A Phase II Trial of Non-Myeloablative Conditioning and Transplantation of Partially HLA-Mismatched/Haploidentical Related or Matched Unrelated Bone Marrow for Patients with Refractory Severe Aplastic Anemia and Other Bone Marrow Failure Syndromes

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TREATMENT SCHEMA

Days -9	Thymoglobulin 0.5 mg/kg IV with pre-meds
	↓
Days -8,-7	Thymoglobulin 2 mg/kg IV qd with pre-meds
	↓
Days -6, -5	Fludarabine 30 mg/M^2 iv qd
	Cyclophosphamide (CTX) 14.5 mg/kg IV qd*
	Start steroid taper from ATG
	↓
Days –4→ -2	Fludarabine 30 mg/M^2 iv qd, adjusted for CrCl
	↓
Day –1	TBI 200 cGy
↓	
Day 0	Infuse bone marrow per institutional standards
	Start appropriate antibiotic prophylaxis
	↓
Days 3, 4	CTX 50 mg/kg iv q d
	Mesna 40 mg/kg iv q d**
	(First dose of CTX must be administered 48-72 hr after START of infusion of marrow)
	↓
Day 5	Begin tacrolimus (section 5.6)** and
	MMF 15 mg/kg po tid with maximum daily dose 3 gm/d
	(24h after the last dose of CY)
	↓
Day 30	Assess Chimerism in peripheral blood
	↓
Day 35	Discontinue MMF
	↓
Day 60	Assess Chimerism in peripheral blood and bone marrow (with biopsy)
	↓
Day 180	Evaluate disease
	Assess Chimerism in peripheral blood
	↓
Day 365	Discontinue tacrolimus if no GVHD and full chimera***
	Evaluate disease
	Assess Chimerism in peripheral blood and bone marrow
	↓
2 yrs	Evaluate disease
	Assess Chimerism in peripheral blood

* Refer to Section 5.3 for complete dosing instructions.
** Or as per institutional standards.
*** Refer to Section 5.6 for complete dosing instructions.

1.0 Primary Objective

Our primary objective is to determine if it is feasible for SAA patients to be transplanted using non-myeloablative conditioning and post transplantation cyclophosphamide with partially HLA-mismatched donors.

2.0 Secondary Objectives

2.1 To estimate overall survival at one year.
2.2 To estimate full donor chimerism by day 60
2.3 To estimate the cumulative incidence of non–relapse-related mortality following transplant.
2.4 To estimate the incidences of primary and secondary graft failure following transplant.
2.5 To estimate the cumulative incidences of grade II-IV and grade III-IV acute graft versus-host disease (GVHD).
2.6 To estimate the cumulative incidence of chronic graft versus-host disease (GVHD).
2.7 To estimate the cumulative incidence of ANC and platelet recovery.
2.8 To estimate GVHD free relapse free survival (GRFS).
2.9 To summarize major transplant related toxicities and to estimate transplant related mortality (TRM).

3.0 BACKGROUND

Bone marrow failure (BMF) syndromes (acquired and inherited) are a heterogeneous group of disorders marked by ineffective hematopoiesis[76]. They confer a significant risk of morbidity and death, due to their progressive natural history and complications of suboptimal therapy.[2, 76] Acquired severe aplastic anemia (SAA) is a rare, life-threatening hematopoietic stem cell disorder that manifests with pancytopenia and a hypocellular bone marrow.[6, 24] In most cases, bone marrow failure results from autoimmune destruction of hematopoietic stem cells. Without definitive treatment, mortality from SAA approaches 70% at two years. Fungal infections are the leading cause of death; however, hemorrhage, evolution to clonal disease (myelodysplastic syndromes [MDS], leukemia, and paroxysmal nocturnal hemoglobinuria [PNH]), and transfusional iron overload are other causes of severe morbidity and mortality. Inherited bone marrow failure syndromes (IBMFS) from disorders such as short telomeres (dyskeratosis congenital [DKC]), Fanconi anemia and other congenital SAA also carries the risk of infection and clonal evolution to MDS. These patients have long pursued BMT as a therapeutic avenue but are limited still by availability of matched sibling donor.

SAA affects all ages, but is most common in children and young adults. Blood or marrow transplantation (BMT) from an HLA-matched sibling donor can cure most patients with SAA, but fewer than 30% of patients have a suitable HLA-matched sibling. Moreover, the best results with allogeneic BMT are in children; adults, especially those over age 40, do less well due to complications from graft-versus-host disease (GVHD). Alternative donor transplants (mismatched and unrelated donors) also have the potential to cure SAA, but are currently reserved for second-line therapy because of their high rates of morbidity and mortality. For SAA patients who lack matched sibling donors or are not good candidates for BMT, immunosuppressive therapy can also be highly effective. Patients with acquired SAA, not responsive to initial IST, or inherited AA lacking other treatments options require additional therapeutic options that are not limited by age or donor status. Patient age and the type of allograft (HLA-matched sibling, unrelated, or mismatched donors) are the most important factors influencing the timing of BMT and outcomes post-transplant. To improve

survival in patients who either do not response to IST or do not have matched sibling donor, there needs to be an available transplant option for all that is feasible and safe. An approach to BMT using post-transplant CY has allowed allogeneic BMT from matched, mismatched, unrelated or haplo-identical donors in other diseases.[38, 39, 41] **Transplant-related mortality, graft-failure rates and risk of GVHD have been very low with this approach with non-myeloablative conditioning.** Here we use alternative donors with CY post BMT to decrease GVHD to expand the donor pool in AA.

Immunosuppressive therapy for SAA
Antithymocyte globulin and cyclosporine (ATG/CSA) immunosuppressive therapy (IST) is generally front-line therapy for SAA patients who lack matched sibling donors or are not good candidates for BMT. The hematopoietic response rate after ATG/CSA is 60-70% and the probability of survival at 5 years ranges from 60% to 85%.[17-20] However, up to 40% of patients eventually relapse and an additional 10 to 40% develop a secondary clonal disease.[17;18;21;22]

High-dose cyclophosphamide (High-CY) High-CY is also highly immunosuppressive and has also been used to successfully treat SAA.[5] A pilot study of 10 patients treated with high-dose cyclophosphamide demonstrated that this approach may have the potential to cure SAA.[7] The incidence of clonal evolution after HiCY is likely less than with ATG/CsA but the response rates remain the same. Immunosuppressive therapy, either ATG/CSA or high-CY is used as initial therapy for older patients or younger patients who lack an HLA-matched sibling donor but only 60-80% of these patients respond and have late complications with clonal evolution and relapse.[10]

Bone marrow transplantation for SAA
Allogeneic BMT from an HLA-matched sibling donor is the treatment of choice at most centers for young patients with SAA. A major advantage of BMT over standard IST is a marked reduction in the risk of relapse and the outgrowth of late clonal disorders such as MDS/AML and PNH[21]. The overall transplant related mortality (TRM) attributable to HLA-identical sibling BMT is 20-30%, with approximately half of these deaths occurring in the first 100 days. The incidence of severe, grade III-IV acute GVHD is approximately 13-17% after HLA-identical sibling BMT.

Historically, unrelated donors and mismatched or haplo- transplants have almost twice the transplant-related mortality and risk of GVHD as matched sibling donor transplants in SAA[2]. Despite the fact that only 30% of people have matched donors and the average person has 4.5 haplo-identical donors, these statistics have limited the use of these alternative donors. The best results with unrelated and mismatched transplants are seen in patients under 21 with disease duration of less than one year. The International Bone Marrow Transplant Registry reported on the results of 318 alternative donor transplants in patients with SAA between 1988 and 1998[18].

Regimen for BMT in SAA
The ideal BMT regimen is one that results in sustained engraftment, minimal toxicity from the regimen, lack of acute or chronic GVHD and allows the majority of patients (old and young) to proceed efficiently to this potentially curative option.

Conditioning: Various conditioning regimens have been employed over the years for transplants to patients with SAA with failure to engraft and GVHD as the main obstacles to success. The current approach used here is one that has been successfully used as

above in sickle cell disease.[4] Historically, TBI- based conditioning regimens reduced the risk of graft rejection but increased GVHD and other late effects.[19] Standard ATG-based conditioning regimens are employed to aid in engraftment but have also been associated with up to a 30% incidence of cGVHD.[8, 14] Fludarabine has been used in conditioning for patients with both acquired and constitutional aplastic anemia with good results.[11, 13, 17, 23]

Choice of Stem Cells: This trial will utilize bone marrow as the stem cell source. The EBMT reviewed outcomes in nearly 700 patients with SAA receiving transplants from HLA-matched siblings. In patients younger than 20 years of age, rates of chronic GVHD (relative risk 2.82; p = 0.002) and overall mortality (relative risk 2.04; p = 0.024) were higher after transplantation of peripheral blood progenitor cell grafts than after transplantation of bone marrow. In younger patients, the 5-year survival was 85% after marrow transplants but only 73% after peripheral blood progenitor cell grafts. These data suggest that bone marrow grafts are preferable in this age group[3, 20]. Furthermore, reports suggest that G-SCSF mobilization may a reasonable approach to decrease GVHD in SAA.[9, 22] GCSF stimulation of the donor will not be mandated with this protocol but we will adhere to the instutitutional standards for donor preparation at the time of transplant.

GVHD prophylaxis: An approach to BMT at Hopkins using post-transplant cyclophosphamide has allowed for transplantation of allogeneic BMT from matched, mismatched, unrelated or haplo-identical donors in both malignant and non-malignant diseases.[4, 15, 16] The administration of a properly timed, high dose of CY *after* BMT inhibits both graft rejection and GVHD[19-22]. It is customary for IST for GVHD to continue post-transplant through one year in SAA.[1]

In conclusion, the major challenge in treating SAA (inherited and acquired) is the management of patients who are refractory to IST, have relapsed after IST, or who have acquired a secondary clonal disorder (MDS/PNH) after IST. BMT is the only curative option for these patients, but many are ineligible because they lack a suitable donor or are too high risk for BMT due to the risk GVHD. Here we seek to increase options for these patients by developing novel therapeutic strategies to treat refractory SAA with expansion of the donor pool through minimization of the post-transplant complication of GVHD. Present above are promising results in the non-myeloablative haplo-identical setting for hematologic malignancies and sickle cell disease[4, 15, 16] with a low incidence of engraftment failure, severe acute GVHD, extensive chronic GVHD, and NRM utilizing post-transplantation CY. This trial will employ non-myeloablative conditioning regimen along with post-transplantation CY on days +3 and +4 for patients with SAA. We anticipate HiCY will likely ameliorate the GVHD while the combination of ATG, fludarabine and TBI will allow for engraftment. Furthermore, this approach allows us to greatly expand the donor pool since any patient shares exactly one HLA haplotype with each biological parent or child and half of siblings, an eligible haplo-identical donor can be identified rapidly in the majority of patients. This is of great benefit to aplastic patients as time to treatment will be shorter. The purpose of the current trial is to improve response rates and cure for in SAA above the standard 70% from immunosuppressive therapy for acquired disease.

3.0 DRUG INFORMATION

3.1 Fludarabine

Fludarabine phosphate is commercially available.

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 30 mg/m^2/day on days -6 to -2.

Fludara® will be dispensed by the Oncology Pharmacy and is produced by Berlex Pharmaceuticals.

3.2 Cyclophosphamide (Cytoxan®)

Cyclophosphamide is commercially available.

Cyclophosphamide is an alkylating agent which prevents cell division primarily by cross-linking DNA strands. Cyclophosphamide is cell cycle non-specific.

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).

Cyclophosphamide will be dispensed by the Oncology Pharmacy and is produced by Mead Johnson Pharmaceuticals.

3.3 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 minutes (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.

3.4 **Tacrolimus** (FK 506)

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.

3.5 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.

3.6 Rabbit antithymocyte globulin (ATG)

Thymoglobulin® [Anti-thymocyte Globulin (Rabbit)] is a purified, pasteurized, gamma immune globulin, obtained by immunization of rabbits with human thymocytes. This immunosuppressive product contains cytotoxic antibodies directed against antigens expressed on human T-lymphocytes. This drug is commonly used to treat graft rejection in kidney transplantation. It is also commonly used in bone marrow transplantation as part of the conditioning regimen to avoid graft failure and to prevent graft-versus-host disease.

Thymoglobulin is a sterile, freeze-dried product for intravenous administration after reconstitution with Sterile Water for Injection, USP (SWFI). Each 10 mL vial contains 25 mg anti-thymocyte globulin (rabbit) as well as 50 mg glycine, 50 mg mannitol, and 10 mg sodium chloride. After reconstitution with 5 mL SWFI, each vial of reconstituted product contains approximately 5 mg/mL of Thymoglobulin, of which >90% is rabbit gamma immune globulin (IgG). The reconstituted solution has a pH of 7.0 ± 0.4. Human red blood cells are used in the manufacturing process to deplete cross-reactive antibodies to non-T-cell antigens. The manufacturing process is validated to remove or inactivate potential exogenous viruses. All human red blood cells are from US registered or FDA licensed blood banks. A viral inactivation step (pasteurization, i.e., heat treatment of active ingredient at 60°C/10 hr) is performed for each lot. Each Thymoglobulin lot is released following potency testing (lymphocytotoxicity and E-rosette inhibition assays), and cross-reactive antibody testing (hemagglutination, platelet agglutination, anti-human serum protein antibody, antiglomerular

Adverse side effects include immunodeficiency, infusion related toxicities such as hypertension, chills, rigors, tachycardia, capillary leak syndrome, hyperglycemia, cytopenias, transient hepatitis, anaphylaxis, serum sickness, myalgias, sensory changes including hearing loss, headaches, renal toxicity, dyspnea and bronchial spasm, fevers. The drug is potentially teratogenic and is unknown if it can be passed to children in breastfeeding.

Thymoglobulin will be dispensed by the Oncology Pharmacy and is produced by Genzyme. ATG–rabbit must be infused through a 0.22 micro filter with premedications: acetaminophen 650 mg orally and diphenhydramine 25mg orally as well as a steroid taper (see Section 6.3). The dose to be used is 0.5 mg/kg on day -9 and 2 mg/kg/day on days -8 and -7. Note: Keep anaphylaxis kit at bedside during ATG administration.

4.0 PATIENT SELECTION

4.1 Criteria for recipient eligibility

4.1.1 Patients with relapsed or refractory SAA or very SAA defined:

- Bone marrow (< 25% cellular)
- Peripheral cytopenias *(at least 2 of 3)*
  - ANC < 500 per ml
  - Platelets < 20,000 per ml
  - Absolute retic < 60,000 or corrected retic < 1%
- Very severe: as above, but ANC < 200
- Disease may be designated as *acquired* or *inherited* if previous counts known (these other bone marrow failure disorders that are characterized by aplastic anemia may go by additional names such as dyskeratosis congenita or PNH)
- Failed at least one course of immunosuppressive therapy (if presumed acquired disease). Patients with inherited disease will be characterized as refractory and do not require immunosuppressive first.

4.1.2 Age 0- upper age limit as determined by current institutional standards

4.1.3 Good performance status (ECOG 0 or 1; Karnofsky and Lansky 70-100)

4.1.4 Patients and donors must be able to sign consent forms (or if a minor the parent will sign). Donors should be willing to donate.

4.1.5 Patients must be geographically accessible and willing to participate in all stages of treatment.

4.1.6 Adequate end-organ function as measured by:

a. Left ventricular ejection fraction $\geq$ 35%, or shortening fraction > 25% (For pediatric patients, a normal ejection fraction is required)
b. Bilirubin $\leq$ 3.0 mg/dL (unless due to Gilbert's syndrome or hemolysis), and ALT and AST $\leq$ 5 x ULN
c. FEV_1 and FVC $\geq$ 40% of predicted; or in pediatric patients, if unable to perform pulmonary function tests due to young age, oxygen saturation >92% on room air

4.2 Criteria for recipient ineligibility

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

4.2.2 Prior transfusions from selected donor (as this could have cause recipient alloimmunization against the donor)

4.2.3 Women of childbearing potential who currently are pregnant (HCG^+) or who are not practicing adequate contraception.

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

4.2.5 Uncontrolled viral, bacterial, or fungal infections (HIV infection permitted if viral load undetectable)

4.3 Criteria for donor eligibility

4.3.1 Donors must meet the selection criteria as defined by the Foundation for the Accreditation of Hematopoietic Cell Therapy (FAHCT) and will be screened per the American Association of Blood Banks (AABB). (AABB guidelines and the recipients will be informed of any deviations.)

4.3.2 Weight ≥ 20kg and patient or guardian able to provide consent

4.3.3 HLA mismatched or haploidentical related donors (including 1st degree relatives and half siblings).

4.3.3.1 The donor and recipient must be identical at at least one allele of each of the following genetic loci: HLA-A, HLA-B, HLA-Cw, HLA-DRB1, and HLA-DQB1. A minimum match of 5/10 is therefore required, and will be considered sufficient evidence that the donor and recipient share one HLA haplotype.

4.3.4 Matched unrelated donors

4.3.4.1 Unrelated volunteer donor matched for HLA-A, -B, -C and -DRB1 defined by high resolution molecular typing.

4.3.4.1.1 Mismatched unrelated volunteer donors may be considered if no other suitable donor is available.

4.3.5 When more than one donor is available, the donor with the lowest number of HLA allele mismatches will be chosen, unless there is HLA cross-match incompatibility or a medical reason to select otherwise, in which case donor selection is the responsibility of the PI, in consultation with the immunogenetics laboratory. In cases where there is more than one donor with the least degree of mismatch, donors will be selected based on the most favorable combination of (i) HLA compatibility in cross-match testing and (ii) ABO compatibility. We will prioritize the lowest number of mismatches in the HVG direction (to potentially minimize graft rejection risk)

Donor selection criteria, in decreasing order of priority:

1. **Donor must be medically, socially, and psychologically fit to donate.**

2. **HLA-identical sibling**.

3. **For a partially HLA-mismatched transplant, the patient must lack antibodies against donor HLA molecules**. Specifically, complement dependent cytotoxicity and flow cytometric crossmatch assays must be negative, and the mean fluorescence intensity (MFI) of any anti-donor HLA antibody by solid phase immunoassay should be <3000. Consult with Immunogenetics for the clinical significance of any anti-donor antibody. *Desensitization to remove anti-donor antibody should only be performed for patients who have no other donor options*

If there is more than one donor with the least amount of HVG allele mismatches, the following prioritization will be used: (Will always minimize HVG mismatch as highest priority)

4. **ABO compatibility (in order of priority).**

    a. Compatible or minor ABO incompatibility
    b. Major ABO incompatibility

5. **CMV status**

The CMV status of the pair donor-recipient is frequently employed to select a potential donor. This is a controversial issue and the data available is somewhat limited. The following guidelines are recommended:

    a. For a CMV seronegative recipient, use a CMV seronegative donor
    b. For a CMV seropositive recipient, use a CMV seropositive donor

In CMV- patients with CMV+ stem-cell donors, primary CMV infection/reactivation develops in about 30%. Data from the European Registry shows the following: Seropositive patients receiving grafts from CMV+ HLA-identical sibling donors had the same survival as patients grafted from CMV- donors. However, MUD recipients receiving grafts from CMV+ donors had an improved 5-year survival, an improved event-free survival, and a reduced transplant-related mortality. There was no influence on the relapse incidence. The effects of donor CMV status remained in multivariate analyses. The effect of donor status was different among different disease categories. In patients with chronic myelogenous leukemia, T-cell depletion abrogated the beneficial effect of donor status, suggesting that the effect is mediated through transfer of donor immunity. These data suggest that donor CMV status influences outcome of unrelated SCT.

6. **Other donor characteristics**

Donor parity and sex mismatch, have also been associated with an increased risk of aGVHD and decreased survival in some but not all studies(4-8). Donor age and weight should be also taken into consideration.

Suggestions (<u>in no order of priority</u>):

a. Younger (18 years of age or older) and lighter donors should be preferred.

If all else is equal, male donors may be preferred over nulliparous female donors who may be preferred over multiparous female donors.

Other factors such as donor age and health history will be integrated into the donor selection process per standard practice and may be prioritized over HLA, ABO and CMV status. Children donors may be used if appropriate.

5.0 TREATMENT PLAN

5.1 Indwelling central venous catheter

A central venous catheter may be placed for administration of IV medications and transfusion of blood products as per BMT standard.

5.2 Pre-treatment Evaluation

All patients will require documentation of a detailed history and physical examination and standard evaluation of cardiac, pulmonary, liver and renal function.

Baseline disease evaluation will be performed by collecting the following laboratory tests: complete blood count, reticulocyte count, LDH, PNH panel, bone marrow biopsy (please see Table 1)

5.3 Preparative regimen

Thymoglobulin will be infused through a 0.22 micro filter with premedications: acetaminophen 650 mg orally and diphenhydramine 25mg orally. Keep anaphylaxis kit at bedside during ATG administration. The dose will be 0.5 mg/kg IV on day -9 over 6 hours and 2mg/kg IV on days -8 and -7 over 4 hours. A steroid taper will be given to prevent reactions to ATG as follows (or per institutional standards): Solumedrol 1mg/kg IV 1 hour prior ATG on days -9 to -7. This dose may be repeated once 3 hours after the first dose. On day -6 and -5, solumedrol 0.75 mg/kg/ IV as a single dose; on days -4 and -3, solumedrol 0.5 mg/kg/ IV as a single dose; on day -2 solumedrol 0.25 mg/kg/ IV as a single dose.

Fludarabine 30 mg/m^2/day (adjusted for renal function) is administered over a 30-60 minute IV infusion on Days –6 through –2 (maximum cumulative dose, 150 mg/m^2).

The body surface area (BSA) for fludarabine dosing is based on actual body weight.

For patients $\geq$ 18 years old, CrCl will be estimated by the Cockcroft Formula, based on weight:

$$\text{CrCl} = \frac{(140 - \text{age}) \times \text{weight (kg)}}{P_{Cr} \times 72} \times 0.85 \text{ for females}$$

When calculating CrCl: if Actual Body Weight is less than Ideal Body Weight, Actual Body Weight will be used; if Actual Body Weight is between 100-120% of Ideal Body Weight, Ideal Body Weight will be used; and if Actual Body Weight is > 120% of Ideal Body Weight, 25% Adjusted Body Weight

For decreased creatinine clearance (CrCl), fludarabine dosage is reduced as follows:
CrCl ≥ 70 ml/min – fludarabine 30 mg/m2
CrCl 40-70 ml/min - fludarabine 24 mg/m2
CrCl 20-40 ml/min – fludarabine 20 mg/m2
CrCl < 20 ml/min – fludarabine 15 mg/m2

For patients <18 years old, CrCl will be estimated by the Schwartz equation:

Schwartz equation: CrCl (mL/min/1.73m^2)= [length (cm) x k] / serum creatinine

k = 0.45 for infants 1 to 52 weeks old
k = 0.55 for children 1 to 13 years old
k = 0.55 for adolescent females 13-18 years old
k = 0.7 for adolescent males 13-18 years old

A measured CrCl or a glomerular filtration rate may be substituted to determine CrCl.

CrCl may change during the days fludarabine is given. The dose should be adjusted accordingly; however a fludarabine dose may be given based on the preceding day's estimated CrCl .

Cyclophosphamide will be administered as an IV infusion over 1 hour for the pretransplant CY and over 2 hours for post-transplant CY on D-6 and D-5. The dose of pre-transplantation cyclophosphamide is 14.5 mg/kg/day. Dose is calculated based on the ideal body weight or actual body weight whichever is less. (Refer to Appendix 2.) Body weight and height are measured directly. An approximate weight for height would be calculated from a standard table or equations that reflect ideal "values".

IBW is calculated by the following equation:

$$IBW = \sqrt{\frac{[ht\ (cm) x\ wt\ (kg)]}{3600}}$$

Note: Mesna will be utilized for the Day 3 and Day 4 post BMT cyclophosphamide doses, not for the pre-BMT cyclophosphamide doses.

Total body irradiation: 200 cGy AP/PA with 4MV or 6MV photons at 8-12 cGy/min at the point of prescription (average separation of measurements at mediastinum, abdomen, hips) will be administered in a single fraction on day -1.

Hydration and antiemetic administration will follow standard operating procedures.

5.4 Bone marrow transplantation and graft information

Bone Marrow (T cell replete graft) will be harvested and infused on day 0. The BM will be infused the same day of collection. The nucleated cell target range will be between 8 to 16 x 10^8/kg of recipient ideal body weight with the volume not to exceed 20 mL/kg of donor's weight once the minimal target of 8 x 10^8/kg has been reached. Given that there is always uncertainty about the feasibility of harvesting certain patients, the harvesting team will have the ability to stop the surgery if the minimum target of 8 x 10^8 cells has not been reached and it is believed that will be unsafe or technically unfeasible to reach the mentioned target if the surgery was to continue after a reasonable effort has been made. In this case, the study PI will be notified immediately and efforts should be made to assure at least a cell count of 2-4 x 10^8/kg of recipient ideal body weight.

Major incompatible ABO graft will have red blood cell depleted by buffy coat preparation. Minor ABO incompatible graft will have plasma removed. Guidelines for the infusion of bone marrow have been established and are outlined in the ABO compatible/minor mismatched allo BMT or the ABO incompatible allo BMT standing orders.

5.5 Post-transplantation cyclophosphamide

Cyclophosphamide [50mg/kg] will be given on D+3 post-transplant (within 48-72 hr of start of marrow infusion as per institutional standards) and on D+4 post-transplant. Cyclophosphamide will be given as an iv infusion over 1- 2 hr (depending on volume).

Mesna will be given in divided doses iv 30 min pre- and at 3, 6, and 8 hr post-cyclophosphamide or administered 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.

It is crucial that no immunosuppressive agents are given from the time of the transplant until at least 24 hours after the completion of the post-transplant Cy. This includes steroids as anti-emetics.

5.6 GVHD prophylaxis

On day 5, patients will begin prophylaxis with Tacrolimus (PO or IV as per institutional standards for starting this prophylaxis) and Mycophenolic Acid Mofetil (MMF).

Tacrolimus begins on Day 5, at least 24 hours after completion of post-transplantation Cy.

The tacrolimus starting dose will be given per institutional standards for adult or pediatric patients. The starting dose of tacrolimus may be increased with PI or co-PI permission should institutional practice guidelines change.. Dose is adjusted to maintain a serum trough level of **10 – 15 ng/Ml**. If there is tacrolimus toxicity at these troughs, lower troughs will be permitted to allow for tolerance of the drug after consultation with the PI. If tacrolimus is completely impossible for the patient to tolerate, alternative immunosuppression may be chosen after consultation with the PI or Co-PI.

***Tacrolimus is discontinued after the last dose on Day 365 without taper, or may be continued if GVHD is present or if patient is still a mixed chimera. At PI or co-PI discretion, cyclosporine (target concentration 200-400 ng/Ml) may be substituted for tacrolimus if the patient is significantly intolerant of tacrolimus.

***Tacrolimus may be discontinued earlier than Day 365 in the context of relapse, graft failure, or prohibitive toxicity. It is suggested that patients with suspected graft failure remain on tacrolimus until at least the ~Day 60 chimerism assessment. Decisions regarding early discontinuation of immunosuppression will be made on a case-by-case basis in consultation with the PI or co-PI.

Mycophenolic acid mofetil will be given at a dose of 15 mg/kg po/ IV TID (based upon actual body weight) with the maximum total daily dose not to exceed 3 grams (1 g po TID).

MMF prophylaxis will be discontinued after the last dose on D35 and Tacrolimus prophylaxis will be discontinued after the last dose around day 365.

5.7 Infection prophylaxis and therapy

During pre-transplant evaluation patients will be screened for respiratory 16yncytial virus, influenza A, B and parainfluenza viruses if symptomatic or during the flu season. Assays of these viruses must be negative for symptomatic patients to be admitted for transplant. Strong consideration should be given to institution of ribavirin therapy if positive for adenovirus or nalidixic acid if positive for BK virus.

Oral hygiene will be maintained according to institutional standards.

Prophylactic anti-microbial therapy will be given per the BMT unit standards.

Empiric therapy with broad-spectrum antibiotics will be instituted for the first neutropenic fever (specific agents as per current practice).

Antifungal prophylaxis will be administered according to institutional preference. It is important to follow tacrolimus levels on patients receiving azoles as the combination of both drugs can raise the levels of the immunosuppressant to toxic levels. If the patient on tacrolimus is started on azoles, a dose reduction of tacrolimus is required and levels should be obtained to be sure the levels are not in the toxic range.

Pneumocystis jiroveci pneumonia (PCP) prophylaxis will be administered according to institutional preference starting around Day 21 (or later if WBC not recovering) and should continue for at least the first year following BMT and while on immunosuppression. If the patient cannot tolerate po then a comparable dose of Bactrim will be given iv. Patients intolerant of Bactrim will receive dapsone, atovaquone, or pentamidine as PCP prophylaxis.

Viral prophylaxis for HSV/ VZV will be administered according to institutional preference and should continue for at least the first year following BMT and while on immunosuppression.

CMV viremia (by PCR) or antigenemia (by ELISA) should be documented weekly or every other week beginning once the WBC>1000 and until discharge. Monitoring of CMV viremia or antigenemia is recommended to continue on a weekly or every other week basis until day 100, then bi-weekly until day 180. Patients who are viremic or antigenemic will be treated according to institutional preference.

5.8 Growth factor support

GCSF (filgrastim) begins on Day 5 at a dose of 5 mcg/kg/day (actual body weight) IV or subcutaneously (rounding to the nearest vial dose is allowed), until the absolute neutrophil count (ANC) is ≥ 1,000/mm^3 over the course of three days. Additional GCSF may be administered as warranted. Pegfilgrastim (Neulasta®) and GM-CSF are not permitted.

5.9 Transfusion support

Packed red cell transfusions and platelet transfusions will be given per current institutional recommendations. If the patient becomes alloimmunized, the blood bank service will select the best blood products to transfuse and will coordinate with the medical floor the transfusion needs.

5.11 Anti-ovulatory treatment

Menstruating females should be started on an anti-ovulatory agent prior to the initiation of the preparative regimen. The treatment administered will be at the discretion of the treating physician.

5.0 PATIENT MONITORING

The following parameters will be obtained according to this schedule: (for details of these evaluations, see text sections 6.1-6.3)

TABLE 1:

	Initial	Allowable time frame from date of consent**	Day < 60	≈Day 30 ± 7	≈Day 60 ± 7	Day 180 ± 30	Day 365 and Day 730 ± 60
History and Physical	X	within 30 days		X	X	X	X
Performance status (ECOG or Karnofsky)	X	within 30 days			X	X	X
Disease staging (including bone marrow biopsy) Unless clinically indicated by CBC or physician discretion	X	within 30 days					
CBC & Diff.	X	within 7 days	*weekly	X	X	X	X
Comprehensive Metabolic Panel	X	within 7 days	weekly	X	X	X	X
reticulocyte count, LDH, PNH panel,	X						
CXR (May be Chest CT if clinically indicated)	X	within 30 days					
Sinus CT	X	within 30 days					
Pregnancy test (women, childbearing age)	X	within 30 days					
Peripheral blood chimerism, both total leukocyte (unsorted) and T-cell sorted)	X			X	X	X	X
PT, PTT	X	within 30 days					
EKG	X	within 60 days					
ECHO (or MUGA)	X	within 60 days					
Hep B Surface Ag, HBC Ab, HCV Ab,	X	within 30 days					

HSV IgG, CMV IgG, RPR, HIV, VZV IgG (if possible)							
Toxicity assessment	X			X	X	X	X
HLA typing/lymphocytotoxic screen	X	Must be done at JHH					
PFTs (Spirometry and DLCO)	X	within 30 days					
GVHD questionnaire				X	X		

* Once ANC >100, this will be obtained daily until ANC >500 for three days or two consecutive measurements over a three day period, then weekly.
**Baseline laboratory tests and radiology studies time frame will follow BMT standards.
*** In order to minimize the need for research-only in-person visits, telemedicine visits may be substituted for in-person clinical trial visits or portions of clinical trial visits where determined to be appropriate and where determined by the investigator not to increase the participant's risks. Prior to initiating telemedicine for study visits the study team will explain to the participant, what a telemedicine visit entails and confirm that the study participant is in agreement and able to proceed with this method. Telemedicine acknowledgement will be obtained in accordance with the Guidance for Use of Telemedicine in Research. In the event telemedicine is not deemed feasible, the study visit will proceed as an in-person visit. Telemedicine visits will be conducted using HIPAA compliant method approved by the Health System and within licensing restrictions.

6.1 Pre-transplant Evaluation

These represent the basic baseline studies required on all patients prior to starting their preparative regimen. Additional investigations may be clinically indicated in certain individuals.

6.1.1. Complete medical history which should include particular attention to the following details:

a) previous medical conditions
b) previous transfusions and transfusion reactions
c) previous serious infections
d) allergies
e) current medications
f) assessment of performance status
g) focused history for evidence of constitutional aplastic anemia

6.1.2. Thorough general medical evaluation which should include:

a) a careful physical examination
b) evaluation for placement of a central venous access device, if the patient does not already have such a catheter.

6.1.3. Baseline investigations including:

a) Hematologic

i. CBC with platelets, differential, reticulocyte count, LDH, PNH Panel
ii. PT, PTT
iii. ABO and Rh typing
iv: bone marrow aspirate and biopsy

b) Chemistries

i. Comprehensive chemistry panel
ii. Routine and microscopic urinalysis with C&S

c) Cardiac

i. EKG
ii. Echocardiogram or MUGA scan with Left Ventricular Ejection Fraction (LVEF) + Right Ventricular Systolic Pressure and evaluation for pulmonary hypertension

d) Pulmonary

i. Chest X-ray (CT scan may be performed as clinically indicated but some form of chest imaging is required in advance of BMT)
ii. Sinus CT scan
iii. Pulmonary function tests including at least spirometry with FEV1 and FVC and DLCO. (pediatric patients under the age of 8 are excluded from this test)

e) Immunologic / Infections

i. HBsAg, anti-HBC, anti-HCV
ii. RPR
iii. HIV antibody
iv. Serology for CMV and HSV (plus VZV – blood samples permitting)
v. HLA typing/lymphocytotoxic antibody screen

f) Chimerism studies will be drawn as a baseline for subsequent engraftment studies including myeloid (unsorted) and CD3 chimerism.

6.2 Post-transplant Evaluation

6.2.1. Day 0 through Day 60 (± 7 days) evaluation. These represent the minimum required. More frequent determinations and additional investigations may be indicated by the clinical condition of the patient.

1. CBC daily with a WBC differential once the total WBC is greater than 100 until ANC > 500 for three days or two consecutive measurements over a three day period; then CBC weekly with differential.
2. Comprehensive metabolic panel once a week.
3. Patients will have evaluations for infectious complications as clinically indicated. Surveillance cultures according to JHOC BMT program standards are recommended.

4. Evaluations by history and physical examination for GVHD will be performed as per BMT unit standards. (see also section 6.2). For study purposes, weekly GVHD summaries will be taken from these standard examinations from day 14 through day 60.

6.2.2 Evaluations on day ~ 30 (±7 days)

1. History and physical examination.
2. donor chimerism on peripheral blood (including myeloid (unsorted) and CD3 chimerism).
3. Disease evaluation.
4. CBC and differential, comprehensive panel.
5. GVHD questionnaire.

6.2.3 Evaluations on day ~60 (±7 days)

1. History and physical examination.
2. Disease evaluation.
3. Studies for donor cell chimerism on peripheral blood.
4. CBC and white blood cell differential, reticulocyte count, comprehensive panel.
5. GVHD questionnaire.

6.2.4 Evaluations for suspected GVHD:

1. Comprehensive panel
2. Biopsies if needed.

In the event that the patient is unable to return to Johns Hopkins for these visits, every attempt will be made to obtain data from the patient and referring physician. After completion of the trial at ≈Day 60 ± 7, patient will be followed in accordance with the JHOC BMT Policy and Procedure Manual.

7.0 POST-BMT EVALUATION

Patients will be followed during (i) the initial post-BMT period (ii) IPOP care and (iii) after discharge to the referring physician as per standard practice.

7.1 Chemotherapy toxicities

The agents being used in the study are FDA approved. These agents are used extensively in the Bone Marrow Transplant setting and have well defined toxicity profiles. In addition, there are many expected toxicities related to a bone marrow transplant. For these reasons, toxicities will be captured and recorded/graded if the adverse event interferes with the subject's daily function and are considered clinically significant. We will capture and grade all these events structured around the categories of the Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0 for the first 60 days post BMT.

Since this trial is an IPOP, inpatient and out-patient trial, the definition of an adverse event 'interfering with daily function' and 'clinically significant' will be

events that require hospitalization (outside of institutional standards for IPOP needs or pediatric re-hospitalization standards for this ambulatory care based on the outpatient clinic resources and availability). Due to the nature of marrow transplantation, readmissions to the hospital are expected; therefore hospitalization (either inpatient or IPOP) during the first 60 days post-transplant will not be considered a SAE. For example, if a patient has a neutropenic fever that requires hospitalization, then 'neutropenic fever' will be captured and graded as an adverse event. An example of a non-captured event is if a patient has hypotension that is corrected by fluid administration in the outpatient setting. This will not be captured as an adverse event unless the patient requires a hospital admission for further treatment of the hypotension.

Once the patient becomes hospitalized, the above definition of 'requiring hospitalization' cannot be used to capture adverse events. For these already hospitalized patients, events will only be recorded once the event is greater than a grade 3 or 4 as stated below.

List of categories that will be recorded only if the event becomes a grade 3 or grade 4 or meets the criteria of a SAE

Cardiac disorders
Congenital, familial and genetic disorders
Ear and labyrinth disorders
Endocrine disorders
Eye disorders
Gastrointestinal disorders
General disorders and administration site conditions
Hepatobiliary disorders
Investigations
Metabolism and nutrition disorders
Musculoskeletal and connective tissue disorders
Nervous system disorders
Pregnancy, puerperium and perinatal conditions
Psychiatric disorders
Renal and urinary disorders
Reproductive system and breast disorders
Respiratory, thoracic and mediastinal disorders
Skin and subcutaneous tissue disorders
Vascular disorders

The Blood/Bone Marrow category is captured as endpoints to the study. Thus for this category, we will not record data according to the NCI Common Toxicity Criteria.

7.2 GVHD

A major toxicity of allogeneic BMT from an unrelated or mismatched donor is GVHD. Acute graft-versus-host disease (GVHD) shall be graded clinically according to the criteria developed by the consensus conference on acute GVHD[14] (Appendix 1). *All suspected cases of acute GVHD must be confirmed histologically*

by biopsy of an affected organ (skin, liver, or gastrointestinal tract). For purposes of reporting, a pathologist at SKCCC will be ultimately responsible for determining whether a patient does or does not have histologic evidence of GVHD. Diarrhea and/or hyperbilirubinemia in a patient with histologically documented skin GVHD may be assumed to be a manifestation of visceral GVHD and will be graded as such. All patients with histologically documented, clinical grade ≥2 acute GVHD should receive initial treatment with corticosteroids (or a corticosteroid containing regimen if a protocol is available) according to institutional preference. If skin GVHD resolves with treatment but suspected visceral GVHD does not, biopsy of the affected organ (liver or gastrointestinal tract) should be obtained to rule out other causes of hyperbilirubinemia and/or diarrhea. Steroid refractory acute GVHD will be treated according to institutional preferences.

Acute GVHD: Acute GVHD is graded by standard clinical criteria (Appendix 1). All suspected cases of acute GVHD must be confirmed histologically by biopsy of an affected organ (skin, liver, or gastrointestinal tract). Date of symptom onset, date of biopsy confirmation of GVHD, maximum clinical grade, and dates and types of treatment will be recorded. Dates of symptom onset of grade II or higher GVHD and grade III-IV GVHD will be recorded.

Chronic GVHD: Chronic GVHD is graded by NIH consensus criteria[12]. Date of onset, date of biopsy proof (if any), dates and types of treatment, and extent will be recorded. The cumulative incidence of chronic GVHD (overall, and according to extent) will be determined through competing risk analysis.

The occurrence and severity of acute and chronic GVHD after Day 60 will be captured at the patients six month and annual evaluations.

7.3 Non relapse mortality (NRM)

Causes of NRM, i.e., death in the absence of relapse, will be documented as important indicators of procedure-associated toxicity, particularly as these causes relate directly or indirectly to GVHD. Analysis will stratify mortality with respect to the peri-transplant period (<100 d post-BMT) or later times post-BMT.

7.4 Disease Evaluation

Disease evaluations will be performed at ~Day 30, ~Day 60, ~Day 180, ~Day 365, year 2 and then yearly thereafter per Johns Hopkins standards. (Dates are approximated based on patient availability) Disease evaluations will be conducted as per table 1.

8.0 STUDY PARAMETERS

8.1 Transplant-related mortality

Transplant-related mortality, which is defined as death in the absence of relapse or progression, will be characterized at 100 days, 6 months and at one year after BMT.

8.2 Hematologic toxicity

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 laboratory 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 laboratory 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.

8.3 Donor Chimerism

Definitions:

Donor chimerism: Mixed donor chimerism is defined as ≥ 5%, but < 95%, donor. Full donor chimerism is defined as ≥ 95% donor.

Graft failure: < 5% donor chimerism in blood and/or bone marrow on ~Day 30 or after and on all subsequent measurements.

- Primary graft failure: < 5% donor chimerism in blood and/or bone marrow by ~ Day 56
- Secondary graft failure: achievement of ≥ 5% donor chimerism, followed by sustained <5% donor chimerism in blood and/or bone marrow.

< 5% donor T cell chimerism, but with ≥ 5 % donor chimerism in total leukocytes, is not considered graft failure.

Donor chimerism will be measured in the peripheral blood around day 30 and day 60. Patients with >5% donor chimerism around day 60 will be considered as having engrafted. Chimerism determinations will be made on peripheral blood by a number of different methods depending on the specific patient. Methods may include (i) the usual standard of restriction fragment length polymorphism (RFLP) if the donor and recipient RFLPs are informative, (ii) fluorescence in-situ hybridization (FISH) for Y-chromosome markers on PBMC if the donor is male, (iii) cytogenetic analysis, (iv) flow cytometric analysis of HLA-A, B or DR on lymphocytes in the peripheral blood if haploidentical and suitable reagents exist or (v) PCR analysis of variable nucleotide tandem repeats (VNTR) in PBMC if informative. Mixed donor chimerism is defined as ≥ 5%, but < 95%, donor. Full donor chimerism is defined as ≥ 95% donor.

8.4 Disease Status

Disease status will be evaluated through the following laboratory tests: complete blood count, reticulocyte count. These disease evaluations will be performed at ~Day 30, ~Day 60, ~Day 180, ~Day 365, and then yearly per Johns Hopkins

standards. Relapse will be defined as the loss of donor chimerism given a graft loss is analogous to relapse in AA.

8.5 GVHD

Patients will be followed for development of acute and chronic GVHD using standard criteria. Chronic GVHD is assessed according to standard criteria. [12] Treatment of GVHD is outlined in Section 6.2.

To allow for flexibility in patient scheduling, all time points may be approximated.

9.0 DATA MANAGEMENT AND COLLECTION GUIDELINES

Data will be maintained on uniform case report forms and appropriate Graft Engineering Laboratory spreadsheets provided by the SKCCC at Johns Hopkins. The research team at each site will make assessments of GVHD. GVHD assessment will be evaluated and scored by the GVHD team, the Research Nurse, the attending BMT physician and PI. Hematopoietic engraftment will be assessed by the BMT attending and the PI at each site. The PI at each site will be responsible for evaluation of chimerism data and weekly overall toxicities.

For outside sites, please notify Donna Dorr when a patient has been enrolled to the study (within 3 days of consent). The CRFs are to be completed as required by protocol and faxed or transmitted by the Internet to JH SKCCC within 30 days of Day 0, and then in a timely fashion for all additional study-related visits, to:

Donna Dorr, RN, MSN
Ph 410-502-2547
Fax 410-955-0185
Email: ddorr1@jhmi.edu

9.1 Data and Safety Monitoring

At the Sidney Kimmel Comprehensive Cancer Center (SKCCC) at Johns Hopkins, the Associate Director for Clinical Research, Clinical Research Review Committee (CRC), SKCCC Safety Monitoring Committee (SMC), CRO Quality Assurance Group, and the PI share monitoring responsibilities.

9.1.1 Internal Data Monitoring

The PI at each site will review data to assure the validity of data, as well as, the safety of the subjects. The PI will also monitor the progress of the trial. The PI will review safety reports and clinical trial efficacy endpoints and to confirm that the safety outcomes favor continuation of the study.

The PI at each site will be responsible for maintaining the clinical protocol, reporting adverse events, assuring that consent is obtained and documented, reporting of unexpected outcomes, and reporting the status of the trial in the continuing renewal report submitted to the IRB and to the trial monitoring

review group. Content of the continuing renewal report at a minimum should include year-to-date and full trial data on: accrual and eligibility, protocol compliance, treatment administration, toxicity and ADR reports, response, survival, regulatory compliance, compliance with prearranged statistical goals. The report should be submitted in a timely manner according to the schedule defined by Johns Hopkins Medicine Institutional Review Board.

Adverse Event reporting – Serious Adverse Events that will be reported should include: any death within the first 100 days post BMT, any graft failures associated with failure of neutrophil recovery to >500/mm³ by day ~60 after transplantation, and any unexpected events as deemed significant by the PI. The PI will be responsible for reporting events to their local CRO/IRB, as well as to the primary study site and other investigators.

An external Data Safety and Monitoring Board (DSMB), comprised of three independent external experts, will convene as requested by the PI to review serious toxicities and adverse events for the purpose of determining whether the trial should be modified or stopped. Triggers for referral to the DSMB are described in the Stopping Rules Criteria of section 10.0. Each site where the trial is being conducted will have its own local DSMB.
Monthly conference calls between the coordinating center and outside site, and in-person meetings every 6 months, will be conducted to review enrollment, data, and any safety issues, and to discuss study progress and the need for any protocol amendments.

9.1.2 External Data Monitoring and Auditing

This is a Level I study under the SKCCC Data and Safety Monitoring Plan. The SKCCC Clinical Research Office, Quality Assurance Group will perform periodic study audits. All trial monitoring and reporting will also be reviewed annually by the SKCCC Safety Monitoring Committee.

9.1.3 Safety Monitoring

The SKCCC Safety Monitoring Committee (SMC) performs an annual review of this Level I study.

The SMC is charged with ensuring the safety of participants and the validity and integrity of the data and the appropriate closure of studies for which significant benefits or risks have been uncovered. The Committee is responsible for continuous, ongoing review of the conduct of the trial, including adherence to study design, documentation of appropriate monitoring, and proper reporting of protocol problems and events. Inherent in this process is the goal of enhancing the quality of the research by providing the investigator with constructive criticism. The SMC membership includes physicians and other representatives from various Center Programs, biostatistics, data management, nursing, and quality assurance.

10.0 STATISTICAL CONSIDERATIONS

Overall Study Design

This is a single-arm study of non-myeloablative conditioning and post transplantation cyclophosphamide with partially HLA-mismatched donors in patients with SAA. Without BMT, the one year survival for refractory SAA is very poor. Seventy percent of Caucasians and 10% of African Americans and patients of mixed racial backgrounds will have a partially HLA-mismatched donor. In children and young adults with this type of transplant the one year survival is at best 70% and in other series as low as 40%. In this trial, we hope to improve survival, but even if survival was unchanged we would markedly advance the field by expanding the donor pool for these patients.

The primary goal of this study is to determine whether this type of transplantation for SAA is feasible and safe. There will be continuous monitoring for feasibility and for safety. Feasibility will have been met with these benchmarks: the patient has the transplant, is assessed for the safety endpoint, and survives one year. The safety monitoring plan is included to monitor graft failure (day 60), grade 2-4 acute graft versus host disease (day100), 6 month mortality (day 180), and chronic graft versus host disease (day 180).

10.1 Primary Objective

Our primary objective is to determine if it is feasible for SAA patients to be transplanted using non-myeloablative conditioning and post transplantation cyclophosphamide with partially HLA-mismatched donors.

10.2 Secondary Objectives

10.3.1 To estimate overall survival at one year.
10.3.2 To estimate full donor chimerism by day 60
10.3.3 To estimate the cumulative incidence of non–relapse-related mortality following transplant.
10.3.4 To estimate the incidences of primary and secondary graft failure following transplant.
10.3.5 To estimate the cumulative incidences of grade II-IV and grade III-IV acute graft versus-host disease (GVHD).
10.3.6 To estimate the cumulative incidence of chronic graft versus-host disease (GVHD).
10.3.7 To estimate the cumulative incidence of ANC and platelet recovery.
10.3.8 To estimate GVHD free relapse free survival (GRFS).
10.3.9 To summarize major transplant related toxicities and to estimate transplant related mortality (TRM).

10.3 Early stopping guideline for feasibility

Feasibility of non-myeloablative, partially HLA-mismatched transplantation in SAA will be based on three benchmarks: the patient has the transplant, is assessed for the safety endpoint, and survives one year. We will use a probability-based decision rule for the study to decide if the probability of successfully proceeding through the study is convincingly less than 0.70. We expect*, a priori*, the feasibility to be high and that 80% of patients will meet the feasibility benchmarks. The monitoring rule will therefore use an *a priori* optimistic Beta(8,2) prior distribution. This distribution corresponds to an assumption that 8 out of 10 patients will proceed successfully through the study as planned and 90% certainty that feasibility is between .57 and .96. This stopping rule will hold enrollment if, given the data, there is at least 90% probability that fewer than 70% of patients are meeting the feasibility criterion.

The table below gives the numbers of patients out of the number on study that would cause the study to be reviewed for feasibility. For example, If only two of the first nine patients are able to meet feasibility criterion and complete the study as expected, the study would be paused.

Stop if number of patients successful	0	1	2	3	4	5	6	7	8	9
in N patients	5	7	9	10	12	13	15	16	18	20

Operating characteristics for feasibility: The operating characteristics of this feasibility rule have been calculated based on 5000 simulations. If the posterior certainty that feasibility is 70% or less, based on Bayes rule and the assumption of a Beta(8.2) prior, is 90% or higher (≥ 9:1 odds against the patients proceeding through the study as planned), further study will be reconsidered. For data simulated with known probabilities of feasibility (θ), the table shows the percent of time that the feasibility rule will determine that the underlying proportion of patients who continue successfully through is below 70%.

True feasibility (θ)	0.30	0.40	0.50	0.60	0.70	0.75	0.80	0.85
% studies stopped	96.5 %	82.8 %	51.0 %	20.3 %	3.5%	1.2%	0.2%	0.1%

10.4 Sample size and accrual

We estimate two to three years of accrual to enroll 20 patients. At the end of the study, according to the stopping rule, at least 10 patients will have met the feasibility benchmarks for the study to complete without a pause. We would probably not recommend this treatment if, given the data, there is a 90% probability that fewer than 70% of patients could continue successfully through the study. If 11 out of 20 patients fail to complete the study successfully, there would be less than 7% probability that the underlying feasibility is 70% or higher.

10.5 Stopping Rules for safety

Mortality, graft failure, and acute and chronic graft versus host disease will be monitored after every patient. We will use probability-based decision rules with assumptions outlined in the table below. Independence was assumed between the four types of toxicities to establish the stopping boundaries. Historical probabilities of these events were obtained from Bacigalupo[1] and Eapen[2]. The prior probabilities for this study are modeled by beta distributions which have means that correspond to the historical estimates. The spread of the prior distributions is described in column 5 as intervals over which we are 90% certain that the estimates of the historical means are within. The last two columns give thresholds for our stopping rule and the certainty with which we must be that the threshold is exceeded before we pause for a review of safety. For all of the endpoints, if there are 3:1 odds that the safety threshold has been exceeded, the study will be paused for a review.

Endpoint	Time (day)	Ref.*	Prior	Spread of prior (90% certainty)	Threshold	Certainty required for stopping
Graft fail	60	17%	Beta(2, 10)	3.3 - 36.4%	25%	75%
aGVHD	100	31%	Beta(3, 6.5)	10.4 - 57.4%	35%	75%
Mortality	180	20%	Beta(2, 8)	4.1 – 42.9%	25%	75%
cGVHD	180	50%	Beta(5, 5)	25.1 – 74.9%	50%	75%

* Historical estimates taken from references.

Stopping rule for graft failure:

Stop if GF	4	5	6	7	8
and N patients	4-6	7-9	10-13	14-17	18-20

Stopping rule for acute graft versus host disease:

Stop if aGVHD	3	4	5	6	7	8	9	10
and N patients	3-4	5-6	7-9	10-11	12-14	15-16	17-19	20

Stopping rule for death:

Stop if death	3	4	5	6	7	8
and N patients	3-4	5-8	9-11	12-15	16-19	20

Stopping rule for chronic graft versus host disease:

Stop if cGVHD	3	4	5	6	7	8
and N patients	3	4-5	6-7	8-9	10	11-12

Stop if cGVHD	9	10	11	12
and N patients	13-14	15-16	17-18	19-20

Operating characteristics of safety stopping rule:
We have assumed independence for establishing our safety monitoring stopping rules. Operating characteristics for each event, assuming the others do not censor or otherwise mask the event, are given below. In practice, these endpoints are not independent and the study stopping criterion could be anti-conservative. While this is a limitation, the lack of preliminary data precluded realistic simulations based on cumulative incidence or multinomial probabilities.

True event prob. (θ)	0.10	.15	.20	.25	.30	.35	.40	.50	.55	.60
Graft failure stopped (%)	0.4	1.7	6.5	15.7	31.7	48.2	64.7	90.0	96.1	98.4
aGVHD stopped (%)	0.4	2.0	5.2	9.9	19.8	32.9	48.9	77.1	88.0	94.1
Death Stopped (%)	0.9	4.3	12.3	26.7	43.1	59.3	75.5	93.4	97.2	99.2
cGVHD Stopped (%)	0.1	0.6	1.6	3.4	6.6	12.5	19.5	44.5	58.8	74.1

10.6 Analysis of Primary Endpoint

10.6.1 The following table shows the 90% credible intervals for the underlying probability of feasibility, based on different numbers of patients successfully meeting the feasibility benchmarks, using a Beta(8,2) prior. The analysis plan is two-sided (5% in each tail), allowing for the full range of possible outcomes, while sample size is based on a one-sided consideration (10% in the upper tail). As an example of the final inference, if 17 out of 20 patients meet the feasibility criterion, we will be fairly confident that feasibility is 70% or higher.

Outcome and 90% Credible Interval		Outcome and 90% Credible Interval	
10 out of 20	(45.1, 74.1)	16 out of 20	(67.1, 90.6)
11 out of 20	(48.6, 77.1)	17 out of 20	(71.2, 93.0)
12 out of 20	(52.1, 80.0)	18 out of 20	(75.4, 95.1)
13 out of 20	(55.7, 82.8)	19 out of 20	(79.8, 97.1)
14 out of 20	(59.4, 85.5)	20 out of 20	(87.2, 100.0)
15 out of 20	(63.2, 88.1)		

10.7 Analysis of Secondary Endpoints

10.7.1 Standard life table methods will be used to report OS. We will report the six-month, one, and two year OS.

10.7.2 We will estimate full donor chimerism by day 60 with an exact 90% binomial confidence interval.

10.7.3 NRM: To estimate the cumulative incidence of non–relapse-related mortality following transplant, a cumulative incidence curve will be produced. Incidence of NRM will be estimated at 60 days, 100 days, six months, and one year along with 90% confidence intervals. Relapse or death will be considered as competing events.

10.7.4 Graft failure: To estimate the day 60 and overall incidence of primary and secondary graft failure following transplant. Exact binomial 90% confidence intervals will be reported.

10.7.5 Acute GVHD: To estimate the cumulative incidence of grade II-IV and grade III-IV acute GVHD from day of transplant. The first day of acute GVHD onset for a given grade will be used to estimate the cumulative incidence curves. Overall cumulative incidence will be estimated along with a 90% confidence interval as well as at 100 days post-transplant. Graft failure, disease progression or death prior to occurrence of acute GVHD will be considered as competing events.

10.7.6 Chronic GVHD: To estimate the cumulative incidence and severity of chronic GVHD from day of transplant, the first day of clinical onset of chronic GVHD will be used to estimate a cumulative incidence curve. Incidences of chronic GVHD at one and two years post-transplant will be estimated along with 90% confidence intervals. Death, disease progression, or graft failure prior to occurrence of chronic GVHD will be considered as competing events.

10.7.7 The cumulative incidence of ANC and platelet recovery will be reported. Death before count recovery will be considered a competing event. Platelet recovery to both 20K and 50K will be reported.

10.7.8	GRFS is defined as the interval from Day 0 to date of first grade 3-4 aGVHD, or chronic GVHD, or relapse, death from any cause, or last patient evaluation. Patients without grade 3-4 aGVHD, chronic GVHD, or who have not progressed or died will be censored at the last date they were assessed. Standard life table methods will be used to report GRFS. We will report the six-month, one, and two year GRFS.
10.7.9	Transplant related toxicities will be summarized descriptively with proportions and exact binomial confidence intervals. TRM: Estimate the probability of death due to causes unrelated to the underlying disease. Standard life table methods will be used to report TRM.

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11.0 RISKS AND BENEFITS

11.1 Risks and toxicity

The major toxicity of using bone marrow from HLA-mismatched donors is GVHD. Using non-myeloablative conditioning regimens and stem cell products from peripheral blood, we would expect the incidence of GVHD in this study to be in the 40-50% range using unmanipulated bone marrow as the source of stem cells.

Another significant risk is failure-to-engraft due to rejection by host lymphocytes. However, because of the non-myeloablative nature of the conditioning regimen we would expect patients to have full autologous, hematologic recovery.

Infection is a major cause of morbidity and mortality in the peri-transplant period (<100d post-BMT). However, given current supportive care and the intensive infection prophylaxis of this protocol, we expect the risk to be acceptable. Prolonged neutropenia may increase this risk in the case of graft rejection, however.

Other risks that may be associated with fludarabine chemotherapy include prolonged immunosuppression of T-lymphocytes increasing the incidence of PCP and viral infections. The extent of this risk is unclear at present. Patients will receive appropriate PCP prophylaxis and will be monitored carefully for evidence of infection by viruses such as CMV, BK and adenovirus. Major risks associated with cyclophosphamide chemotherapy include hemorrhagic cystitis and congestive heart failure.

Relapse of the underlying disease also may occur.

11.2 Benefits

The potential benefits of this trial are prolongation of overall survival.

12.0 INFORMED CONSENT

Patients eligible for marrow grafting are completely evaluated and then presented and approved for transplant at the Bone Marrow Transplant group conference. The group's recommendations are discussed with the patient. If the patient is approved for BMT, the marrow processing procedure itself, the risks of the preparative regimen, risks of BMT complications including infection and GVHD and alternate forms of therapy are presented as objectively as possible. For pediatric

patients (<18 yr of age) assent is obtained from the patient and informed consent is obtained from all parents. Informed consent is obtained from the recipient using the forms approved by the IRB.

13.0 ON-STUDY DATE:

Date of consent signing.

14.0 OFF-STUDY DATE:

Upon completion of "Day 60" evaluations, patients have completed their treatment except for tacrolimus, which continues until day 365. Patient follow-up beyond day 60 will consist of collecting information regarding ongoing engraftment, disease status, late effects of this protocol, acute and chronic graft-vs-host disease, immune reconstitution, additional therapies, and survival. Patients will go off study early in the event of:

1. Death
2. Patient decision (or decision by a parent or guardian on behalf of a minor)

There will be long term follow up, regardless of disease status, to all patients for a minimum of two years.

An eligibility form must be completed for every subject and must be kept in the research chart.

Appendix 1. Consensus conference clinical grading of acute GVHD

Clinical Staging

Stage	**Skin**	**Liver: Total Bilirubin**	**Intestinal Tract: Diarrhea**
0	No rash	<2.0 mg/dL	≤500 ml/day
1	<25% of skin surface	2.0-3.0	500-1000 ml/day
2	25-50%	3.1-6.0	1001-1500 ml/day
3	Erythroderma	6.1-15.0	>1500 ml/day
4	Erythroderma with bullae and desquamation	>15.0	Severe abdominal pain with or without ileus

Clinical Grading

Grade	**Skin***	**Liver**	**GI**
I	1-2	0	0
II	3	1	1
III	-	2-3	2-4
IV	4	4	-

*Each column identifies minimum stage for organ grade

Appendix 2. Ideal Body Weight and Adjusted Ideal Body Weight Calculations

Ideal Body Weight Formula

Males: 50 kg + (2.3 x the number of inches > 5 feet)
Females: 45 kg + (2.3 x the number of inches > 5 feet)

Adjusted Ideal Body Weight Formula

[(actual weight – ideal weight) x 25%] + ideal weight

Note: If actual weight < ideal, use actual weight.
If actual weight > ideal, use corrected ideal.

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