adjusted body weight calculated as noted above.

Fludarabine will be given by 1hour IV infusion.

5.3 Busulfan

Busulfan IV will be administered in preparative regimen #1 as noted above.

Busulfan IV will start at approximately 9 am and be given over 3 hours via IV infusion.

Busulfan pharmacokinetics (PK) study will be done on day 5 (test dose)

Busulfan PK will be adjusted to target AUC of 20,000 μM·min.

5.4 Melphalan

Melphalan IV will be administered in preparative regimen #4 as noted above.
Melphalan IV will be infused over 30 to 40 minutes.
Melphalan will be dosed on actual BSA unless actual weight is greater than adjusted IBW.
If actual weight is greater than adjusted IBW then melphalan will be dosed using adjusted IBW.

5.5 Anti-Seizure prophylaxis

Anti-seizure prophylaxis will be administered as per standard practice.

5.6 GVHD prophylaxis

GVHD prophylaxis consists of post-transplant cyclophosphamide, mycophenolate mofetil (MMF), and tacrolimus. Patients will not receive immunosuppressive therapy between days 0 and +5 for concern of mitigating the immunosuppressive effect of post-transplant Cytoxan, which is administered on Day +3, unless determined by treating physician that there is a need based on clinical status.

5.6.1 *Cyclophosphamide*

Cytoxan will be administered on Day +3 at 50mg/kg IV over 2 hours single dose.

5.6.2 *Mycophenolate Mofetil*

MMF will be administered starting day +5. Dosing will start at 15 mg/kg Q8h IV with a maximum dose of 1g Q8h IV, then change to PO when patient can tolerate PO intake. MMF will be continued till day +35 unless there is active GVHD.

5.6.3 *Tacrolimus*

Tacrolimus will be administered starting on day +5. Dosing will follow therapeutic standards for GVHD prophylaxis. Tacrolimus will be continued till day +100 with taper starting on +100 unless there is active GVHD.

5.7 Filgrastim

Filgrastim (G-CSF) will be administered beginning Day +5 at approximately 5 mcg/kg rounding to the nearest vial size according to the ranges listed below. Treatment will continue until engraftment.

Weight Range	G-CSF dose
Up to 60kg	Use 300mcg Vial
61 kg to 96 kg	Use 480mcg Vial
97 kg to 120 kg	Use two 300mcg vials
121 kg to 156 kg	Use one 300 mcg vial and one 480 mcg vial
157 kg to 192 kg	Use two 480 mcg vials

5.8 Infection Prophylaxis

Antibacterial, antiviral and antifungal coverage during neutropenia until engraftment will be at the discretion of the transplant physician's clinical practice standards.

The following is a suggested prophylaxis regimen:

5.8.1 ***Recommended fungal prophylaxis***

Patients will receive Voriconazole 200 mg PO BID until day +75 post-transplant. For patients who develop elevated liver enzymes (AST or ALT > 2.5 x ULN or total bilirubin > 2.5 x ULN), micafungin 50mg IV/day may replace voriconazole until AST, ALT and/or total bilirubin decline to grade I level (<2.5x ULN).

5.8.2 ***HSV / VZV Prophylaxis***

Herpes simplex virus / Herpes zoster virus (HSV/VZV) infection prophylaxis will be done using Acyclovir or valganciclovir daily until day +365 post-transplant.

5.8.3 ***CMV (Cytomegalovirus) preemptive therapy***

In all cases in which either the patient or donor are seropositive for CMV pre-transplant, CMV-antigenemia or quantitative PCR testing will be obtained from blood weekly, starting from the time of neutrophil engraftment until at least day 100. Patients whose CMV PCR or antigenemia assays become positive post-transplant shall be treated per the transplant physician's clinical practice standard.

5.8.4 ***PCP (Pneumocystic pneumonia) prophylaxis***

PCP prophylaxis should be initiated approximately day +30 post-transplant, provided the patient has met the engraftment criteria for neutrophils and platelets. Primary PCP prophylaxis shall be trimethoprim-sulfamethoxazole (TMP-SMX) single strength daily for 5 days a week or double strength (Bactrim DS) PO once a day for 2 or 3 days a week (preferred PCP prophylaxis). If intolerant to TMP-SMX, pentamidine 300 mg inhaler (or IV) every 4 weeks, dapsone 100 mg once a day or atovaquone (Mepron) 1500 mg PO daily may be used.

5.8.5 ***PCR testing***

PCR testing for HHV6 and Adenovirus will be sent weekly (+/- 3 days) starting approximately Day +20 and continue until day 100.
PCR testing for EBV will be sent every 2 weeks (+/- 3 days) starting approximately Day +20 until day 100.

6 DRUG INFORMATION

6.1 Cyclophosphamide (Cytoxan)

Chemical Characteristics

Cyclophosphamide monohydrate (cyclophosphamide) is a synthetic antineoplastic drug chemically recognized as 2-[bis(2-cholorethyl) amino] tetrahydro-2H-1,3,2-oxazaphosphorine 2-oxide monohydrate. The molecular formula of cyclophosphamide is $C_7H_{15}Cl_2N_2O_2P \cdot H_2O$ with a molecular weight of 279.1.

Available Forms

IV cyclophosphamide is available as a sterile white crystalline powder. Cyclophosphamide is stored in a single dose vial with 500mg of the powder.

Storage and Handling

Unopened vials should be stored at or below 25°C (77°F).

Toxicity

Common side effects of cyclophosphamide include: nausea, vomiting, alopecia, leucopenia, interstitial pneumonitis, interstitial pulmonary fibrosis, anaphylactic reactions, amenorrhea, hemorrhagic cystitis, oligospermia or azoospermia and suppression of immune responses. Less common side effects include: secondary malignancies in patients diagnosed with a primary malignancy, fetal harm when administered to pregnant women, acute cardiac toxicity, congestive heart failure, hemorrhagic myocarditis, hemopericardium and possible cross sensitivity with other alkylating agents.

Administration

Cyclophosphamide for parenteral use must be prepared by either adding 0.9% sodium chloride solution, if injected directly, or sterile water, if infused. Constituted in water, cyclophosphamide is hypotonic hence should not be injected directly. Solutions of cyclophosphamide with sodium chloride solution may be injected, intravenously, intramuscularly, intraperitoneally, or intrapleurally.

Constituted cylophosphamide is physically and chemically stable for 24 hours at room temperature or six days refrigerated. Prepared solutions do not contain any microbial preservative; hence sterility of the solutions should be monitored.

6.2 Fludarabine (Fludara)

Chemical Characteristics

Fludarabine phosphate (fludarabine) is an antimetabolite with chemical name 9H-Purin-6-amine, 2-fluoro-9-(5-0-phosphono- 0-D-arabino-furanosyl) (2-fluoro-ara-AMP). The molecular formula is $C_{10}H_{13}FN_5O_7P$ with molecular weight of 365.2.

Available Forms

IV Fludarabine is packaged as a white lyophilized solid cake in a vial. Each vial has 50mg fludarabine phosphate and only one vial is enclosed per carton.

Storage and Handling

Unopened vials of fludarabine should be stored at 20° to 25°C (68° TO 77°F); excursions permitted between 15° to 30°C (59° TO 86°F).

Toxicity

Toxicities reported as more than 10% incidence are:

Myelosuppression (neutropenia, thrombocytopenia, and anemia), fever and chills, infections, nausea and vomiting, pain, weakness, cough, pneumonia, dyspnea, diarrhea, anorexia, rash, edema.

Toxicities with expected incidences between 1% and 10% are:

Malaise, stomatitis, myalgia, paresthesia, visual disturbance, gastrointestinal bleeding, upper respiratory infection, diaphoresis, dysuria, urinary infection, sinusitis, hearing loss, hyperglycemia, headache, pharyngitis, hemoptysis, esophagitis, mucositis, hematuria, osteoporosis, alopecia, anaphylaxis, hemorrhage, dehydration, sleep disorder, depression, cerebellar syndrome, impaired mentation, allergic pneumonitis, epitaxis, bronchitis, hypoxia, liver failure, abnormal liver function, cholelithiasis, ARDS, respiratory distress, pulmonary hemorrhage, pulmonary fibrosis, respiratory failure, constipation, dysphagia, pruritus, seborrhea, renal failure, abnormal, renal function test, protenuria, hesitancy, angina, congestive, heart failure, arrhythmia, supraventricular tachycardia, myocardial infarction, deep venous thrombosis, phlebitis, transient ischemic attack, aneurysm, cerebrovascular accident, arthralgia, tumor lysis syndrome.

Administration

IV fludarabine is prepared by adding sterile water to the white solid cake. Reconstituted in 2mL of sterile water, the solid cake produces a solution with approximate concentration of 25mg/mL fludarabine phosphate. Follow the institutional guidelines for further preparation and administration procedures of fludarabine.

Reconstituted IV fludarabine contains no antimicrobial preservative hence should be utilized within 8 hours of reconstitution. DO NOT infuse concomitantly with another intravenous solution of unknown compatibility.

6.3 Busulfan (Busulfex)

Chemical Characteristics

Busulfan is a bifunctional alkylating agent known chemically as 1,4-butanediol, dimethanesulfonate with a molecular formula of $CH_3SO_2O(CH_2)_4OSO_2CH_3$ and a molecular weight of 246 g/mole.

Available Forms

IV busulfan (Busulfex) is supplied as a clear, colorless, sterile solution in 10-mL single-use glass vials each containing 60 mg of busulfan at a concentration of 6 mg/mL for intravenous use.
IV busulfan is packaged in a tray pack of 8 vials.

Storage and Handling

Unopened vials of Busulfex must be stored under refrigerated conditions between 2°-8°C (36°-46°F).

Toxicity

Toxicities reported as more than 10% incidence are:
Tachycardia, hypertension, edema, thrombosis, chest pain, vasodilation, hypotension,
insomnia, fever, anxiety, headache, chills, pain, dizziness, depression, confusion, rash, pruritus, alopecia, hypomagnesemia, hyperglycemia, hypokalemia, hypocalcemia, hypophosphatemia, nausea, mucositis/stomatitis, vomiting, anorexia, diarrhea, abdominal pain, dyspepsia, constipation, xerostomia, rectal disorder, abdominal fullness, myelosuppression, neutropenia, thrombocytopenia, lymphopenia, anemia, hyperbilirubinemia, ALT increase, veno-occlusive disease, jaundice, injection site inflammation, injection site pain, weakness, back pain, myalgia, arthralgia, creatinine increased, oliguria, rhinitis, lung disorder, cough, epistaxis, dyspnea, pneumonia, hiccup, pharyngitis, infection, allergic reaction.

Toxicities with expected incidences are 1% to 10% are:

Arrhythmia, cardiomegaly, atrial fibrillation, ECG abnormality, heart block, heart failure, pericardial effusion, tamponade, ventricular extrasystoles, hypervolemia, lethargy, hallucinations, agitation, delirium, encephalopathy, seizure, somnolence, cerebral hemorrhage, vesicular rash, vesiculobullous rash, skin discoloration, maculopapular rash, acne, exfoliative dermatitis, erythema nodosum, hyponatremia, ileus, weight gain, hematemesis, pancreatitis, prothrombin time increase, hepatomegaly, hematuria, dysuria, hemorrhagic cystitis, BUN increase, asthma, alveolar hemorrhage, hyperventilation, hemoptysis, pleural effusion, sinusitis, atelectasis, hypoxia.

Administration

IV busulfan must be diluted prior to use with either NS or D5W. The diluent quantity should be 10 times the volume of Busulfex, so that the final concentration of busulfan is approximately 0.5 mg/mL.

Infusion pumps should be used to administer the diluted busulfan solution. DO NOT infuse concomitantly with another intravenous solution of unknown compatibility. Warning: Rapid infusion of IV busulfan has not been tested and is not recommended.

Follow the institutional guidelines for more detailed preparation and administration procedures of busulfan.

6.4 Melphalan

Chemical Characteristics

Melphalan is a phenylalanine derivative of nitrogen mustard. Melphalan is a bi-functional alkylating agent that is active against selected human neoplastic diseases. It is known chemically as 4-[bis(2-chloroethyl)amino]-L -phenylalanine. The molecular formula is $C_{13}H_{18}Cl_2N_2O_2$.

Available Forms

Melphalan for Injection is supplied in a carton containing one single-use clear glass vial of freeze-dried melphalan hydrochloride equivalent to 50 mg melphalan and one 10-mL clear glass vial of sterile diluent (NDC 0173-0130-93).

Storage and Handling

Store at controlled room temperature 15° to 30°C (59° to 86°F) and protect from light. Procedures for proper handling and disposal of anticancer drugs should be considered.

Toxicity

The most common side effect is bone marrow suppression leading to leukopenia, thrombocytopenia, and anemia. Gastrointestinal disturbances such as nausea and vomiting, diarrhea, and oral ulceration occur infrequently. Hepatic disorders ranging from abnormal liver function tests to clinical manifestations such as hepatitis and jaundice have been reported. Hepatic veno-occlusive disease has been reported.

Other reported adverse reactions include skin hypersensitivity, skin ulceration at injection site, skin necrosis rarely requiring skin grafting, maculopapular rashes, vasculitis, alopecia, hemolytic anemia, allergic reaction, pulmonary fibrosis (including fatal outcomes), and interstitial pneumonitis. Temporary significant elevation of the blood urea has been seen in the early stages of therapy in patients with renal damage. Subjective and transient sensation of warmth and/or tingling.

Administration

Parenteral drug products should be visually inspected for particulate matter and discoloration prior to administration whenever solution and container permit. If either occurs product should not be used. Care should be taken to avoid possible extravasation of melphalan and in cases of poor peripheral venous access, consideration should be given to use of a central venous line.

Melphalan for injection must be reconstituted by injecting 10 mL of the supplied diluent directly into the vial of lyophilized powder. This provides a 5-mg/mL solution of melphalan.
Dose to be administered in diluted in 0.9% Sodium Chloride Injection, USP, to a concentration not greater than 0.45 mg/mL. Administer the diluted product over a minimum of 15 minutes.

6.5 Tacrolimus (Prograf)

Chemical Characteristics

Tacrolimus is a macrolide immunosuppressant produced by Stretocyces Tsukubaensis. Tacrolimus has an empirical formulation of $C_{44}H_{69}NO_{12}$-H_2O and a formula weight of 822.05. Tacrolimus appears as white crystals or crystalline powder. It is practically insoluble in water, freely soluble in ethanol, and very soluble in methanol and chloroform. Tacrolimus inhibits T-lymphocyte activation, although the exact mechanism of action is not known. Experimental evidence suggests that tacrolimus binds to an intracellular protein, FKBP-12. A complex of tacrolimus-FKBP-12, calcium, calmodulin, and calcineurin is then formed and the phosphatase activity of calcineurin inhibited. This effect may prevent the dephosphorylation and translocation of nuclear factor of activated T-cells (NF-AT), a nuclear component thought to initiate gene transcription for the formation of lymphokines (such as interleukin-2, gamma interferon). The net result is the inhibition of T-lymphocyte activation (i.e., immunosuppression).

Available Forms

Tacrolimus is available for oral administration as capsules (tacrolimus capsules) containing the equivalent of 0.5 mg, 1mg and 5mg of anhydrous tacrolimus. Inactive ingredients include lactose, hydroxypropyl methylcellulose, croscarmellose sodium, and magnesium stearate. The 0.5 mg and 1 mg capsule shell contains gelatin and titanium dioxide, and the 5-mg capsule shell contains gelatine, titanium dioxide, and ferric oxide. Tacrolimus is also available as a sterile solution (tacrolimus injection) containing the equivalent of 5mg anhydrous tacrolimus in 1 ml for administration by IV infusion only. Each mL contains polyoxyl 60 hydrogenated castor oil (HCO-60), 200 mg, and dehydrated alcohol, USP, 80.0% v/v.

Storage and Handling

Tacrolimus capsules should be stored at room temperature between 15° and 30°C (59° and 86°F). Tacrolimus injection should be stored between 5° and 25°C (41° and 77°F).

Toxicity

Possible side effects of tacrolimus include: depressed kidney function, high blood sugar, high blood potassium, skin rash, headache, nausea, vomiting. Less common side effects are loss of appetite, sleep disturbances, vivid dreams, hallucinations, high blood pressure, seizure, decreased

level of consciousness, anemia, agitation, tremors, irritability, slurred speech, tingling in the hands and feet, pain in the palms and soles of the feet, weakness, and abnormal blood cell levels. All of these side effects are reversible by reducing the dose or discontinuing the drug. Rare fatal cases of severe allergic reactions have been reported in patients receiving cyclosporine and it is possible that similar reactions could also occur in patients receiving tacrolimus.

Administration

Tacrolimus (Prograf injection) must be diluted with NS or D5W before use. Tacrolimus is administered as a continuous infusion. Oral preparation will be administered on empty stomach every 12 hours.

6.6 Mycophenolate Mofetil (MMF, Cellcept)

Chemical Characteristics

CellCept (mycophenolate mofetil) is the 2-morpholinoethyl ester of mycophenolic acid (MPA); an immunosuppressive agent; inosine monophosphate dehydrogenase (IMPDH) inhibitor. The chemical name for mycophenolate mofetil (MMF) is 2-morpholinoethyl (E)-6-(l,3-dihydro-4-hydroxy-6-methoxy-7- methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate. It has an empirical formula of $C_{23}H_{31}NO_7$ a molecular weight of 433.50.

Mycophenolate mofetil is a white to off-white crystalline powder. It is slightly soluble in water (43 µg/mL at pH 7.4); the solubility increases in acidic medium (4.27 mg/mL at pH 3.6). It is freely soluble in acetone, soluble in methanol, and sparingly soluble in ethanol. The apparent partition coefficient in 1-octanol/water (pH 7.4) buffer solution is 238. The pKa values for mycophenolate mofetil are 5.6 for the morpholino group and 8.5 for the phenolic group.

Mycophenolate mofetil hydrochloride has a solubility of 65.8 mg/mL in D5W. The pH of the reconstituted solution is 2.4 to 4.1.

Available Forms

CellCept is available for oral administration as capsules containing 250 mg of mycophenolate mofetil, tablets containing 500 mg of mycophenolate mofetil, and as a powder for oral suspension, which when constituted contains 200 mg/mL mycophenolate mofetil

Toxicity

Toxicities reported as more than 20% incidence are:

Hypertension, hypotension, peripheral edema, chest pain, tachycardia, pain, headache, insomnia, fever, dizziness, anxiety, rash, hyperglycemia, hypercholesterolemia, hypomagnesemia, hypokalemia, hypocalcemia, hyperkalemia, abdominal pain, nausea, diarrhea, constipation, vomiting, anorexia, dyspepsia, urinary tract infection, leukopenia, anemia, leukocytosis, thrombocytopenia, liver function tests abnormality, ascites , back pain, weakness, tremor, paresthesia, abnormal kidney function, dyspnea, respiratory tract infection, pleural effusion, cough, lung disorder, sinusitis, infection, sepsis, lactate dehydrogenase increase.

Toxicities with expected incidences are 3% to 20% are:

Angina, arrhythmia, arterial thrombosis, atrial fibrillation, atrial flutter, bradycardia, cardiac arrest, cardiac failure, CHF, extrasystole, facial edema, hyper-/hypovolemia, pallor, palpitation, pericardial effusion, peripheral vascular disorder, postural hypotension, supraventricular extrasystoles, supraventricular tachycardia, syncope, thrombosis, vasodilation, vasospasm, venous pressure increased, ventricular extrasystole, ventricular tachycardia, chills with fever, confusion, delirium, depression, emotional lability, hallucinations, hypoesthesia, malaise, nervousness, psychosis, seizure, somnolence, thinking abnormal, vertigo , acne, alopecia, bruising, cellulitis, fungal dermatitis, hirsutism, petechia, pruritus, skin carcinoma, skin hypertrophy, skin ulcer, vesiculobullous rash, acidosis, alkalosis, Cushing's syndrome, dehydration, diabetes mellitus, gout, hypercalcemia, hyper-hypophosphatemia, hyperlipemia, hyperuricemia, hypochloremia, hypoglycemia, hyponatremia, hypoproteinemia, hypothyroidism, parathyroid disorder, abdomen enlarged, dysphagia, esophagitis, flatulence, gastritis, gastroenteritis, gastrointestinal hemorrhage, gastrointestinal moniliasis, gingivitis, gum hyperplasia, ileus, melena, mouth ulceration, oral moniliasis, stomach disorder, stomach ulcer, stomatitis, xerostomia, weight gain/loss, impotence, nocturia, pelvic pain, prostatic disorder, scrotal edema, urinary frequency, urinary incontinence, urinary retention, urinary tract disorder, coagulation disorder, hemorrhage, neutropenia, pancytopenia, polycythemia, prothrombin time increased, thromboplastin time increase, alkaline phosphatase increased, bilirubinemia,

cholangitis, cholestatic jaundice, GGT increased, hepatitis, jaundice, liver damage, transaminases increase, abscess, arthralgia, hypertonia, joint disorder, leg cramps, myalgia, myasthenia, neck pain, neuropathy, osteoporosis, amblyopia, cataract, conjunctivitis, eye hemorrhage, lacrimation disorder, vision abnormality, deafness, ear disorder, ear pain, tinnitus, albuminuria, creatinine increased, dysuria, hematuria, hydronephrosis, oliguria, pyelonephritis, renal failure, renal tubular necrosis, apnea, asthma, atelectasis, bronchitis, epistaxis, hemoptysis, hiccup, hyperventilation, hypoxia, respiratory acidosis, pharyngitis, pneumonia, pneumothorax, pulmonary edema, pulmonary hypertension, respiratory moniliasis, rhinitis, sputum increased, voice alteration, thirst.

Administration

Oral dosage formulations (tablet, capsule, suspension) should be administered on an empty stomach to avoid variability in MPA absorption. The oral solution may be administered via a nasogastric tube (minimum 8 French, 1.7 mm interior diameter); oral suspension should not be mixed with other medications. Delayed release tablets should not be crushed, cut, or chewed.

Intravenous solutions should be administered over at least 2 hours (either peripheral or central vein); do not administer intravenous solution by rapid or bolus injection.

6.7 Filgrastim (Neupogen)

Chemical Characteristics

Neupogen is the trademark name for filgrastim, representing recombinant methionyl human granulocyte colony-stimulating factor (r-methHuG-CSF). Neupogen is a 175 amino acid protein produced by recombinant DNA technology utilizing *Escherichia coli (E. coli)*. Neupogen has molecular weight 18,800 daltons and an amino acid sequence similar to that of natural human DNA except for the additional methonine at the N-terminal, necessary for expression in *E. coli*.

Available Forms

Neupogen is marketed as a sterile, clear, colorless preservative-free liquid. It is packaged in vials and prefilled syringes. The vials are single-dose, preservative-free and contain 300 mcg/mL of filgrastim. The prefilled syringes are single-dose, preservative free and hold 600mcg/mL of filgrastim.

Storage and Handling

Neupogen must be stored refrigerated at 2° to 8°C (36° TO 46°F). Avoid shaking.

Preceding injection, neupogen must reach room temperature for at most 24 hours. Discard any vials or prefilled syringes left at room temperature for more than 24 hours. Avoid use if particulate matter or discoloration is observed prior to administration

Toxicity

Common side effects include: nausea, vomiting, skeletal pain, spontaneous reversible elevations in uric acid, lactate dehydrogenase and alkaline phosphatase, alopecia, palpable splenomegaly and petechiae. Other side effects include: diarrhea, neutropenic fever, mucositis, fever, fatigue, thrombocytopenia, anorexia, dyspnea, headache, epistaxis, transfusion reaction, cough, skin rash, chest pain, generalized weakness, sore throat, stomatitis, pain, myocardial infarctions, constipation, arrhythmias, hypotension, hemorrhagic events, renal insufficiency, capillary leak syndrome, hepatomegaly, arthralgia, osteoporosis, cutaneous vaculitis, hematuria/proteinuria, exacerbation of some pre-existing skin disporders, splenic rapture, sickle cell crisis, sweet's syndrome and decreased bone density.

Administration

Neupogen may be administered as an IV or a subcutaneous infusion. It is recommended that neupogen be administered at least 24 hours after bone marrow infusion, with dosage modifications determined by neutrophil response.

If necessary, neupogen maybe diluted in 5% dextrose with addition of Albumin(human) to prevent absorption to plastic materials. Dilution to final concentration less than 5mcg/mL is not recommended at any time. Do not dilute with saline as product may precipitate.

When using either vials or prefilled syringes, do not save unused drugs for later administration. Dispose of all unused portions.

6.8 Total Body Irradiation (TBI)

Toxicity

TBI will be administered per standard of care procedure as implemented by radiation oncologists. TBI alone for post-pubescent patients with dose/fractionation not exceeding 2 Gy x 6 is well within the tolerance of most normal organs for < 5% risk of severe late toxicity (organ failure or major dysfunction) by 5 years. Notable exceptions are risk of cataract development, bone marrow suppression, ovarian and testicular dysfunction. Also, there is a small risk of second malignancy. The most common acute effects include nausea, vomiting, diarrhea and painful swelling of the parotid glands.

When TBI is given in conjunction with other therapies in the transplant setting, there is additional risk of side effects including loss of appetite, dry mouth, difficult or painful swallowing , headache, stomatitis (sore throat/mouth), altered skin integrity, hair loss, swelling, increased risk for infection and/or bleeding, possible lung failure, dry cough, fatigue, anxiety, fever, possible liver failure, lung scarring, loss of vision, shortness of breath, sterility, heartburn, cystitis, sleep disturbances altered gastrointestinal and genitourinary function, neuropathy, fistulas, altered endocrine function, pericarditis and increased risk of a second cancer. Overall, the incidence of most major toxicity when radiation is given in conjunction with other therapy as outlined above is still low, rare, serious side effects are possible.

7 ALLOGENEIC STEM CELL INFUSION

The day of the stem cell/marrow infusion will be defined as day 0. If more than one day of infusion is required, then these days are defined as day 0a, day 0b accordingly. The first day after the last stem cell infusion will be defined as day +1.

Besides the use of steroid use for pre-medication (per our program standards), patients will not receive any immunosuppression, including steroid, tacrolimus, sirolimus, or mycophenolate mofitle (unless definitely indicated per the attending physician) between day 0 and day 5 for concern of mitigating the immunosuppressive effect of post-transplant Cytoxan (given on day +3).

7.1 Stem Cell Source

The source of donor stem cells will be peripheral blood stem cells (PBSC) or bone marrow (BM). PBSC will be preferred. Cord blood will not be allowed.

7.2 PBSC Mobilization and Collection

PBSC mobilization and collection procedures and BM collection procedures will follow institutional practice.

7.3 Stem Cell Dosing

For PBSC infusions: The recommended stem cell dose is 4-8 X 10^6 CD34 cells/kg recipient weigh. The minimum accepted stem cell dose is 2 X 10^6 CD34 cells/kg recipient weight.

For marrow infusions: The recommended stem cell dose is 4-8 X 10^8 mononuclear cells/kg recipient weigh. The minimum accepted stem cell dose is 2 X 10^8 mononuclear cells/kg recipient weight.

8 REQUIRED OBSERVATIONS

Please see appendix B for study calendars.

8.1 Pre-Transplant Observations (within 35 days prior to study registration / day of final transplant evaluation).

- History and physical exam (include Karnofsky Score within 14 days prior to registration)
- Creatinine, AST, ALT, Alk Phos., and Total bilirubin
- Echocardiogram or MUGA
- Pulmonary function testing: FVC, FEV1, DLCO (corrected for hemoglobin).
- Unilateral bone marrow aspirate and biopsy, morphology and cytogenetics. (ALL and AML will be performed within 28 days prior to registration)
- Cerebrospinal fluid examination, if required, will be based on the transplant physician's clinical practice standards.

8.2 Post-Transplant Required Observations and Follow-up Plan

- Bone marrow aspirate and biopsy specimen shall be collected for morphology examination and cytogenetics at day +30(±7), +100(±14), and 1 year (±45) post-transplant for all patients who have not demonstrated disease progression by that time point. In addition, a unilateral marrow aspirate will also be collected whenever a relapse is suspected.

- Immune Reconstitution and Chimerism studies will be performed per BMT standard and/or as clinically indicated. The recommended timing of the labs is at Day +30 (±7), Day +100 (±14), Day +180 (±21) and at 1 year (±45) post-transplant.

- Patients need to be seen in clinic at least once a week until day 100 post-transplant and have acute GVHD assessment weekly (±3 days). Then they need to be seen at

least once a month (±14 days) until 1 year after transplant to have chronic GVHD assessment.

- The patient will be followed at least for 2 years after transplant. The follow up interval is determined as clinically necessary.
- The patient who relapsed after transplant will be followed only for survival.

8.3 Assessment of GVHD

Acute GVHD typically occurs after engraftment of the stem cells. Clinical assessment for acute GVHD will start after infusion of the stem cells. There are 2 grading systems for acute GVHD; the modified Keystone (Glucksberg) consensus criteria (grades I-IV)[25, 26] and CIBMTR criteria (grades A-D)[27]. Both systems were shown to equally predict survival.[28, 29]

The Glucksberg criteria will be followed in assessment of acute GVHD and the highest grade will be recorded (appendix C).

Chronic GVHD is graded based on clinical manifestations of organ systems based on the NIH Consensus data and CIBMTR grading guidelines utilizing UAB BMTCT form for recording. (appendix D)

9 STATISTICAL CONSIDERATIONS

9.1 Sample Size

This is a single-center phase II study whose primary aim is to show an improvement in incidence of acute GVHD grade II-IV after myeloablative MUD/MMUD all HCT from 57 %. The base line (historical) incidence of 57% is based on the data of a systematic analysis of CIBMTR (Center of International Blood and Marrow transplant Research) data that showed the incidence of grade II-IV acute GVHD after myeloablative MUD/MMUD all HCT to be 50-63%.[30] With complete follow-up of all patients (which is expected), a maximum of 45 patients would supply 80% power and a significance level of .05 for the null hypothesis of a GVHD rate of 57% if the true rate is 37%. We have augmented the sample size to 48 to account for patients who become non-evaluable or may lose follow-up. Non-evaluable patients are those who were registered for the study but did not receive stem cell transplant. It is expected the enrollment may be completed within 36 months.

9.2 Analysis Methods

A Simon two stage optimum design will be used. In the first stage 17 patients will be assessed. If nine or more of them experience grade II - IV GVHD the study will be stopped with the conclusion that the GVHD rate is not less than 57%. Otherwise, an additional twenty-eight patients will be accrued for a total of 45. If twenty-one or more of them experience GVHD we will conclude that the GVHD is not less than 57%. Otherwise we will conclude that the GVHD rate is less than 57%. This plan will provide a probability of .05 of concluding that the GVHD rate less than 57% if it is 57% and a probability of 80% of concluding that the rate is less than 57% if it is actually 37%. The expected sample size will be 24.8 under the null and 41.2 under the alternative hypothesis. If the null hypothesis is true and the GVHD rate is 57%, we will have a 72% probability of stopping early after 17 patients are assessed. The method of Koyama and Chen will be used to compute estimated GVHD rates and a 95% confidence interval. This method takes proper account of the Simon two stage design.

10 ADVERSE EVENT REPORTING

Adverse events occurring following study registration, but prior to beginning transplant therapy will not be reported. Protocol related therapy does not begin until patients are admitted for their bone marrow transplant. Post-transplant, regimen related toxicity / adverse events will be reported through day 100. Following day 100, patients will still be followed for relapse and overall survival.

10.1 Definitions of Adverse Events with Commercially Available Agents

This trial utilizes commercially available agents for transplant therapy for patients with hematological malignancies. Commercial agents are those agents not provided under an IND but obtained instead from a commercial source. In some cases, an agent obtained commercially may be used for indications not included in the package label. In this case, the agent is still considered to be a commercial agent and the procedures described below should be followed.

An adverse event (AE) is any untoward medical occurrence in a patient or clinical investigation subject whether or not it may have a causal relationship with this treatment. An AE includes significant exacerbation of any baseline medical condition including, but not limited to, the disease under study. Reporting requirements may include the following considerations: 1) the

characteristics of the adverse event including the grade (severity); 2) the relationship to the study therapy (attribution); and 3) the prior experience (expectedness) of the adverse event.

Expected events are those that have been previously identified as resulting from administration of the agent. For commercially available agents, an adverse event is considered unexpected, for reporting purposes only, when either the type of event or the severity of the event is not listed in:

The current NCI Agent-Specific Adverse Event List (provided in the Drug Information Section of this protocol); or
The drug package insert (for treatments with commercially available agents).

Except where otherwise specified, Common Terminology Criteria for Adverse Events (CTCAE) v.4 will be used to grade adverse events in this study.

10.2 Required Adverse Events Reporting

Therapy for hematological malignancies, with or without stem cell transplantation, is associated with significant toxicity. These toxicities are generally viewed as an anticipated consequence of therapy rather than an adverse event. To summarize, adverse events with severity grades 1, 2, 3, and all expected grade 4 toxicities will not be reported to the IRB, as they are expected in patients undergoing stem cell transplantation for hematological malignancies. Only unexpected grade 4 non-hematologic toxicity events with a possible, probable or definite relation to the study and all grade 5 events will be reported to IRB.

10.3 Hematologic Toxicity and Definition of Primary Engraftment Failure

Failure to achieve a neutrophil count > 500/μL within 35 days of the stem cell infusion will be defined as primary engraftment failure and reported as an adverse event. If primary engraftment failure occurs, an action to obtain neutrophil recovery (such as the use of growth factors or stem cell boost) will be allowed. Neutrophil count recovery will continue to be monitored in these patients until they reach statistical endpoint.

10.4 Serious Adverse Event Reporting Procedures

All serious adverse events (SAE) which require reporting must be reported immediately (i.e. within 24 hours of awareness) to the Principal Investigator or designee, followed by written documentation to the IRB from the PI (including the PI's or designee's medical summary of the SAE) within 7 days of the PI's knowledge of occurrence. A serious adverse event (SAE) is defined as**:** Any adverse event (experience) occurring that results in **ANY** of the following outcomes:

1) Death.

2) A life-threatening adverse drug experience.

3) Inpatient hospitalization or prolongation of existing hospitalization.

4) A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.

5) A congenital anomaly/birth defect.

6) Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition (FDA, 21 CFR 312.32; ICH E2A and ICH E6).

The research staff of Bone Marrow Transplant Cell Therapy (BMTCT) Program will coordinate the reporting process between the investigators and IRB as well as other applicable reporting agencies (FDA, CTEP, and NIH). Copies of all correspondence and reporting documents will be maintained in a regulatory file held by the research staff of BMTCT Program.

11 DATA SAFETY AND MONITORING PROCEDURES

11.1 Data and Safety Monitoring Procedures

The Data and Safety Monitoring Board (DSMB) of The University of Alabama at Birmingham Comprehensive Cancer Center is the DSMB for this study. This committee is responsible for the quarterly review and monitoring the study's scientific progress, accrual rate and any serious adverse events.

In addition to the Cancer Center DSMB, BMTCT Program will form a Data and Safety Monitoring Committee (DSMC) for the study. This committee will be composed of the PI, co-investigator(s), data manager, or study coordinator, and other members of the study staff involved in the conduct of the trial. During the committee's quarterly meeting, the PI will discuss matters related to:

- Enrollment rate relative to expectations, characteristics of participants
- Safety of study participants (Serious Adverse Event & Adverse Event reporting, Day 100 all-cause mortality, and incidence of engraftment failure.)
- Adherence to protocol (protocol deviations)
- Completeness, validity and integrity of study data
- Retention of study participants

These meetings are to be documented by the data manager or study coordinator using the Protocol Specific Data and Safety Monitoring Report (DSMR), signed by the PI or co- investigator. The completed DSMR is to be sent to DSMB.

Similarly, protocol deviations are to be documented using the Notice of Protocol Deviation Form and requires the signatures of either data manager or study coordinator and the PI or co-investigator.

11.2 Quality Assurance and Audits

The Quality Assurance Review Committee (QARC) of The University of Alabama at Birmingham Comprehensive Cancer Center performs quality assurance audits of investigator- initiated clinical trials. Audits provide assurance that trials are conducted in compliance with the protocol. Further, they ensure that study data are collected, documented and reported in compliance with Good Clinical Practices (GCP) Guidelines and regulatory requirements.

A QARC audit of each clinical trial is conducted annually. Audits occur within the month of the study's initial IRB approval (provided the trial is open, and study accrual is greater than five subjects).

All audit findings are reported by QARC to the DSMB. These findings are followed-up by the DSMB until they have been resolved.

The DSMB can also request QARC for a 'for cause' audit of the trial if the board identifies a need for a more rigorous evaluation of study-related issues.

A regulatory authority (e.g. FDA) may also wish to conduct an inspection of the study, during its conduct or even after its completion. If an inspection has been requested by a regulatory authority, the PI must immediately inform the IRB/DSMB that such a request has been made.

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Appendix A: Performance Scores

Karnofsky Performance Score

100% – normal, no complaints, no signs of disease
90% – capable of normal activity, few symptoms or signs of disease
80% – normal activity with some difficulty, some symptoms or signs
70% – caring for self, not capable of normal activity or work
60% – requiring some help, can take care of most personal requirements
50% – requires help often, requires frequent medical care
40% – disabled, requires special care and help
30% – severely disabled, hospital admission indicated but no risk of death
20% – very ill, urgently requiring admission, requires supportive measures or treatment
10% – moribund, rapidly progressive fatal disease processes
0% – death

Appendix B: Study Calendars

Study Calendar for Fludarabine/Busulfan Conditioning Regimen

Procedures	Screening Period Day −35 prior to Study Registration	Tx.		Follow-Up Phase										
		-5	-4	-3	-2	-1	Day 0	+3	+5	Day 30 (±7)	Day 100 (±14)	Day 180 (±21)	Day 365 (±45)	2 Years
Inclusion/ Exclusion criteria, Informed Consent, Medical and Treatment History	X													
Physical Exam, and Vital Signs	X									X	X	X	X	
Pulmonary Function Test and Echocardiogram or MUGA	X													
HIV1 and HIV2 labs and Pregnancy Test	X													
Karnofsky status[1]	X													
Bone marrow aspirate and biopsy[4], morphology and cytogenetics	X[2]									X	X		X	
Immune Reconstitution and Chimerism Studies										X	X	X	X	
CBC/Diff [5]; Liver Function Panel, , Renal Function Panel	X									X	X	X	X	
Stem cell Infusion[3]							X							
HHV6 PCR, EBV PCR, Adenovirus PCR and CMV PCR[8]							X							
Infection Prophylaxis[10]							X							
GvHD Assessments [6, 7]							X							
Mycophenolate Mofetil[9]									X					
Tacrolimus[9]									X		X			
Cyclophosphamide								X[9]						
Fludarabine[11]		X	X	X	X									
Busulfan[12]		X	X	X	X									
Filgrastim (G-CSF) [13]									X					
Assess for Disease Progression and Survival Status														X

Study Calendar for Fludarabine/Busulfan Conditioning Regimen

1. Karnofsky Performance Score will be assessed within 14 days prior to registration.
2. A unilateral bone marrow aspirate and biopsy will be performed within 28 days of registration to assess morphology and cytogenetics.
3. If stem cell infusion requires more than one day, then the first day after the last stem cell infusion will be defined as day 1.
4. A unilateral bone marrow aspirate will be collected whenever relapse is suspected. Patients that relapse will be followed for survival.
5. Post Transplant - Neutrophil engraftment is defined as the first of 3 consecutive days with an absolute neutrophil count (ANC) > 500/μL.
6. Acute GVHD will be assessed weekly (±3 days) till 100 days post-transplant.
7. Chronic GVHD will be assessed monthly (±14 days) till 1 year post transplant.
8. Starting approx. Day +20 post transplant collect and send the following. HHV6 PCR and Adenovirus PCR once a week until Day 100. EBV PCR once every 2 weeks until day 100. CMV PCR once a week until Day 100 for pts. when either the pt. or the donor is seropositive for CMV pre-transplant.
9. GVHD prophylaxis will consist of Cyclophosphamide (50mg/kg IV) starting on day +3. Mycophenolate Mofetil (15mg/kg Q8h IV) and Tacrolimus drip (dose dependent on Voriconazole use) both starting on day +5. MMF will stop by day +35. Patients will convert to oral tacrolimus when tolerated. Tacrolimus will begin taper at approximately day +100 to end by Day +100 if no signs of active GVHD.
10. Recommended infection prophylaxis: Voriconazole 200 mg PO BID starting on day 0 and continue through day 75. Acyclovir starting day 0 until at least day +365. Bactrim starting day +30 and continuing until off immunosuppression or at the discretion of the attending physician.
11. Fludarabine dosed at 40mg/m2 IV
12. Busulfan dosing will be based on pharmacokinetics.
13. G-CSF (at approx. 5mcg/kg) will begin on Day 5 and continue until engraftment.

Study Calendar for TBI / Cytoxan Conditioning Regimen

Procedures	Screening Period Day −35 prior to Study Registration	Conditioning Phase Day						Tx.	Follow-Up Phase						
		-6	-5	-4	-3	-2	-1	Day 0	+3	+5	Day 30 (±7)	Day 100 (±14)	Day 180 (±21)	Day 365 (±45)	2 Years
Inclusion/ Exclusion criteria, Informed Consent, Medical and Treatment History	X														
Physical Exam, and Vital Signs	X										X	X	X	X	
Pulmonary Function Test and Echocardiogram or MUGA	X														
HIV1 and HIV2 labs and Pregnancy Test	X														
Karnofsky status[1]	X														
Bone marrow aspirate and biopsy[4], morphology and cytogenetics	X[2]										X	X		X	
Immune Reconstitution and Chimerism Studies											X	X	X	X	
CBC/Diff[5]; Liver Function Panel, GGT, Renal Function Panel	X										X	X	X	X	
Stem cell Infusion[3]								X							
HHV6 PCR, EBV PCR, Adenovirus PCR and CMV PCR[8]								X							
Infection Prophylaxis[10]								X							
GvHD Assessments [6, 7]								X							
Mycophenolate Mofetil[9]										X					
Tacrolimus[9]										X					
Cyclophosphamide					X[11]	X[11]			X[9]						
TBI[12]		X	X	X											
Filgrastim (G-CSF) [13]										X					
Assess for Disease Progression and Survival Status															X

Study Calendar for TBI / Cytoxan Conditioning Regimen

1. Karnofsky Performance Score will be assessed within 14 days prior to registration.
2. A unilateral bone marrow aspirate and biopsy will be performed within 28 days of registration to assess morphology and cytogenetics.
3. If stem cell infusion requires more than one day, then the first day after the last stem cell infusion will be defined as day 1.
4. A unilateral bone marrow aspirate will be collected whenever relapse is suspected. Patients that relapse will be followed for survival.
5. Post-Transplant - Neutrophil engraftment is defined as the first of 3 consecutive days with an absolute neutrophil count (ANC) > 500/μL.
6. Acute GVHD will be assessed weekly (±3 days) till 100 days post-transplant.
7. Chronic GVHD will be assessed monthly (±14 days) till 1 year post transplant.
8. Starting approx. Day +20 post-transplant collect and send the following. HHV6 PCR and Adenovirus PCR once a week until Day 100. EBV PCR once every 2 weeks until day 100. CMV PCR once a week until Day 100 for pts. when either the pt. or the donor is seropositive for CMV pre-transplant.
9. GVHD prophylaxis will consist of Cyclophosphamide (50mg/kg IV) starting on day +3. Mycophenolate Mofetil (15mg/kg Q8h IV) and Tacrolimus drip (dose dependent on Voriconazole use) both starting on day +5. MMF will stop by day +35. Patients will convert to oral tacrolimus when tolerated. Tacrolimus will begin taper at approximately day +100 to end by Day +180 if no signs of active GVHD.
10. Recommended infection prophylaxis: Voriconazole 200 mg PO BID starting on day 0 and continue through day 75. Acyclovir starting day 0 until at least day +365. Bactrim starting day +30 and ending continuing until off immunosuppression or at the discretion of the attending physician.
11. Cyclophosphamide as a conditioning regimen will be administered on day-3(35mg/kg IV) and day -2 (35mg/kg IV).
12. TBI 200cGY/fraction x2 each day
13. G-CSF (at approx. 5mcg/kg) will begin on Day 5 and continue until engraftment.

Study Calendar for TBI / Fludarabine Conditioning Regimen

Procedures	Screening Period Day −35 prior to Study Registration	Conditioning Phase Day							Tx.	Follow-Up Phase						
		-7	-6	-5	-4	-3	-2	-1	Day 0	+3	+5	Day 30 (±7)	Day 100 (±14)	Day 180 (±21)	Day 365 (±45)	2 Year
Inclusion/ Exclusion criteria, Informed Consent, Medical and Treatment History	X															
Physical Exam, and Vital Signs	X											X	X	X	X	
Pulmonary Function Test and Echocardiogram or MUGA	X															
HIV1 and HIV2 labs and Pregnancy Test	X															
Karnofsky status[1]	X															
Bone marrow aspirate and biopsy[4], morphology and cytogenetics	X[2]											X	X		X	
Immune Reconstitution and Chimerism Studies												X	X	X	X	
CBC/Diff[5]; Liver Function Panel, GGT, Renal Function Panel	X											X	X	X	X	
Stem cell Infusion[3]									X							
HHV6 PCR, EBV PCR, Adenovirus PCR and CMV PCR[8]									X							
Infection Prophylaxis[10]									X							
GvHD Assessments [6, 7]									X							
Mycophenolate Mofetil[9]											X					
Tacrolimus[9]											X		X			
Cyclophosphamide										X[9]						
Fudarabine[11]		X	X	X	X											
TBI[12]						X-2	X-2	X-1								
Filgrastim (G-CSF) [13]											X					
Assess for Disease Progression and Survival Status																X

Study Calendar for TBI / Fludarabine Conditioning Regimen

1. Karnofsky Performance Score will be assessed within 14 days prior to registration.
2. A unilateral bone marrow aspirate and biopsy will be performed within 28 days of registration to assess morphology and cytogenetics.
3. If stem cell infusion requires more than one day, then the first day after the last stem cell infusion will be defined as day 1.
4. A unilateral bone marrow aspirate will be collected whenever relapse is suspected. Patients that relapse will be followed for survival.
5. Post-Transplant - Neutrophil engraftment is defined as the first of 3 consecutive days with an absolute neutrophil count (ANC) > 500/μL.
6. Acute GVHD will be assessed weekly (±3 days) till 100 days post-transplant.
7. Chronic GVHD will be assessed monthly (±14 days) till 1 year post transplant.
8. Starting approx. Day +20 post-transplant collect and send the following. HHV6 PCR and Adenovirus PCR once a week until Day 100. EBV PCR once every 2 weeks until day 100. CMV PCR once a week until Day 100 for pts. when either the pt. or the donor is seropositive for CMV pre-transplant.
9. GVHD prophylaxis will consist of Cyclophosphamide (50mg/kg IV) starting on day +3. Mycophenolate Mofetil (15mg/kg Q8h IV) and Tacrolimus drip (dose dependent on Voriconazole use) both starting on day +5. MMF will stop by day +35. Patients will convert to oral tacrolimus when tolerated. Tacrolimus will begin taper at approximately day +100 to end by Day +180 if no signs of active GVHD.
10. Recommended infection prophylaxis: Voriconazole 200 mg PO BID starting on day 0 and continue through day 75. Acyclovir starting day 0 until at least day +365. Bactrim starting day +30 and ending continuing until off immunosuppression or at the discretion of the attending physician.
11. Fludarabine dose 40mg/m^2/day.
12. TBI 200cGy/fraction x 2 on days -3 and -2. TBI 200cGy/fraction x1 on day -1.
13. G-CSF (at approx. 5mcg/kg) will begin on Day 5 and continue until engraftment.

Study Calendar for Fludarabine / Melphalan Conditioning Regimen

Procedures	Screening Period Day −35 prior to Study Registration	Conditioning Phase Day						Tx.		Follow-Up Phase				
		-5	-4	-3	-2	-1	Day 0	+3	+5	Day 30 (±7)	Day 100 (±14)	Day 180 (±21)	Day 365 (±45)	2 Years
Inclusion/ Exclusion criteria, Informed Consent, Medical and Treatment History	X													
Physical Exam, and Vital Signs	X									X	X	X	X	
Pulmonary Function Test and Echocardiogram or MUGA	X													
HIV1 and HIV2 labs and Pregnancy Test	X													
Karnofsky status[1]	X													
Bone marrow aspirate and biopsy[4], morphology and cytogenetics	X[2]									X	X		X	
Immune Reconstitution and Chimerism Studies										X	X	X	X	
CBC/Diff [5]; Liver Function Panel, , Renal Function Panel	X									X	X	X	X	
Stem cell Infusion[3]							X							
HHV6 PCR, EBV PCR, Adenovirus PCR and CMV PCR[8]							X							
Infection Prophylaxis[10]							X							
GvHD Assessments [6, 7]							X							
Mycophenolate Mofetil[9]									X					
Tacrolimus[9]									X					
Cyclophosphamide								X[9]						
Fludarabine[11]		X	X	X	X									
Melphalan[12]						X								
Filgrastim (G-CSF) [13]									X					
Assess for Disease Progression and Survival Status														X

Study Calendar for Fludarabine/Melphalan Conditioning Regimen

1. Karnofsky Performance Score will be assessed within 14 days prior to registration.
2. A unilateral bone marrow aspirate and biopsy will be performed within 28 days of registration to assess morphology and cytogenetics.
3. If stem cell infusion requires more than one day, then the first day after the last stem cell infusion will be defined as day 1.
4. A unilateral bone marrow aspirate will be collected whenever relapse is suspected. Patients that relapse will be followed for survival.
5. Post-Transplant - Neutrophil engraftment is defined as the first of 3 consecutive days with an absolute neutrophil count (ANC) > 500/μL.
6. Acute GVHD will be assessed weekly (±3 days) till 100 days post-transplant.
7. Chronic GVHD will be assessed monthly (±14 days) till 1 year post transplant.
8. Starting approx. Day +20 post-transplant collect and send the following. HHV6 PCR and Adenovirus PCR once a week until Day 100. EBV PCR once every 2 weeks until day 100. CMV PCR once a week until Day 100 for pts. when either the pt. or the donor is seropositive for CMV pre-transplant.
9. GVHD prophylaxis will consist of Cyclophosphamide (50mg/kg IV) starting on day +3. Mycophenolate Mofetil (15mg/kg Q8h IV) and Tacrolimus drip (dose dependent on Voriconazole use) both starting on day +5. MMF will stop by day +35. Patients will convert to oral tacrolimus when tolerated. Tacrolimus will begin taper at approximately day +100 to end by Day +180 if no signs of active GVHD.
10. Recommended infection prophylaxis: Voriconazole 200 mg PO BID starting on day 0 and continue through day 75. Acyclovir starting day 0 until at least day +365. Bactrim starting day +30 and ending continuing until off immunosuppression or at the discretion of the attending physician.
11. Fludarabine dose 40mg/m^2/day.
12. Melphalan dose 140mg/m2 on day -1 .
13. G-CSF (at approx. 5mcg/kg) will begin on Day 5 and continue until engraftment.

Appendix C: Grading of Acute Graft versus Host Disease

(Per modified Glucksberg criteria = Keystone criteria)[26]

Stages of organ involvement		
Skin	1	Maculopapular eruption involving less than 25% of the body surface
	2	Maculopapular eruption involving 25-50% of the body surface
	3	Generalized erythroderma
	4	Generalized erythroderma with bullous formation.
GI	1	> 500 ml of liquid stool/day **or** biopsy-proven upper GI involvement
	2	> 1,000 ml of stool/day
	3	> 1,500 ml of stool/day
	4	Severe abdominal pain with or without ileus
Liver	1	Bilirubin is 2 -2.9 mg/dl
	2	Bilirubin is 3 - 5.9 mg/d1
	3	Bilirubin is 6 - 14.9 mg/dl
	4	Bilirubin is > 15 mg/dl

Overall grading of acute GVHD by organ staging.			
	Skin	**GI**	**Liver**
I	1-2	None	None
II	3 or	1 or	1
III	---	2-4	2-3
IV*	4	---	4

*If Karnofsky performance status < 30%, then grade IV.

Appendix D: Grading of Chronic Graft versus Host Disease

Pt. Name:_____ **MR#**_____ ☐ **Inpt** ☐ **Outpt**

Transplant Date: ____/_____/_____ **Research Study:** *(if any)* _____
☐ **MRD** ☐ **MUD** ☐ **PBSC** ☐ **Marrow**

Date of last GvHD Assessment: _____/____/_____ **Has Chronic GVHD Occurred this period?** ☐ **Yes** ☐ **No**
Date of GvHD Assessment: _____/_____/_____ **If yes, Date of onset:** _____ **Site:**_____

Has Chronic GVHD Resolved this period ☐ **Yes** ⇨ **Date :** _____ ☐ **No** ⇨ **Continue Form**

Was a specific therapy used to treat or prevent cGVHD ? ☐ **Yes** ☐ **No** ⇨ **Skip to Clinical Assessments**
☐ **Prophylaxis -** Prophylaxis is defined as standard preventive immunosuppression ; still considered prophylaxis if continued after GVHD diagnosis
☐ **Treatment -** Treatment is defined as local or systemic therapy started with the onset of signs and symptoms of GVHD
Treatment Started: ___/____/_____ **Was Treatment Dose Increased?** ☐ **No** ☐ **Yes** ⇨ **Date:** _____
Tapering Therapy ? ☐ **No** ☐ **Yes** ⇨ ☐ **Taper Started on:**_____ ☐ **Ongoing or Stop Date:**_____

- ☐ **CSA (Cyclosporin)**
- ☐ **FK 506 (Tacrolimus, Prograf)**
- ☐ **ECP (extra-coporeal photopheresis)**
- ☐ **MMF (Mycophenolate Mofetil , Cellcept)**
- ☐ **Corticosteroids (Systemic)**
- ☐ **MTX (Methotrexate)**
- ☐ **PUVA**
- ☐ **Other** _____

Assessments

Onset of cGVHD was:
☐ **Progressive** (acute progressed directly to chronic)
☐ **Interrupted** (acute resolved; then chronic developed)
☐ **de novo** (never developed acute GVHD)
☐ **Chronic Flare** (symptoms reactivated within 30 days of drug tapering or discontinuation)

Stage of cGVHD:
☐ **Limited:** Localized skin involvement resembling localized scleroderma with or without liver involvement; no other organ involvement.
☐ **Extensive:** Generalized skin and/or multiple organ involvement. – generalized skin involvement; or, – liver histology showing chronic aggressive hepatitis, bridging necrosis or cirrhosis; or, – involvement of eye: Schirmer's test with < 5 mm wetting; or – involvement of minor salivary glands or oral mucosa demonstrated on labial biopsy; or – involvement of any other target organ

Overall Severity of cGVHD
☐ **Mild** – signs and symptoms of cGVHD do not interfere substantially with function and do not progress once appropriately treated with local therapy or standard systemic therapy (first line therapy)**.**
☐ **Moderate –** signs and symptoms of cGVHD interfere somewhat with function despite appropriate therapy **or** are progressive through first line systemic therapy.
☐ **Severe –** signs and symptoms of cGVHD limit function substantially despite appropriate therapy **or** are progressive through second line therapy.

KPS: 100 90 80 70 60 50 40 30 20 10 0
Normal→ → → → → → → → → → Death

Biopsy Performed: ☐ **No** ☐ **Yes** ⇨	**Tissue:**	**Date:**	**Results:** ☐ **Positive** ☐ **Negative**

_____
Clinical Signature Date