TITLE: Regenerative Medicine to Restore Normal Hematopoiesis in Aplastic Anemia

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

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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 daily with pre-meds
	↓
Days -6, -5	**Fludarabine** 30 mg/M^2 IV daily (adjusted for renal function)
	Cyclophosphamide (CTX) 14.5 mg/kg IV daily
	Start steroid taper from ATG
	↓
Days –4→ -2	**Fludarabine** 30 mg/M^2 IV daily (adjusted for renal function)
Day –1	**TBI** 400 cGy
	↓
Day 0	Infuse bone marrow per institutional standards
	Begin antibiotic prophylaxis
	↓
Days 3, 4	**Cyclophosphamide** 50 mg/kg IV daily
	Mesna 40 mg/kg IV daily
	(First dose of cyclophosphamide must be administered 60-72 hr after START of infusion of marrow)
	↓
Day 5	Begin **tacrolimus** (section 2.5.3.2)
	Begin **MMF** 15 mg/kg PO TID (maximum daily dose 3 g/day)
	(At least 24 hours after the last dose of CY)
	↓
Day 28	Assess Chimerism in peripheral blood, assess GVHD
	↓
Day 35	Discontinue MMF
	↓
Day 56	Assess Chimerism in peripheral blood and bone marrow (with biopsy)
	Assess GVHD
	↓
Day 84	Evaluate disease
	Assess Chimerism in peripheral blood, assess GVHD
	↓
Day 112	Evaluate disease
	Assess Chimerism in peripheral blood, assess GVHD
	↓
Day 180	Evaluate disease
	Assess Chimerism in peripheral blood, assess GVHD
	↓
Day 365	Discontinue tacrolimus if no GVHD and full chimera
	Evaluate disease
	Assess Chimerism in peripheral blood and bone marrow

Contents

1. 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.[5, 20] In most cases (acquired), bone marrow failure is believed to result from autoimmune destruction of hematopoietic stem cells[5]. 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. Goals of upfront therapy should include availability to all patients and avoidance of possibility of clonal evolution.

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 the vast majority of 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 historically have been 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 effective. Currently, patients with acquired SAA, not responsive to initial IST, or inherited AA lacking other treatments options require have required alternative donor transplants. This is becoming more and more feasible and safe. The goal now is to possibly eliminate the need for IST in all patients by moving more efficiently and safely to up front BMT. 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 and move alternative donors to the upfront setting.

1.1. 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[18]. The overall transplant related

mortality (TRM) attributable to HLA-identical sibling BMT is low (10%) and the incidence of severe, grade III-IV acute GVHD is approximately 10-20% after HLA-identical sibling BMT.[6, 15]

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.[15]

1.2. 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.[16] 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, 11] Fludarabine has been used in conditioning for patients with both acquired and constitutional aplastic anemia with good results.[9, 10, 14, 19]

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, 17].

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, 12, 13] 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]

Using the regimen specified in this proposal (with 4.5 mg/kg of Thymoglobulin® over three days) developed at Johns Hopkins, ten refractory or relapsed patients to date have been treated. The median age of these patients at the time of transplant was 35 years (range 17-54). All are currently alive with median follow up of 12.5 months (range 3-44). All patients engrafted with the median time to ANC >500 of 15 days (14-20). Median time to red cell independence was 23.5 days (range 16-58) and median time to platelet engraftment (defined as greater than 50,000 platelets for seven days without transfusion) was 29.5 days (range 22-108). All patients were 100% donor chimerism by day 100. Two patients had grade 1-2 skin aGVHD (22%). Two patients also had cGVHD (22%) grade 2 of the skin and mouth. One patient was able to come off immunosuppression by 15 months and the other by 18 months.

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. The time lost with ineffective IST as well as the risk of acquired secondary clonal disorders as well as more rapid immune reconstitution can be a benefit of upfront BMT for all SAA patients. Given that BMT is the only curative option for these patients, the proposed regimen eliminates patientineligibility 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 rSAA 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 relapsed and refractory SAA with a low incidence of engraftment failure, severe acute GVHD, extensive chronic GVHD, and NRM utilizing post-transplantation CY. This trial will employ this non-myeloablative conditioning regimen along with post-transplantation CY on days +3 and +4 for patients with SAA in the upfront, treatment naïve setting. 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 also allows older patients to use a matched sibling donor upfront or a patient with an inherited marrow failure syndrome to use an unrelated donor with lower rates of GVHD. This is of great benefit to aplastic patients as time to treatment will be shorter and presumed avoidance of longer term complications post IST such as clonal evolution. The purpose of the current trial is to improve response rates and cure for in SAA similar to matched sibling transplant upfront.

2. Study Design

2.1. Study Overview

2.2. This study is a prospective phase II study to assess if it is feasible for previously untreated SAA patients to be transplanted using non-myeloablative conditioning and post transplantation cyclophosphamide.

2.3. Hypotheses and Objectives

2.3.1. Primary Hypothesis Using ATG dose (4.5 mg/kg) together with fludarabine/cyclophosphamide/ TBI and post-BMT cyclophosphamide,we will determine if it is feasible for previously untreated SAA patients to be transplanted using non-myeloablative conditioning and post transplantation cyclophosphamide.

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2.4. Primary Objective

Our primary objective is to determine if it is feasible for previously untreated SAA patients to be transplanted using non-myeloablative conditioning and post transplantation cyclophosphamide.

2.5. Secondary Objectives

2.5.1. To estimate overall survival at one year.

2.5.2. To estimate full donor chimerism by day 60

2.5.3. To estimate the cumulative incidence of non–relapse-related mortality following transplant.

2.5.4. To estimate the incidences of primary and secondary graft failure following transplant.

2.5.5. To estimate the cumulative incidences of grade II-IV and grade III-IV acute graft versus-host disease (GVHD).

2.5.6. To estimate the cumulative incidence of chronic graft versus-host disease (GVHD).

2.5.7. To estimate the cumulative incidence of ANC and platelet recovery.

2.5.8. To estimate GVHD free relapse free survival (GRFS).

2.5.9. To summarize major transplant related toxicities and to estimate transplant related mortality (TRM).

2.6. Eligibility Criteria for Enrollment

2.6.1. Inclusion Criteria

Patients will be enrolled regardless of gender or age. Additional inclusion criteria are:

1. Confirmed diagnosis of SAA (acquired or inherited), either from initial diagnosis or follow-up assessments, defined as:[7]

    a. Bone marrow hypocellularity is required and relative to patient's age (normocellularity is 100- patient age in years).

    (Often marrow cellularity <50% but with <30% residual hematopoietic cells may be applied where appropriate at the discretion of the PI)

    b. Two out of three of the following (in peripheral blood): neutrophils <0.5 $x10^9$/L, platelets <20 $x10^9$/L (without transfusions), reticulocyte count <60 $x10^9$/L.

2. No suitable fully matched related (6/6 match for HLA-A and B at intermediate or high resolution and DRB1 at high resolution using DNA based typing) donor if under age 25 years.

3. Available donor as follows:

    a. HLA haploidentical relative of the patient. A unidirectional mismatch in either the graft versus host or host versus graft direction is considered a mismatch. The donor and recipient must be identical at least one allele (high resolution DNA-based typing) at the following genetic loci: HLA-A, -B, -C, and DRB1. See section 2.4 for additional information.
    b. If patient over age 25 years, may use HLA identical sibling donor.
    c. If patient has inherited bone marrow failure syndrome (IBMFS) and clear evidence of same disorder in potential related donors, unrelated donor may be used. Unrelated donor must be a 10/10 match using HLA-A, -B, -C, DRB1, and DPB1. Unrelated donor may also be used in case of donor specific antibodies to related donors or other clinical causes of unsuitable related donors.

4. Patient and/or legal guardian must sign informed consent for BMT.

5. The potential donor must be willing to donate bone marrow.

6. Adequate organ function defined as:

    a. Cardiac:
        i. Left ventricular ejection fraction (LVEF) at rest ≥40%. For patients aged <13 years, shortening fraction (SF) ≥26% by echocardiogram or LVEF by MUGA may be used.

b. Hepatic:
    i. Total bilirubin <3.0 x the upper limit of normal (ULN) for age (patients who have been diagnosed with Gilbert's Disease are allowed to exceed this limit)
    ii. AST and ALT <5.0 x ULN for age
c. Renal:
    i. For patients ≥13.0 years of age at the time of enrollment: estimated creatinine clearance >50 mL/minute (using the Cockcroft-Gault formula and actual body weight).
    ii. For patients ≥1.0 year of age but <13.0 years of age at the time of enrollment: GFR estimated by the updated Schwartz formula ≥90 mL/min/1.73 m^2. If the estimated GFR is <90 mL/min/1.73 m^2, then renal function must be measured by 24-hour creatinine clearance or nuclear GFR, and must be >50 mL/min/1.73 m^2.
d. Pulmonary
    i. For patients ≥ 8 years of age (or otherwise able to complete pulmonary function testing per established American Thoracic Society standards), DLCO (corrected/adjusted for hemoglobin) >40% *and* FEV1 >50% predicted (without administration of bronchodilator) *and* FVC >50% predicted.
    ii. For patients <8years of age unable to perform PFTs due to age or developmental ability: (1) no evidence of dyspnea at rest *and* (2) no need for supplemental oxygen *and* (3) O2 saturation >92% on room air.

7. Karnofsky or Lansky performance status ≥ 70%

8. Females and males of childbearing potential must agree to practice 2 effective methods of contraception at the same time, or agree to abstinence.

2.6.2.Exclusion Criteria

1. Patient with previous administration of immunosuppressive therapy for SAA.

2. Patients with Fanconi anemia. At a minimum, the diagnosis of Fanconi anemia must be excluded by diepoxybutane (DEB) or equivalent testing on peripheral blood or marrow in patients younger than 30 years of age. (Additional mutational testing may have been performed in a clinical or research capacity on a per patient basis but is not considered an exclusion criteria)

3. Clonal cytogenetic abnormalities consistent with pre-myelodysplastic syndrome (pre-MDS) or MDS on marrow examination (e.g. Monosomy 7).

4. Presence of anti-donor HLA antibodies (positive anti-donor HLA antibody is defined as a positive cross-match test of any titer by complement-dependent cytotoxicity or flow cytometric testing or the presence of anti-donor HLA antibody to the high expression loci HLA-A, B, C, or DRB1 with mean fluorescence intensity (MFI) >1000 by solid phase immunoassay).

5. Prior allogeneic stem cell transplant.

6. Prior solid organ transplant.

7. Uncontrolled bacterial, viral, or fungal infection at the time of enrollment. Uncontrolled is defined as currently taking medication and with progression or no clinical improvement on adequate medical treatment.

8. Seropositivity for the human immunodeficiency virus (HIV).

9. Active Hepatitis B or C determined by serology and/or NAT

10. Female patients who are diagnosed as pregnant by beta BCG testing (per institutional practice) or breast-feeding.

11. Prior malignancies except resected basal cell carcinoma or treated cervical carcinoma in situ. Cancer treated with curative intent > 5 years previously will be allowed. Cancer treated with curative intent ≤ 5 years previously will not be allowed unless approved by the PI.

2.7. Donor Selection

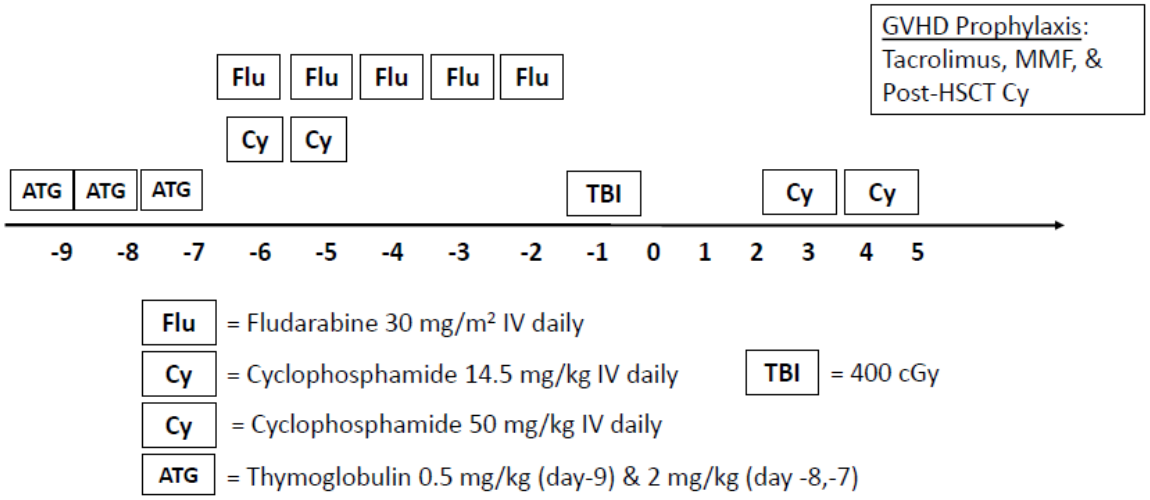
1. Donor must be medically, socially, and psychologically fit to donate.

2. Haploidentical donor will be prioritized where medically appropriate.

    a. Donors must be at least 5/10 HLA-matched relative. Donor selection is multi-faceted and takes into many variables such as donor health, donor age, and donor and recipient gender parity, CMV status, and ABO compatibility (see BMT Policy and Procedures Manual).

    b. Unrelated donors, where used, will also be chosen based on current institutional standards and must be 10/10 matches. (See BMT Policy and Procedures Manual).

2.8. Treatment Plan



2.8.1. Conditioning for Bone Marrow Recipients

2.8.1.1. Thymoglobulin

Thymoglobulin® preparation of ATG will be dosed by actual body weight. On day -9, thymoglobulin 0.5 mg/kg is administered and 2 mg/kg on day -8 and day -7. 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. Premedication should follow local institutional practice, but should include a minimum of 1 mg/kg methylprednisolone in children or 100 mg in adults prior to the infusion (equivalent steroid allowed), preferably repeated in 3-4 hours during the infusion.

2.8.1.2. Fludarabine

Fludarabine 30 mg/m^2 IV daily for 5 days, from day -6 to day -2 (total dose received 150 mg/m^2), will be dosed on actual body weight. Fludarabine will be dosed according to the recipient's actual body weight. Fludarabine should be dose-reduced based on creatinine clearance according to institutional standard of care. Fludarabine dosing is based on the last creatinine clearance prior to the start of conditioning. Fludarabine dose should not change on Days -6 to -2, unless the patients creatinine clearance changed but the Cockgroft Gault calculation by greater than 30% from baseline creatinine clearance.

2.8.1.3. Pre-BMT Cyclophosphamide

Hydration prior to cyclophosphamide may be given according to institutional standards. Cyclophosphamide 14.5 mg/kg IV daily for 2 days from day -6 to day -5 and administered as a 1-2 hour infusion (total dose received 29 mg/kg). Doses of cyclophosphamide and mesna are based on the patient's IBW unless actual body weight is less than IBW, in which case use actual body weight. If the patient weighs more than 125% of IBW, cyclophosphamide will be doses according to the adjusted IBW. Hydration, as well as doses and schedule for uroprotective agents (i.e. mesna) may be administered per institutional practice.

2.8.1.4. Total Body Irradiation

Total body irradiation (TBI) will be delivered in a single dose of 400cGy on day -1. TBI may be delivered from either linear accelerator or Cobalt sources (per institutional practice).

2.8.2. Donor Marrow Product Handling and Infusion

Patients will receive unmanipulated marrow unless there is a major ABO incompatibility, in which case red blood cells will be depleted from the donor marrow using institutional practices. Institutional practices will determine if there will be processing for minor ABO incompatibilities. Donor bone marrow will be harvested with a target yield of 4 x10^8 nucleated cells per kilogram of recipient IBW and a minimum yield of 2.5 x10^8 nucleated cells per kilogram of recipient IBW. If the minimum yield is not obtained, patient will be removed from the study. It is recommended that no more than 10 mL per aspirate be taken. In addition to calculating the total nucleated cell dose per kilogram, a sample of the product to be infused will be sent for flow cytometry to determine the content of CD34+ cells. The use of cryopreserved marrow is not permitted.

Note: The patient is not to receive steroids as an antiemetic or any other immunosuppressive agent from day 0 until at least 24 hours after completions of day +4 cyclophosphamide dose unless used for adrenal support or during medical emergency (e.g., treatment of anaphylaxis).

2.8.3.GVHD Prophylaxis

2.8.3.1. Post-BMT Cyclophosphamide

Cyclophosphamide 50 mg/kg IV daily will be given for 2 days, days +3 to +4 (total dose received 100 mg/kg) after transplantation. Doses of cyclophosphamide and mesna are based on IBW unless actual body weight is less than IBW, in which case use actual body weight. If the patient weighs more than 125% of IBW, cyclophosphamide will be doses according to the adjusted IBW. Hydration, as well as doses and schedule for uroprotective agents (i.e. mesna) should follow local institutional practice. (Mesna has generally been utilized for the Day 3 and Day 4 post BMT cyclophosphamide doses, but not for the lower pre-BMT cyclophosphamide doses)

2.8.3.2. Tacrolimus

For adult patients, Tacrolimus IV or PO (per institutional standards) should be started on day +5 (24 hours after the end of the infusion of the CY) and administered per institutional standards to maintain a level of 10-15 ng/mL. Tacrolimus will be continued through day 365 and then stop without taper.

2.8.3.3. MMF

MMF 15 mg/kg PO TID up to 1 gm TID (or IV equivalent) starting on day +5, (24 hours after the end of the infusion of the CY)will be discontinued after the last dose on day +35. MMF dosing may continue if active GVHD is present.

2.8.3.4. Growth factor use

G-CSF will be given IV or SQ starting on day +5 at 5 mcg/kg/day until ANC is >1000 for 3 days. Additional GCSF may be administered as warranted if the counts nadir again later in the course. Pegfilgrastim (Neulasta)® and GM-CSF are not permitted.

2.8.4. GVHD Treatment

In the event of the development of either acute or chronic GVHD, therapy will be at the discretion of treating centers.

2.8.5. Supportive Care

2.8.5.1. Infectious disease prophylaxis

Antifungal prophylaxis will be administered according to institutional practices. It is important to follow levels of tacrolimus for patients receiving one of the azole antifungal medications. The combination of both drugs can raise the levels of the immunosuppressant to toxic levels. If a patient on tacrolimus is started on an azole antifungal medication, a dose reduction of the tacrolimus is required and levels should be obtained to ensure they are not in the toxic range.

Pneumocystis jiroveci pneumonia (PJP) prophylaxis will be administered according to institutional practices. Recommendations include starting approximately one month post-BMT (or later if WBC not recovering) and continuation until at least three months off of all immunosuppressive medications.

Viral prophylaxis for herpes simplex virus (HSV) and varicella zoster virus (VZV) will be administered according to institutional practices. Recommendations include continuation for at least one year post-BMT and/or while on immunosuppressive medications.

Prophylactic and empiric antibiotics as well as intravenous immunoglobulin (IVIG) will be administered according to institutional practices. Re-immunization may be performed according to institutional practices.

2.8.5.2. Infectious disease monitoring

CMV viremia as tested by DNA PCR will be monitored as per institutional standard. Patients who are viremic or show evidence of end organ CMV disease may be treated according to institutional practices.

EBV viremia as tested by DNA PCR will be monitored per clinician discretion. If the patient becomes viremic with a DNA PCR level of 1000 copies/mL or higher on two consecutive occasions, recommendation includes the use of Rituximab (375 mg/m^2 IV x1). If the patient develops persistent EBV viremia or signs/symptoms of EBV-related post-transplant lymphoproliferative disease (PTLD) despite rituximab administration, they may be treated according to institutional practices.

HHV-6 viremia as tested by DNA PCR is suggested to be monitored weekly until day +60. If the patient becomes viremic, they may be treated according to institutional practices.

2.8.5.3. Management of graft failure

Patients experiencing primary or secondary graft failure may be managed according to institutional practices.

2.8.5.4. Management of ATG Intolerance

Patients experiencing a new, severe or life-threatening reaction to ATG and therefore unable or unwilling to receive the full planned cumulative dose will continue on study, but their conditioning regimen may then be altered as per institutional preference or practice with documentation of the deviation.

2.8.6. Risks and Toxicities

2.8.6.1. General

The agents being used in the study are used extensively in the BMT setting and have well-defined toxicity profiles. In addition, there are many expected toxicities of allogeneic BMT. The following are examples of toxicities that are serious but not unexpected: Grade 4 cytopenias; neutropenic fever and sepsis; bacterial, fungal, or viral (including CMV, BK virus) infection; severe mucositis; severe GVHD; hepatic veno-occlusive disease; pulmonary toxicities; hemorrhagic cystitis; bleeding without hemodynamic compromise; death.

2.8.6.2. Drug Information

2.8.6.2.1. Fludarabine

Fludarabine is a purine analog antimetabolite. Side effects of fludarabine include:

1. Neurotoxicity: Agitation or confusion, blurred vision, loss of hearing, peripheral neuropathy or weakness have been reported. Severe neurologic effects, including blindness, coma, and death may occur; severe CNS toxicity is rarely seen with doses in the recommended range for nontransplant therapy. The dose used in this study is approximately 1.5 times the usual one-course dose given in non-transplant settings. Doses and schedules similar to those used in this study have been used in adult and pediatric patients without observed increase in neurotoxicity.

2. Anemia: Life-threatening and sometimes fatal autoimmune hemolytic anemia has been reported after one or more cycles of therapy in patients with or without a previous history of autoimmune hemolytic anemia or a positive Coombs test and who may or may not be in remission. Corticosteroids may or may not be effective in controlling these episodes. The majority of patients re-challenged developed a recurrence of the hemolytic process.

3. Cardiovascular: Deep venous thrombosis, phlebitis, transient ischemic attack, and aneurysm (1%) are reported.

4. Fever: 60% develop fever.
5. Rash: 15% develop a rash, which may be pruritic.
6. Digestive: Gastrointestinal side effects include: nausea/vomiting (36%), diarrhea (15%), stomatitis (9%), anorexia (7%), GI bleeding and esophagitis (3%), mucositis (2%), liver failure, abnormal liver function test, constipation, dysphagia (1%) and mouth sores.
7. Some other effects include: Chills (11%), peripheral edema (8%), myalgias (4%), osteoporosis (2%), pancytopenia, arthralgias (1%), dysuria (4%), urinary tract infection and hematuria (2%); renal failure, abnormal renal function test, and proteinuria (1%); and, very rarely, hemorrhagic cystitis and pulmonary toxicity.

Dose adjustments of fludarabine are required for renal insufficiency (sections 2.5.1).

2.8.6.2.2. Cyclophosphamide & Mesna

Cyclophosphamide is an alkylating agent whose metabolites form cross-links with DNA resulting in cell cycle-nonspecific inhibition of DNA synthesis and function. Cyclophosphamide side effects include: nausea, vomiting, diarrhea, headache, dizziness, hemorrhagic cystitis, fluid weight gain/edema, SIADH, transaminitis, cardiomyopathy, pericarditis, rash, mucositis, alopecia, cytopenias, sterility, and rarely, secondary myelodysplastic syndrome and anaphylaxis.

Dose adjustments for cyclophosphamide will not be made.

Mesna (sodium-2-mercapto ethane sulphonate) 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.

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, potential adverse effects include nausea and vomiting, diarrhea, abdominal pain, altered taste, rash, urticaria, headache, joint or limb pain, hypotension, and fatigue.

2.8.6.2.3. Thymoglobulin®

Thymoglobulin is a rabbit preparation of anti-thymocyte globulin (ATG). Common side effects include nausea, fever, chills, diarrhea, rash, dizziness, headache and tiredness. More serious side effects can include severe allergic reaction, serum sickness, easy bleeding/bruising, fast/irregular heartbeat, joint/muscle pain, stomach/abdominal pain, and weakness. Because this drug works by weakening the immune system, it lowers the ability to fight infections. No dose adjustments are required.

2.8.6.2.4. Mycophenolate Mofetil (MMF)

MMF is an ester prodrug of the active immunosuppressant mycophenolic acid (MPA).

Side effects include: pancytopenia, infection (including sepsis, CMV, HSV, VZV, and Candida), nausea, vomiting, diarrhea, allergic reactions, hypertension, headache, dizziness, insomnia, hyperglycemia, electrolyte imbalances, rash, and leg cramps/bone pain.

Drug interactions: MMF activity is decreased with oral antacids and cholestyramine. There are no pharmacokinetic interactions with co-trimoxazole, oral contraceptives, or cyclosporine. Acyclovir or ganciclovir blood levels may increase due to competition for tubular secretion. High doses of salicylates or other highly protein-bound drugs may increase the free fraction of MPA and exaggerate the potential for myelosuppression.

Dose adjustments: No dose adjustments are required for liver dysfunction. For renal insufficiency, MMF dosing should not be modified unless dialysis is needed, in which case MMF can be reduced to 25-50% of the starting dose.

2.8.6.2.5. Tacrolimus

Tacrolimus is a macrolide immunosuppressant that inhibits lymphocytes through calcineurin inhibition.

Toxicities: There is a spectrum of well-described toxicities of tacrolimus. Toxicities include renal insufficiency, hypertension, hyperglycemia, hypomagnesemia, hypokalemia, nausea, diarrhea, headache, neurologic toxicity including tremor and leukoencephalopathy, infection, and rarely thrombotic thrombocytopenic purpura (TTP).

Drug interactions: Tacrolimus is well absorbed orally. Tacrolimus is extensively metabolized by the cytochrome P-450 (CYP3A4) system and metabolized products are excreted in the urine. Drugs that may increase tacrolimus levels include tri-azole drugs (especially voriconazole and posaconazole), nephrotoxic drugs, calcium channel blockers, cimetidine and omeprazole, metoclopramide, macrolide antibiotics, quinupristin/dalfopristin, danazol, ethinyl estradiol, methylprednisolone, and HIV protease inhibitors. Drugs that may decrease tacrolimus levels include some anticonvulsants (phenobarbital, phenytoin, carbamazepine), caspofungin, rifamycins, and St. John's wort.

Dose adjustments: The tacrolimus dose is adjusted to maintain a serum trough level of 5-15 ng/mL, with a target of 10-15 ng/mL. Patients with hepatic or renal insufficiency should receive doses at the lower end of therapeutic concentrations. No dose adjustments are required in patients undergoing hemodialysis.

Due to extreme interactions with voriconazole and posaconazole, the tacrolimus dose should be empirically lowered when these azoles are initiated at steady state levels of tacrolimus. Guidelines are provided in the table below. Dose adjustments for therapy with other azoles may be indicated. However, the initial tacrolimus dose (on Day 5) remains fixed.

Dosing considerations with concurrent azole therapy: Triazole antifungal medications are expected to increase serum CNI levels; therefore dosages of CNI's should be adjusted accordingly. Guidelines are provided in the table below. Of note, reversal of azole-mediated inhibition of CYP3A4 (and others) and P-glycoprotein is gradual when azoles are stopped. Therefore, immediate significant dose increases in tacrolimus are not advised when azoles are stopped. Rather, tacrolimus dose increases should be cautious and based on more frequent monitoring of levels as appropriate.

Table 1: Suggested preemptive dose reduction of tacrolimus when azoles are initiated at steady state levels of tacrolimus

Antifungal	**Tacrolimus**

	Dose ↓	Comment
Voriconazole	67%	Strongly advised
Posaconazole	67%	Advised
Itraconazole	50%	Advised
Fluconazole	25%	Consider

2.8.6.2.6. Filgrastim (G-CSF)

Administration of G-CSF can cause bone pain, increased levels of liver enzymes and uric acid in the blood, thrombocytopenia, headaches, fatigue, local irritation at injection site, nausea, bleeding, fever, allergic reaction, splenomegaly or rupture of the spleen, worsening of pre-existing skin rashes, temporary hair loss, and inflammation of a blood vessel in the skin.

2.8.6.2.7. Total Body Irradiation (TBI)

TBI can cause nausea and vomiting, diarrhea, parotitis (rapid onset within 24-48 hours, usually self-limited), generalized mild erythema (usually within 24 hours, resolving in 48-72 hours), hyperpigmentation, fever, mucositis, alopecia, and pancytopenia. Late effects include: cataracts (10-20%), hypothyroidism, nephropathy, interstitial pneumonitis, veno-occlusive disease, carcinogenesis, and sterility.

2.8.6.3. Toxicity Grading

Toxicities are graded using the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) Version 4.03.
(http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm)

3. STUDY ENDPOINTS AND DEFINITIONS

3.1. Primary Endpoint

Feasibility: Feasibility will have been met with these benchmarks: the patient has the transplant, achieves engraftment, and survives one year.

3.2. Secondary Endpoints

3.2.1. Overall Survival (OS)

OS at 1 year post-BMT in patients with SAA. OS is defined as the time interval from date of transplant to death or to last follow-up.

3.2.2.Neutrophil Engraftment

Neutrophil engraftment is achieving an ANC >0.5 x10^9/L for three consecutive measurements on different days, with the first of the three days being defined as the day of neutrophil engraftment.

3.2.3.Platelet Engraftment

Platelet engraftment is defined by achieving a platelet count >20 x 10^9/L with no platelet transfusions in the preceding seven days. The first day of the sustained platelet count will be defined as the day of platelet engraftment.

3.2.4. Alive with Sustained Engraftment

Being alive and engrafted is defined as not having experienced death, primary graft failure, or secondary graft failure.

3.2.4.1. Primary Graft Failure

Primary graft failure is defined by the lack of neutrophil engraftment by day 56 post-BMT or <5% donor chimerism on all measurements up to and including day 56.

3.2.4.2. Secondary Graft Failure

Secondary graft failure is defined by initial neutrophil engraftment (ANC >0.5 x10^9/L measured for three consecutive measurements on different days) followed by sustained subsequent decline in ANC to < 0.5 x 10^9/L for three consecutive measurements on different days or initial whole blood or marrow donor chimerism more than 5%, but then declining to <5% on subsequent measurements.

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

The cumulative incidences of acute grade III-IV and grade III-IV GVHD will be determined through competing risk analysis. Treatment of disease relapse/progression/persistence (with the exception of planned maintenance or consolidative therapy), graft failure, and death are considered competing risks for GVHD for study purposes. In addition, GVHD will be reported with only graft failure and death regarded as competing risks.

3.2.6.Chronic GVHD: Chronic GVHD is graded by both NIH consensus criteria and Seattle criteria. Date of onset, date of biopsy confirmation (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.

3.2.7.Immune Reconstitution

Quantitative assessments of peripheral blood CD4, CD19 and CD56 positive lymphocytes will be done through flow cytometric analysis at baseline, 3, 6 and 12 months post transplantation. Results will be tabulated according to time from transplant.

3.2.8.Infection

Infections will be tracked and reported according to section 5.2.

3.2.9 Clonal hematopoiesis

This will be tracked clinically only with conventional karyotyping and PNH testing. Research somatic mutational testing will be assessed from pre BMT samples as well as per Table 3.

3.3. Study Monitoring

3.3.1. Follow-up Schedule

The follow-up schedule for study visits is outlined in Table 2.

Table 2: Follow-Up Schedule

Study Visit	Target Day Post-Transplant
1 week	7 ± 3 days
2 week	14 ± 3 days
3 week	21 ± 3 days
4 week	28 ± 3 days
8 week	56 ± 5 days
12 week	84 ± 7 days
6 month	180 ± 28 days
12 month	365 ± 45 days

3.3.2. Patient Evaluations

Table 3 summarizes patient clinical assessments over the course of the study.

Table 3: Summary of Assessments

Study Assessments / Testing	Baseline	7	14	21	28	56	84	180	365
Weight and height	X								
History and physical exam					X	X	X	X	X
Karnofsky/Lansky Performance Status	X				X	X	X	X	X
CBC with differential and platelet counts[1]	X	X	X	X	X	X	X	X	X
Blood Chemistries[2]	X	X	X	X	X	X	X	X	X
HLA Typing & Anti-Donor Antibody Testing	X								
Infectious Disease Titers[3]	X								
LVEF or Shortening Fraction for <13 years	X								
Pulmonary Function Tests[4]	X								
Bone Marrow Aspirate for Pathology, Quantitative CD34 count and Cytogenetics[5]	X					X			X
Testing for Fanconi Anemia[6]	X								
Mutational testing preBMT where clinically appropriate (May include somatic mutational testing; telomere length analysis or known gene testing)	(X) not required unless clinically indicated by treating physician								X (repeat only when positive initially
Pregnancy Test (females only)	X								
CD4, CD19, and CD56 Counts (by flow cytometry when available, not mandatory)	X						X	X	X
Serum Quantification of IgG, IgM, and IgA	X								X
Peripheral Blood Chimerism[7]	X				X	X	X	X	X

Bone marrow chimerism						X			X
CMV DNA Quantitative PCR Testing[8]					X	X	X		
Toxicity Assessments[9]		X	X	X	X	X	X	X	X
GVHD assessments		X	X	X	X	X	X	X	X

Notes:

[1] CBC performed three times weekly from Day 0 until ANC > 0.5 x 10^9/L for three consecutive measurements on different days. CBC then performed twice per week until Day 28, then weekly until 84 days, then at days 180, and 365.

[2] Blood chemistries include: creatinine, bilirubin, alkaline phosphatase, AST, ALT, ferritin (baseline only), and tacrolimus level. Tacrolimus levels will be measured at least once weekly until Day 84 and then at each follow-up visit until the drug is stopped. Blood chemistries performed twice weekly until Day 30 and then weekly until Day 84, then at days 180, and 365.

[3] Infectious disease titers include: CMV IgG, hepatitis B/C panel (HepB SAb, HepB SAg, HepB Core Ab, HepC Ab), and HIV.

[4] DLCO (corrected for Hb), FEV1, and FVC or pulse oximetry for patients aged <8 years if unable to perform standard PFT.

[5] Bone marrow aspirate and biopsy (cytogenetics is required, local MDS (somatic mutational panel recommended) within 60 days of enrollment.

[6] Results of Diepoxybutane (DEB) testing on peripheral blood or comparable testing on marrow for Fanconi anemia at any time prior to enrollment. Required only for participants < 40 years old at the time of enrollment.

[7] Chimerism to be measured by standard molecular testing of a peripheral whole blood sample.

[8] To be done as per institutional practices. It is recommended to start at Day 28 and continue weekly or every other week monitoring until off all immunosuppression.

[9] Toxicities should be monitored and reported continuously through day 365 as per section 5.2.

[10] 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 participants 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.

4. STATISTICAL CONSIDERATIONS

Overall Study Design

This study is designed as a Phase II trial to assess the safety and efficacy of upfront BMT using primarily related donors (and unrelated donors in cases of clear inherited predisposition to marrow failure), with Thymoglobulin® (ATG) containing preparative regimen in patients with untreated severe aplastic anemia. The target enrollment is 20 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, achieves engraftment, 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).

4.1. Primary Objective

Our primary objective is to determine if it is feasible for previously untreated SAA patients to be transplanted using non-myeloablative conditioning and post transplantation cyclophosphamide.

4.2. Secondary Objectives

4.2.1. To estimate overall survival at one year.

4.2.2. To estimate full donor chimerism by day 60

4.2.3. To estimate the cumulative incidence of non–relapse-related mortality following transplant.

4.2.4. To estimate the incidences of primary and secondary graft failure following transplant.

4.2.5. To estimate the cumulative incidences of grade II-IV and grade III-IV acute graft versus-host disease (GVHD).

4.2.6. To estimate the cumulative incidence of chronic graft versus-host disease (GVHD).

4.2.7. To estimate the cumulative incidence of ANC and platelet recovery.

4.2.8. To estimate GVHD free relapse free survival (GRFS).

4.2.9. To summarize major transplant related toxicities and to estimate transplant related mortality (TRM).

4.3. Early stopping guideline for feasibility

Feasibility of non-myeloablative, partially HLA-mismatched, unrelated (or related if older patient age) transplantation in previously untreated SAA will be based on three benchmarks: the patient has the transplant, achieves engraftment, and survives one year. Estimates of one year survival for upfront non-myeloablative SAA transplants between July 1999 and June 2012 were obtained from the Center for International Blood and Bone Marrow Transplant Research (CIBMTR). For patients ≤ 20 years (N=1126) one year survival was 79% (95% CI: 74-84%) and in patients over the age of 20 (N=975) it was 68% (95% CI: 62-74%).

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%

4.4. 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.

4.5. 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. 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. Historical probabilities of these events in patients transplanted after relapse were obtained from Bacigalupo[1] and Eapen[2]. The patients in this

trial will be treated upfront and therefore the incidence of these events may differ somewhat. 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.

Patients who develop both acute and chronic GVHD will be regarded as having adverse GVHD events for both stopping rules.

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%	15%	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 for patients transplanted after relapse.

Stopping rule for graft failure:

Stop if GF	2	3	4	5
and N patients	2-6	7-11	12-17	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.

True event prob. (θ)	0.10	.15	.20	.25	.30	.35	.40	.50	.55	.60
Graft failure stopped (%)	17.2	36.5	58.1	75.7	86.6	94.2	97.2	99.7	100	100
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

4.6. Analysis of Primary Endpoint

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)		

4.7. Analysis of Secondary Endpoints

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

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

4.7.3. 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

4.7.4. 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.

4.7.5. 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.

4.7.6. 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.

4.7.7. 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.

4.7.8. 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.

4.7.9. 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.

Statistical References:

[1] Bacigalupo, A., Socie', G., Lanino, E., Prete, A., Locatelli, F., Locasciulli, A., Cesaro, S., Shimoni, A., Marsh, J., Brune, M., Van Lint, M., Oneto, R., and Passweg, J. Fludarabine, cyclophosphamide, antithymocyte globulin, with or without low dose total body irradiation, for alternative donor transplants, in acquired severe aplastic anemia: a retrospective study from the EBMT-SAA working party. Haematologica, 95(6): 976–982, 2010.

[2] Eapen, M., Le Rademacher, J., Antin, J.H., Champlin, R.E., Carreras, J., Fay, J., Passweg, J.R., Tolar, J., Horowitz, M.M., Marsh, J.C., Deeg, H.J. Effect of stem cell source on outcomes after unrelated donor transplantation in severe aplastic anemia. Blood, 118(9):2618-21, 2011.

4.7.10. Analysis of Secondary Endpoints

Proportion of Patients Alive and Engrafted:
The proportion of patients alive and engrafted at one year will be estimated along with a 95% confidence interval.

Neutrophil Engraftment
Cumulative incidence of neutrophil engraftment at Day 56 will be estimated with a 95% confidence interval using the cumulative incidence function with death prior to neutrophil engraftment as the competing risk.

Platelet Engraftment
Cumulative incidence of platelet engraftment at Day 100 will be estimated with a 95% confidence interval using the cumulative incidence function with death prior to platelet engraftment as the competing risk.

Primary and Secondary Graft Failure
The frequency and proportion of patients experiencing graft failure by Day 56 and the proportion of patients who have engrafted who subsequently experience secondary graft failure will be described with 95% confidence interval.

Grade II – IV Acute GVHD
Cumulative incidence of Grade II – IV acute GVHD at Day 100 will be estimated with a 95% confidence interval using the cumulative incidence function with death prior to Grade II – IV acute GVHD as the competing risk.

Chronic GVHD
Cumulative incidence of chronic GVHD at one year will be estimated with a 95% confidence interval using the cumulative incidence function with death prior to chronic GVHD as the competing risk.

Immunologic Reconstitution
Immune reconstitution assays including CD4, CD19, and CD56 will be measured pre-HCT, and at 3, 6, and 12 months post HCT. These will be summarized at each time point using descriptive statistics.

Incidence of Infectious Complications
The number of infections and the number of patients experiencing infections will be tabulated. Incidences of CMV viremia and disease, EBV viremia and PTLD will be reported.

5. DATA MANAGEMENT

Data will be maintained on case report forms and appropriate Cell Therapy Laboratory spreadsheets and forms. The research team will make assessments of GVHD. Evaluation by history and physical examination for GVHD will be performed as per BMT unit standards. For study purposes, weekly GVHD summaries will be taken from these standard examination from Day 14-Day 60.Hematopoietic engraftment will be assessed by the BMT attending and the PI. The PI will be responsible for evaluation of chimerism data and weekly overall toxicities.

5.1. 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.

5.2. Internal Data Monitoring and Adverse Event reporting

The PI 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 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.

For study purposes, the following will be recorded and reported as Serious Adverse Events in accordance with IRB requirements:

a. Events that are unexpected and possibly, probably, or definitely related to transplant will be reported in real-time; all other events (e.g. neutropenic fever) will be reported on a yearly basis.
b. Any death before Day 100, and any later death which is possibly, probably, or definitely transplant-related, will be reported to IRB in real-time.
c. Graft failures associated with failure of neutrophil recovery to >500/mm^3 by Day 60 after BMT.
d. 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.

In addition, the following toxicities will be tracked for study purposes:

a. Clinically significant infections during the first year of transplant, with the exception of uncomplicated, culture-negative neutropenic fever. This includes CMV disease, other clinically significant documented viral infections, bacterial infections, and proven or probable invasive fungal infections.
b. CMV reactivation (including asymptomatic reactivation)
c. Hepatic veno-occlusive disease
d. All grade 3-5 AEs by CTCAE 4.03 criteria

5.3. External Data Monitoring and Auditing

The SKCCC Compliance Monitoring Program will provide external monitoring for JHU-affiliated sites in accordance with SKCCC DSMP (Version 6.0, 02/21/2019).

5.4. Safety Monitoring

The SKCCC Safety Monitoring Committee (SMC Subcommittee will determine the level of patient safety risk and level/frequency of monitoring.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.

5.5. Risks and Benefits

5.5.1. 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.

5.5.2. Benefits

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

5.6. 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.

5.7. On-study Date

Date of consent signing.

5.8. Off-study Date

Upon completion of Day 365 evaluations, subjects have completed their participation on this study. Participants will be taken off study early in the event of:

1. Death

2. Participant decision (or decision by a parent or guardian on behalf of a minor)

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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