NORTHSIDE HOSPITAL

A PHASE II TRIAL OF REDUCED INTENSITY CONDITIONING AND TRANSPLANTATION OF PARTIALLY HLA-MISMATCHED PERIPHERAL BLOOD STEM CELLS FOR PATIENTS WITH HEMATOLOGIC MALIGNANCIES

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TABLE OF CONTENTS

Treatment Schema

Days –6 → –2 Fludarabine 30 mg/M^2 IV qd x 5 days (day -6 to -2)

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Day –1 Melphalan 140 mg/M^2 IV x 1 dose (day -1)

↓

Day 0 Infuse unmanipulated PBSCs
(Begin antibiotic prophylaxis)

↓

Days 3, 4 Cyclophosphamide 50 mg/kg IV q d
(hydration/Mesna)

(First dose of Cyclophosphamide must be administered 48-72 hr after infusion of PBSCs)

↓

Day 5 Begin Tacrolimus (target plasma level 5-15)
MMF 15 mg/kg po tid with maximum daily dose 3 gm/d

↓

Day 35 Discontinue MMF

↓

Day 180 Discontinue Tacrolimus

1. OBJECTIVES:

Primary Objectives:

1.1. To estimate the incidence of graft rejection following reduced intensity, human leukocyte antigen (HLA)-mismatched hematopoietic stem cell transplantation (HSCT) in patients with high risk hematologic malignancies.

Secondary Objectives:

1.2. To obtain estimates of overall survival (OS), relapse, graft versus host disease (GVHD), non-relapse mortality (NRM), and event-free survival (EFS) in patients receiving reduced intensity conditioning and transplantation of partially human leukocyte antigen (HLA)-mismatched peripheral blood stem cells (PBSC) from first-degree relatives.

1.3. Characterize additional hematologic and non-hematologic toxicities of reduced intensity haploidentical HSCT.

1.4. Identify the incidence of BK virus cystitis in patients receiving reduced intensity conditioning and transplantation of partially human leukocyte antigen (HLA)-mismatched peripheral blood stem cells (PBSC) from first-degree relatives.

2. BACKGROUND

Allogeneic HSCT is considered a curative modality for many patients with high risk hematologic malignancies. Transplantation using a matched related sibling if available leads to improved outcomes when compared to other graft sources[1]. Unfortunately, only about a third of candidates for HSCT have HLA-matched siblings. For patients who lack HLA-matched siblings, there are other alternative sources of stem cells for HSCT: 1) volunteer unrelated donors; 2) umbilical cord blood; and 3) partially HLA-mismatched, or haploidentical, related donors. Haploidentical HSCT has been historically associated with significant risks of graft rejection and severe GVHD[2], which are manifestations of excessive alloreactivity by host and donor T cells[3], respectively. Earlier study of T cell depletion in the setting of haploidentical HSCT were associated with prolonged immune compromise and increase risk of mortality from infections[4]. Recently, the use of cyclophosphamide (Cy) post infusion of stem cells has become a new standard to reduce the incidence of GVHD without significantly increasing the risk of infections seen with T cell depleted haploidentical transplantation[5].

Post-transplant Cy (PTCY) was studied by the group from Johns Hopkins in a non-ablative conditioning regimen of non-T cell-depleted marrow from haploidentical first degree relatives[6]. This study showed that optimally dosed and timed PTCY, after a conditioning regimen of fludarabine and low dose total body irradiation had an acceptably low risk of graft rejection and GVHD, the two major complications of haploidentical HSCT. All patients received mycophenolate mofetil (MMF) and tacrolimus, beginning 24 hours after PTCY administration for GVHD prophylaxis. The median times to neutrophil and platelet recovery for all patients were 15 and 24 days, respectively. Graft failure occurred in a total of 15/84 patients (18%). All but two patients with graft failure experienced recovery of autologous hematopoiesis with median times to neutrophil and platelet engraftment of 24 days (range 11-48 days) and 44 days (range 15-395 days), respectively.

The cumulative incidences of grades II-IV and III-IV acute GVHD were 35% and 10%. The cumulative incidence of chronic and extensive chronic GVHD in the first year after transplantation for the entire population was 22% and 14%, respectively. The cumulative incidences of NRM at 180 days and 1 year after transplantation were 13% and 19%, respectively. The cumulative incidences of relapse at 1 and 2 years after transplantation were 50% and 57%, respectively. At a median follow-up of survivors of 817 days (range, 112-1808 days), the actuarial overall survival at 1 and 2 years were 45% and 35%, respectively.

From these results, it was concluded that a combination of PTCY, tacrolimus, and MMF were associated with an acceptably low incidence of graft rejection, severe acute GVHD, and extensive chronic GVHD. There was effective clinical immune reconstitution as demonstrated by the low incidence of severe opportunistic infections. Relapse represented the major cause of treatment failure in this poor risk patient population. One explanation for the high rate of relapse, as in other nonmyeloablative HSCT trials, is that the transplantation conditioning was not intense enough to achieve sufficient tumor cytoreduction.

Given that relapse remains the largest impediment to success after nonmyeloablative bone marrow transplantation utilizing PTCY, efforts to reduce relapse remain an important area of clinical investigation. We have recently published 2 studies on the use of myeloablative conditioning with PTCY. The first study with busulfan based conditioning established that myeloablative haploidentical transplantation can yield full donor chimerism at day 30, grade III-IV acute GVHD rate of 10%, and a 1 year OS of 69% and DFS of 50%[7]. The second myeloablative study with total body irradiation (TBI) based conditioning showed full donor chimerism at day 30, and a 2 year DFS of 73%[8]. The limitation of myeloablative regimens is that they cannot be safely administered for elderly patients or patients with poor organ function, increased comorbidities and poor performance status. The need of a more intense regimen than the non-ablative (Fludarabine/low dose TBI) but less intensive than the full myeloablative

conditioning regimens in an effort to decrease relapse among HSCT patients remains an area of clinical investigation.

This trial will evaluate the safety and efficacy of a reduced intensity allogeneic HSCT from partially HLA-mismatched first-degree relatives utilizing PBSC as the stem cell source. The primary objective of the study is to estimate the incidence of graft rejection and acute GVHD. A secondary objective will be to estimate the incidence of the relapse, NRM, OS, chronic GVHD and EFS.

3. Drug Information

3.1 Cyclophosphamide (Cytoxan®)

Cyclophosphamide is an alkylating agent which prevents cell division primarily by cross-linking DNA strands. Cyclophosphamide is cell cycle nonspecific. Cyclophosphamide for injection is available in 2000 mg vials which are reconstituted with 100 ml sterile water for injection. The concentration of the reconstituted product is 20 mg/ml. The calculated dose will be diluted further in 250-500 ml of Dextrose 5% in water. Each dose will be infused over 1-2 hr (depending on the total volume). Clinical toxicities of cyclophosphamide include alopecia, nausea and vomiting, headache and dizziness, hemorrhagic cystitis, cardiotoxicity, immunosuppression, myelosuppression, pulmonary fibrosis, increased hepatic enzymes and syndrome of inappropriate anti-diuretic hormone (SIADH).

3.2 Mesna (sodium-2-mercapto ethane sulphonate)

Mesna is a prophylactic agent used to prevent hemorrhagic cystitis induced by the oxasophosphorines (cyclophosphamide and ifosphamide). It has no intrinsic cytotoxicity and no antagonistic effects on chemotherapy. Mesna binds with acrolein, the urotoxic metabolite produced by the oxasophosphorines, to produce a non-toxic thioether and slows the rate of acrolein formation by combining with 4-hydroxy metabolites of oxasophosphorines. Mesna is available in 200 mg, 400 mg and 1000 mg vials containing a 100 mg/ml solution. Each dose of mesna will be diluted further in 50 ml of normal saline to be infused over 15 min or as per institutional standards. Mesna dose will be based on the cyclophosphamide dose being given. The total daily dose of mesna is equal to 80% of the total daily dose of cyclophosphamide. At the doses used for uroprotection mesna is virtually non-toxic. However, adverse effects which may be attributable to mesna include nausea and vomiting, diarrhea, abdominal pain, altered taste, rash, urticaria, headache, joint or limb pain, hypotension and fatigue. Mesna will be dispensed by the Oncology Pharmacy and is produced by Mead Johnson Pharmaceuticals.

3.3 Melphalan

Melphalan is an alkylating agent that is commonly used in stem cell transplant due to its significant myelosuppressive effects. Melphalan will be infused as a single dose on day -1 prior to infusion of stem cells. Even with the use of hematopoietic growth factors, significant neutropenia is expected. Neutropenic infections and/or need for transfusions of red cells and/or platelets may result. Stomatitis, esophagitis and severe diarrhea are common with high dose melphalan: management with i.v. narcotics and potentially i.v. alimentation may be needed. Other less common toxicities reported include pulmonary fibrosis and interstitial pneumonitis, skin hypersensitivity, vasculitis, alopecia, hemolytic anemia and allergic reaction.

3.4 Fludarabine (Fludara®)

Fludarabine phosphate is purine antimetabolite that, after administration, undergoes rapid conversion in plasma to the nucleoside 2-fluoro ara-A (F-araA). F-araA subsequently enters cells where it is phosphorylated to F-araATP and the monophosphate F-araAMP. Once activated, F-araATP inhibits DNA polymerase and ribonucleotide reductase. The monophosphate F-araAMP, once incorporated into DNA, is an effective DNA chain terminator. Fludarabine monophosphate, 50 mg/vial, is reconstituted with 2 ml of sterile water, resulting in a 25mg/ml solution. The desired dose is further diluted to concentrations of 0.04-1 mg/ml in normal saline or 5% dextrose (50-100ml) for injection and will be administered by IV infusion over 30 minutes or longer. Following IV administration, the drug is metabolized to 2-F-araA and widely distributed in tissues. 2-F-araA is excreted primarily in urine and has a terminal elimination half-life of 7 to 12 hours. Clinical toxicities of fludarabine monophosphate include: myelosuppression, primarily lymphopenia and granulocytopenia, alopecia, rash, dermatitis, nausea, vomiting, anorexia, stomatitis, diarrhea, somnolence, fatigue, peripheral neuropathy, mental status changes, cortical blindness, hepatocellular toxicity with elevation in serum transaminases, and interstitial pneumonitis. These effects are reversible when the drug is discontinued. Fludarabine will be administered by IV infusion over 30 minutes in a dose of 40 mg/m^2/day on days -5 to -2. Fludara® will be dispensed by the Oncology Pharmacy and is produced by Berlex Pharmaceuticals.

3.5 Tacrolimus

Tacrolimus, also known as FK-506, is a macrolide immunosuppressant. It inhibits lymphocytes by forming a complex with FKBP-12, calcium, and calmodulin, leading to the decrease in the phosphatase activity of calcineurin. This drug is used with corticosteroids for prophylaxis of organ rejection in patients receiving allogeneic liver transplants. Its use is also currently being investigated in kidney, bone marrow, cardiac, pancreas, pancreatic islet cell and small bowel transplantation. This drug is well-absorbed orally. It is metabolized in the liver by unknown

mechanisms, but demethylation and hydroxylation have been proposed based on *in vitro* studies. The metabolized products are excreted in the urine. Nephrotoxic drugs, antifungals, calcium channel blockers, cimetidine, danazol, erythromycin, methylprednisone and metoclopramide increase the bioavailabilty of FK-506. In contrast, phenobarbital, phenytoin, rifamycins and carbamazepine decrease FK-506 levels. Adverse reactions include tremor, headache, diarrhea, hypertension, nausea, and renal dysfunction.

3.6 Mycophenolic Acid Mofetil (Cellcept®)

Mycophenolate Mofetil is an ester prodrug of the active immunosuppressant mycophenolic acid (MPA). This active metabolite is a noncompetitive, reversible inhibitor of inosine monophosphate dehydrogenase (IMPDH). There are no pharmacokinetic interactions with ganciclovir, cotrimoxazole, oral contraceptives and cyclosporine. Side effect profiles include diarrhea, leukopenia, sepsis, allergic reactions, and vomiting. There is also an increase in certain types of infection mainly from the herpes virus family (CMV, HSV & VZV) and candida.

3.7 Hematopoietic growth factor: G-CSF

G-CSF may be associated with development of fevers, chills, skin rash, polyserositis, muscle aches, malaise and/or headache. It has also been reported to cause bone aches or pain in a minority of patients

4. PATIENT SELECTION

4.1 Criteria for recipient eligibility

4.1.1 No available matched related or unrelated donor, OR a matched related or unrelated donor will not be available in the time frame necessary to perform a potentially curative transplant.

4.1.2 Availability of a 3/6 – 5/6 matched (HLA-A, B, DR) related donor

- Donor must have negative HLA cross-match in the host vs. graft direction.

4.1.3 Age ≤75 years

4.1.4 Karnofsky status ≥70%

4.1.5 One of the following high-risk malignancies:

4.1.5.1 Chronic Myelogenous Leukemia

- Chronic myelogenous leukemia in chronic phase, resistant or intolerant to available tyrosine kinase inhibitors (OR)
- Chronic myelogenous leukemia in accelerated phase (OR)
- Chronic myelogenous leukemia with blast crisis that has entered into a second chronic phase following induction chemotherapy.

4.1.5.2 Acute Myelogenous Leukemia in first or greater remission

4.1.5.3 Myelodysplastic Syndrome at least one of the following:

- Treatment-related
- Monosomy 7, complex cytogenetics or other high risk karyotype
- IPSS score of 1.0 or greater
- Neutropenia or cytopenia requiring transfusion not responding to therapy
- Peripheral or BM blast count of <10%
- Chronic myelomonocytic leukemia (CMML)

4.1.5.4 Acute lymphocytic leukemia/lymphoblastic lymphoma

- 2nd or subsequent complete remission (OR)
- First complete remission:
  Acute lymphocytic leukemia with poor-risk features: including but not limited to [t (9; 22) or bcr-abl fusion, t(4;11) or other MLL translocation] or hypodiploidy; WBC >20,000/µl at diagnosis, no CR by day 28 of initial induction therapy, extramedullary leukemia or age >30 at diagnosis
- Marrow blasts <5%, but persistence of minimal residual disease by flow cytometry, cytogenetics or FISH

4.1.5.5 Chronic Lymphocytic Leukemia / Prolymphocytic Leukemia

- Previously treated disease that has either relapsed or failed to respond adequately to conventional-dose therapy including purine analogs

4.1.5.6 Hodgkin's or Non-Hodgkin's Lymphoma (including low-grade, mantle cell, and intermediate-grade/diffuse)

- Previously treated disease that has either relapsed or failed to respond adequately to conventional-dose therapy or autologous transplantation

4.1.5.7 Myeloproliferative diseases (myelofibrosis, CMML)

4.1.5.8 Multiple Myeloma with relapse after a prior autologous transplant or eligible for allogeneic HSCT based on other risk factors

4.2 Exclusion Criteria for recipient

4.2.1 Patients will not be excluded on the basis of sex, racial or ethnic background.

4.2.2 Poor cardiac function: left ventricular ejection fraction <40%

4.2.3 Poor pulmonary function: FEV_1 and FVC <50% predicted

4.2.4 Poor liver function: bilirubin $\geq$2 mg/dl (not due to hemolysis, Gilbert's or primary malignancy)

4.2.5 Poor renal function: Creatinine $\geq$2.0mg/dl or creatinine clearance (calculated creatinine clearance is permitted) < 40 mL/min based on Traditional Cockcroft-Gault formula: 140 - age (yrs) x Smaller of Actual Weight vs. Ideal Body Weight (kg) / 72 x Serum creatinine (mg/dl)

4.2.6 HIV-positive

4.2.8 Women of childbearing potential who currently are pregnant or who are not practicing adequate contraception

4.2.9 Patients who have any debilitating medical or psychiatric illness which would preclude their giving informed consent or their receiving optimal treatment and follow-up.

4.3 Criteria for donor selection

4.3.1 Donors will be relatives (parent, child, sibling) of the recipient and be matched at 3/6 – 5/6 loci (HLA-A, B, DR) with the recipient.

4.3.2 Donors will be selected to avoid a positive HLA crossmatch in the host-versus-graft (HVG) direction or high titer donor specific antibodies as determined by the pre-transplant panel reactive antibody (PRA) testing.

5. Treatment Plan

Days –6→ –2 Fludarabine 30 mg/M^2 IV qd x 5 days (day -6 to -2)

↓

Days –1 Melphalan 140 mg/M^2 IV qd x 1 dose (day -1)

↓

Day 0 Infuse unmanipulated PBSCs
(Begin antibiotic prophylaxis)

↓

Days 3, 4 Cyclophosphamide 50 mg/kg IV q d
(hydration/Mesna)

(First dose of Cyclophosphamide must be administered 48-72 hr after infusion of PBSCs)

↓

Day 5 Begin Tacrolimus (target plasma level 5-15)
MMF 15 mg/kg po tid with maximum daily dose 3 gm/d

↓

Day 35 Discontinue MMF

↓

Day 180 Discontinue Tacrolimus

5.1 Peripheral Blood Stem Cell Collection

Peripheral blood stem cells will be collected by institutional guidelines following standard G-CSF-based mobilization and infused on day 0. Cryopreservation of the stem cell product prior to infusion is permissible.

5.2 Stem Cell Processing

Minor ABO incompatible marrow or stem cell grafts will be plasma reduced and washed. Major ABO incompatible stem cell products will not require manipulation.

5.3 Preparative Regimen

5.3.1 **Fludarabine** 30 mg/m^2 infused over 30 minutes once daily on five consecutive days (total dose, 150 mg/m^2): days -6, -5, -4, -3 and -2.

5.3.2 **Melphalan** 140 mg/m^2 infused on day -1.

5.3.3 **Veno-occlusive disease (VOD**) prophylaxis will include:

- Ursodiol 300mg PO bid starting 24 hours prior to starting the preparative regimen until day +30 post-transplant

5.3.4 **Stem Cell Infusion** (Day of Transplant) day 0.

5.4 Post-transplant Cyclophosphamide

5.4.1 **Cyclophosphamide** 50mg/kg will be given on D+3 post-transplant (within 48-72 hr of PBSC infusion) and on D+4 post-transplant. Cyclophosphamide will be given as an IV infusion over 1**-** 2 hr (depending on volume). Hydration and Mesna will be given according the institution's standard of care.

5.5 Chemotherapy Dosing: All chemotherapy should be dosed based on the lesser of actual or ideal body weight for patients who weigh less than or equal to 130% of their IBW. For patients who weigh more than 130% of their IBW, dosing should be based on the adjusted ideal body weight (AIBW).

1. Ideal Body Weight (IBW) Formulas:
   Males IBW = 50 kg + 2.3 kg/inch over 5 feet
   Females IBW = 45.5 + 2.3 kg/inch over 5 feet
   For patients less than 5 feet, subtract 2.3 kg/inch
2. Adjusted Ideal Body Weight (AIBW) Formula:

$$AIBW = IBW + [(0.25) \times (ABW - IBW)]$$

Doses of Fludarabine and Cyclophosphamide will be adjusted as needed according to creatinine clearance:

$$\text{Creatinine Clearance} = \frac{(140\text{-Age}) \times IBW\ (\times 0.85 \text{ for females})}{72 \times \text{Serum Creatinine}}$$

CHEMOTHERAPEUTIC AGENT	**Calculated Creatinine Clearance 51-60**	**Calculated Creatinine Clearance 30-50**
Fludarabine	80% of total dose	80% of total dose
Cyclophosphamide	100% of total dose	75% of total dose

5.6 Antibiotic prophylaxis and other supportive care measures will be implemented according to institutional guidelines.

5.7 Post-transplant immunosuppression:

5.7.1 No immunosuppressive agents are given until 24 hours after the completion of PTCY. This includes steroids as anti-emetics

5.7.2 Tacrolimus 0.03mg/kg/day infuse over 24 hours starting on day+5 (adjusted to maintain a trough level of 5-15 ng/ml) and switched to oral (twice-daily divided dose) on day 21 or when able to tolerate p.o. Tacrolimus will be discontinued on day +180, in the absence of clinically significant GVHD.

5.7.3 Mycophenolate Mofetil (MMF) 15 mg/kg po tid with maximum daily dose 3 gm/d, starting on day+5. MMF will be discontinued on day +35 in the absence of clinically significant GVHD.

5.8 Chimerism testing

5.8.1 Quantitation of chimerism: PCR-based analysis of DNA to detect short tandem repeats (STRs) will be used for quantitative chimerism of post-transplant peripheral blood T-lymphocytes and myeloid cells. The level of chimerism in each of these populations is represented by the percentage of the cells that are of donor origin. Chimerism will be assessed beginning at day +30. If full donor chimerism is reached by day +30, it will be assessed per institutional guidelines for restaging. If full donor chimerism is not reached by day +30, additional assessments will be done at day +60, +90 and then every four weeks (+/- 3 days) until the goal of full donor chimerism is achieved. For the purposes of this protocol *mixed chimerism* will be defined as the detection of > 5% and < 95% cells of donor origin and *full donor chimerism* will be defined as ≥95% cells of donor origin.

5.9 Graft-versus-host disease will be evaluated, graded and managed by current BMT of Georgia GVHD standards found in the BMT of Georgia Supportive Care Manual.

5.10 Restaging evaluations will occur per institutional standards at day +100, at 6 months, 12 months, 18 months, 24 months, and 36 months.

5.11 Treatment failure:

5.11.1 Treatment failure is defined as (1) patients that develop disease relapse and progression in spite of grade II-IV acute GVHD or extensive chronic GVHD, (2) patients with graft failure, or (3) patients with impending graft rejection as defined by low (<20%) or dropping T cell chimerism.

5.11.2 Such patients will be considered a treatment failure and receive any additional treatment off-study according to physician discretion.

6. Adverse Event /Serious Adverse Event Reporting

6.1 Patients will be monitored by the investigators and data maintained by the Clinical Research Program. All Adverse Events will be graded with the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. Adverse events will be monitored for a period of three (3) months post HSCT (approximately D100 restaging) and for study purposes will be defined as

- Grade 2, 3 and 4 **unexpected** reactions that are deemed **related** to study.

6.2 Serious adverse events will be reported by telephone or email to the Institutional Review Board within 24 hours of notification. Written report will follow within 10 working days. Serious adverse events will be reported for a period of six (6) months post HSCT (180 days)

6.2.1 Procedure for reporting Serious and Unexpected Adverse Events

Serious Adverse Events will be reported by telephone or email to the Institutional Review Board within 24 hours of notification of event. Written report will follow within 10 working days. Serious adverse events will be reported for a period of 6 months post-transplant.

Routine hospital admissions for expected side effects/complications of stem cell transplant such as fever, infection, mucositis or GVHD will not be reported to the IRB but will be recorded in the BMT database. Only hospitalizations that are for unexpected side effects of the transplant procedure will be reported to IRB as serious adverse events.

6.2.2 Definition of Serious and Unexpected Adverse Events:

Serious Adverse Events are defined as one of the following:

a. Death

b. Life-Threatening

c. Hospitalization (initial or prolonged)

d. Disability

e. Congenital Anomaly

f. Requires Intervention to Prevent Permanent Impairment or Damage

Unexpected is defined as:
Not previously reported with the agents/devices or procedures being undertaken.
Symptomatically and pathophysiologically related to known toxicity but differs because of greater severity than previously reported.

7. Risks and Toxicities

7.1 Cyclophosphamide after graft infusion

The major risk of participating in this research protocol is that shifting part of the standard HSCT dose of cyclophosphamide after the graft infusion may damage the graft. The

consequences of damaging the graft may include delayed hematologic recovery, graft failure, or treatment-related malignancy in donor cells. The risk of delayed hematologic recovery does not appear to be severe, because patients who have engrafted with donor cells in the setting of a nonmyeloablative preparative regimen incorporating the same post-HSCT dose of cyclophosphamide experienced only approximately two weeks of neutropenia. This protocol is more intense than the non-ablative regimens and if patients fail to engraft, this can be fatal . However, we expect this to be a rare complication for several reasons. First, hematopoietic stem cells express high levels of aldehyde dehydrogenase that detoxifies the active metabolite of cyclophosphamide, 4 hydroxycyclophospamide. Second, patients with acute hematologic malignancies who received a much less intensive preparative regimen that incorporated the same dose of cyclophosphamide after HSCT all engrafted, and intensification of the preparative regimen reduces the likelihood of graft rejection. The risk of treatment-related malignancy in donor cells is difficult to estimate, but is likely to be similar to the 1% risk estimated after limited exposure to cyclophosphamide.

7.2 Acute and Chronic GVHD

The second major risk in participating in this research protocol is the risk of developing acute and/or chronic GVHD given the use of a reduced intensity preparative regimen followed by the infusion of PBSC from haploidentical donors. The degree of GVHD varies from mild cutaneous reactions to extensive widespread and systemic involvement of skin, liver, and gastrointestinal tract. The incidence of fatal infection is greater in patients developing GVHD due to the immunosuppressive nature of GVHD and its associated treatments. The development of grade 3 or higher acute GVHD is considered clinically significant and associated with increased morbidity and non-relapse mortality. The likelihood of surviving severe GVHD is to a large part dependent on the age of the patient and the patient's overall condition. For the majority of the patients eligible for this trial who have high-risk hematologic malignancies, a moderate increase in GVHD may be accompanied by an increased graft-versus-malignancy (GVM) benefit, so that the same long-term relapse free survival is maintained.

7.3 Regimen-related toxicities

Toxicities directly related to the administration of high-dose chemotherapy include gastrointestinal toxicity (nausea, vomiting, mucositis), alopecia, infertility (which may be permanent), interstitial pneumonitis, idiopathic cardiomyopathy, hemorrhagic cystitis, hepatic venoocclusive disease, or multi-organ failure which may be fatal.
Toxicities related to individual chemotherapy drugs are described in section 3.0.

7.4 Infection

Infection is a major cause of morbidity in allo HSCT and is a major concern in these patients. Infections may be bacterial, viral, parasitic, or fungal. Often, these infections are life-threatening, particularly when caused by viral or fungal organisms, and are associated with high mortality in the transplant population.

7.5 Aplasia/Graft Failure

Pancytopenia is an expected side effect of allogeneic HSCT with the use of reduced intensity preparative regimens. Given the previous experience with post-transplant cyclophosphamide in other trials, we would expect the duration of aplasia to be relatively short. Prolonged aplasia can result from the failure of donor PBSC to engraft and is usually fatal.

8. STUDY PARAMETERS

8.1 Non-relapse mortality

Non-relapse mortality, which is defined as death in the absence of relapse or progression, will be characterized at 100 days and at one year after HSCT.

8.2 Relapse

The secondary objective of this protocol is to characterize overall and progression free survival following reduced intensity conditioning and transplantation of PBSCs from partially HLA-mismatched related donors. High clinical suspicion of relapse will most likely lead to a disease-specific evaluation. Cytogenetic studies or decreasing donor chimerism also increase the suspicion of relapse as well and can sometimes detect asymptomatic relapse in routine protocol-related marrow samples. If there is evidence of early relapse and no evidence of GVHD following HSCT, immunosuppressive therapy may be discontinued earlier than indicated in Section 5.7. If there is evidence of low donor chimerism and no evidence of GVHD, patients may be eligible for subsequent donor lymphocyte infusions.

8.3 Hematologic recovery

A secondary endpoint of this Phase II trial is time to recovery of circulating neutrophils and platelets (following chemotherapy). Neutrophil recovery is defined as the first day of three consecutive lab values on different days, after the conditioning regimen-induced nadir of blood counts, that the absolute neutrophil count is > 500/μL. Platelet recovery is defined as the first day of three consecutive lab values on different days, after the conditioning regimen-

induced nadir of blood counts, that the platelet count is ≥ 20,000/μL without platelet transfusion support in the seven days prior.

8.4 Donor chimerism

Chimerism will be assessed beginning at day +30. If full donor chimerism is reached by day +30, it will be assessed per institutional guidelines for restaging. If full donor chimerism is not reached by day +30, additional assessments will be done at day +60, +90 and then every four weeks (+/- 3 days) until the goal of full donor chimerism is achieved. For the purposes of this protocol *mixed chimerism* will be defined as the detection of > 5% and < 95% cells of donor origin and *full donor chimerism* will be defined as ≥95% cells of donor origin.

8.5 GVHD

Patients will be followed for development of acute and chronic GVHD using standard criteria.

9. FOLLOW UP/OFF STUDY CRITERIA

9.1 Patients will be considered to be off study if disease relapse occurs and additional treatment is received. No subsequent adverse or serious adverse event reporting will be done. Further follow up off study will be according to institutional requirements. (For purposes of the study, donor lymphocyte infusions will not be considered "additional treatment.")

9.2 Patients will be considered to have completed study follow up at 6 months post HSCT and will be removed from "on study" status. Further off study follow up will be according to institutional requirements.

10 STATISTICAL CONSIDERATIONS

The primary objective of this phase II clinical trial is to estimate the incidence of graft rejection and severe graft versus-host disease (GVHD) following reduced intensity, HLA-mismatched HSCT for patients with high risk hematologic malignancies. A secondary objective is to obtain estimates of overall survival (OS), relapse, non-relapse mortality (NRM), and event-free survival (EFS) in patients receiving myeloablative conditioning and transplantation of partially human leukocyte antigen (HLA)-mismatched bone marrow from first-degree relatives.

We will plan to accrue 30 patients that will be enough to test the following hypothesis: For the graft failure endpoint we will test the hypotheses H0: p=10% versus Ha: p<10% using a one-sided exact Binomial test. Assume that none of patients undergoing the proposed regimen will

have graft failure, the power for confirming p less than 15% is always 100% if the null hypothesis is rejected. The size of at least 29 patients is needed to achieve rejection of the null hypothesis at the 5% significance level.

Stopping criterion:

10.1 NRM: As outlined in Section 2.0 (Background), the overall nonrelapse mortality on the phase I nonmyeloablative haploidentical HSCT trial utilizing 2 doses of post-transplantation Cy, MMF and tacrolimus was approximately 19% at one year. Other haploidentical HSCT trials, both nonmyeloablative and ablative have shown NRM in the range of 30-40%, mostly from infectious causes. The working hypothesis of this trial is that the overall toxicity of reduced intensity haploidentical HSCT is less than that of haploidentical HSCT after myeloablative conditioning utilizing PTCY. Therefore we adopt the stopping rule that a NRM incidence is greater than 30% after a minimum enrollment of 5 patients. The stopping boundaries are presented in the following table.

Number of enrollment	5	6	7	8	9	10	11	15
Suspend trial if NRM occurs in r patients	$r \geq 2$	$r \geq 2$	$r \geq 3$	$r \geq 3$	$r \geq 3$	$r \geq 4$	$r \geq 4$	$r \geq 5$

10.2 Severe acute GVHD: The incidence of severe aGVHD (Grades III-IV)) on the myeloablative haploidentical HSCT trial utilizing 2 doses of PTCY, MMF and tacrolimus was approximately 23%. The working hypothesis of this trial is that the overall toxicity of reduced intensity conditioning should not be significantly more than that seen with myeloablative conditioning utilizing post-transplantation Cy. Therefore we adopt the stopping rule that an incidence of III-IV aGVHD is greater than 35% after a minimum enrollment of 5 patients. The stopping boundaries are presented in the following table.

Number of enrollment	5	6	7	8	9	10	11	15
Suspend trial if severe acute GVHD occurs in r patients	$r \geq 2$	$r \geq 3$	$r \geq 3$	$r \geq 3$	$r \geq 4$	$r \geq 4$	$r \geq 4$	$r \geq 6$

10.3 Engraftment Failure: As outlined in Section 2.0 (Background), the incidence of engraftment failure on the phase I nonmyeloablative haploidentical HSCT trial utilizing 2 doses of post-transplantation Cy, MMF and tacrolimus was approximately 18%. All patients in the two

myeloablative studies at our center engrafted. It is concerning that with the intensity of flu/mel, patients are not going to recover their own count if they fail to engraft. The working hypothesis of this trial is that the overall toxicity of reduced intensity haplo HSCT is not significantly greater than haploidentical HSCT after nonmyeloablative conditioning utilizing PTCY. Therefore we adopt the stopping rule that an incidence of engraftment failure is greater than 30% after a minimum enrollment of 5 patients. The stopping boundaries are presented in the following table.

Number of enrollment	5	6	7	8	9	10	11	15
Suspend trial if engraftment failure occurs in r patients	$r \geq 2$	$r \geq 2$	$r \geq 3$	$r \geq 3$	$r \geq 3$	$r \geq 4$	$r \geq 4$	$r \geq 5$

If any of the above stopping criteria is met, accrual to the trial will be temporarily halted, until a decision regarding either modification or termination of the trial could be made.

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