A Phase II Trial of Non-Myeloablative Conditioning and Transplantation of Partially HLA-Mismatched and HLA-Matched Bone Marrow for Patients with Sickle Cell Anemia and Other Hemoglobinopathies

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TREATMENT SCHEMA

Days -9 — Thymoglobulin 0.5 mg/kg IV with pre-meds
Start steroid taper

↓

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*

↓

Days –4→ -2 — Fludarabine 30 mg/M^2 iv qd

↓

Day –1 — TBI 400 cGy

↓

Day 0 — Infuse bone marrow and start penicillin

↓

Days 3, 4 — CTX 50 mg/kg iv q d
Mesna 40 mg/kg iv q d**
<u>(First dose of CTX must be administered 48-72 hr after infusion of marrow)</u>

↓

Day 5 — Begin sirolimus (section 6.6)** and
MMF 15 mg/kg po tid with maximum daily dose 3 gm/d

↓

Day 30 — Assess Chimerism in peripheral blood

↓

Day 35 — Discontinue MMF

↓

Day 60 — Assess Chimerism in peripheral blood

↓

Day 180 — Evaluate disease
Assess Chimerism in peripheral blood

↓

Day 365 — Discontinue sirolimus
Evaluate disease
Assess Chimerism in peripheral blood

↓

1 yr, 2 yrs — Evaluate disease
Assess Chimerism in peripheral blood

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

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1.0 OBJECTIVES

1.1 Obtain estimates of transplant-related mortality (TRM) and progression-free survival in patients with severe hemoglobinopathies receiving non-myeloablative conditioning and transplantation of partially human leukocyte antigen (HLA)-mismatched bone marrow (haploidentical) from relatives ("mini-haploBMT") as well as HLA-matched donors.

1.2 Characterize donor hematopoietic chimerism in peripheral blood at days ~30, ~60, and ~180 after mini-haploBMT.

1.3 Characterize hematologic and non-hematologic toxicities of mini-haploBMT.

2.0 BACKGROUND

Allogeneic blood or marrow transplantation (alloBMT) is a curative therapy for a variety of hematologic disorders, including sickle cell disease and other hemoglobinopathies such as thalassemia. Even when it is clear that alloBMT can give to these patients an improvement in their disease[1], myeloablative transplants have important toxicities and mortalities associated. Substantial progress has been made recently in the development of reduced intensity conditioning regimens that facilitate the sustained engraftment of donor marrow with reduced toxicity. Most of these regimens incorporate highly immunosuppressive purine analogues, such as fludarabine, which allow the reduction or elimination of myeloablative agents such as busulfan or total body irradiation without endangering the sustained engraftment of HLA-identical allogeneic stem cells. Preliminary results of non-myeloablative allogeneic stem cell transplantation, or NST, suggest that the procedure can be performed in patients who are ineligible for myeloablative alloBMT, and that sustained remissions of several hematologic malignancies can be obtained.

Despite the encouraging results in hematologic malignancies, the results of nonmyeloablative alloBMT in patients with hemoglobinopathies are less encouraging. Recently, Jacobsohn et al reported on 13 patients with non-malignant conditions undergoing nonmyeloablative alloBMT[2]. Engraftment was poor in patients with hemoglobinopathies as only one patient engrafted out of 4 patients. These findings have been duplicated in other small studies[3,4]. Also, the lack of suitable donors continues to be a limit to access to transplantation. Therefore, developing novel strategies that address the issue of expanding donor pool and have different immune suppression are of paramount relevance for the therapy of sickle cell disease.

In the past five years, we have been developing non-myeloablative conditioning regimens for transplantation of marrow from partially HLA-mismatched, or haploidentical, bone marrow from first-degree relatives. The main goal of J9966 (RPN 99-11-05-01) was to titrate the dose of pre- and post-transplantation cyclophosphamide (CTX), a potent immunosuppressive drug, given in conjunction with pre-transplantation fludarabine and total body irradiation (TBI), to achieve a regimen that had an acceptably low risk of graft rejection and GVHD, the two major complications of haploidentical BMT. All patients received mycophenolate mofetil and tacrolimus, beginning on day 4 or 5 and terminating on days 35 and 50-180, respectively, to reduce the incidence and severity of GVHD. The first cohort of three patients received no pre-transplantation CTX and 50 mg/kg CTX IV on day 3, and two of the patients rejected their grafts. A second cohort of 20 patients received 14.5 mg/kg CTX IV on days –6 and –5 in addition to 50 mg/kg IV on day 3. Of 18 evaluable patients, 13 patients had donor engraftment on day 60, but accrual of patients to this dose level was stopped because 8/13 patients developed severe GVHD, an incidence convincingly in excess of the stopping criterion of 20%. To reduce the incidence of GVHD, a third cohort of patients received an additional dose of CTX 50 mg/kg IV on day 4, and MMF dosing was increased from bid to tid, based upon pharmacokinetic data suggesting the need for more frequent dosing. Of seventeen evaluable patients so far, two patients have had non-fatal graft rejection, and only one patient treated according to the protocol has had severe GVHD (an additional patient developed severe GVHD after withdrawal of immunosuppression to treat relapse). Two patients have died of causes other than relapse: one from GVHD, and the other from disseminated fungal infection. Of the sixteen patients who have been followed up to 100 days for relapse, eight have relapsed at a median of 64 days (range 24-~100) after transplantation, and six patients are alive and disease free at a median of 206 days (range, 100-429 days [as of Feb 8, 2004]) following BMT.

In order to better judge the safety and efficacy of our non-myeloablative BMT protocol, the tables below compare the results of J9966, dose level 3, to the results of the four largest published trials of HLA-identical sibling peripheral blood versus bone marrow transplantation for early stage leukemia.

Engraftment Data

Author	**N (PB/BM)**	**Median age**	**ANC 500/mm^{3}†**	**Plt 20K†**	**Plt 50K†**
Blaise[5]	48/52*	37/36	15/21	13/21	15/26
Bensinger[6]	81/91	42/42	16/21	13/19	NA
Schmitz[7]	163/166	39/37	12/15	15/20	20/26
Couban[8]	109/118	45/44	19/23	16/22	NA
J9966	**17**	**31**	**16**	**24**	**31**

*Numbers represent: recipients of peripheral blood/recipients of bone marrow
†Time from transplantation to designated count, sustained without transfusion

Outcomes data

Author	aGVHD II-IV (%)	aGVHD III-IV (%)	cGVHD (%)	TRM (%)	Relapse (%)
Blaise	45/42*	17/28	55/30	23/21†	6/11
Bensinger	64/57	15/12	46/35	21/30†	14/25
Schmitz	52/39	28/16	66/50	24/24*	12/7
Couban	44/44	26/18	40/30	7/16†	15/20
J9966	**47**	**13**	**NA**	**13**	**50**

*Numbers represent: recipients of peripheral blood/recipients of bone marrow
†Transplant-related mortality (TRM) or relapse over entire study (median f/u ~ 2 years)
†100 day mortality

Compared to the patients receiving HLA-identical sibling bone marrow following myeloablative conditioning, patients on J9966 were younger, took longer to engraft platelets, and had a substantially higher rate of relapse, but were similar in the time to neutrophil recovery, the incidence of GVHD, and transplant-related mortality (TRM). The higher rate of relapse for patients on J9966 may be attributable to a benefit of myeloablative conditioning in reducing the risk of relapse, or that patients on J9966 had advanced, poor prognosis hematologic malignancies, which relapse more frequently than early leukemias after alloBMT.

Since the toxicities of non-myeloablative haploidentical BMT were not known when the trial was written, eligibility for J9966 was restricted to patients with advanced, poor-risk hematologic malignancies, such as chronic myeloid leukemia in 2nd chronic phase, advanced myelodysplasia, acute leukemia in 2nd remission, and lymphoma in relapse after autologous BMT. We have now expanded eligibility to ‘standard risk’ hematologic malignancies in trial J0457, which is still on going.

Between J9966 and J0457, 56 patients with hematologic malignancies received cyclophosphamide 50 mg/kg IV, either once (on day 3) or twice (on days 3 and 4) after non-myeloablative conditioning and haploidentical bone marrow transplant. Most of these patients had advanced disease or failed a previous autologous transplant. All were conditioned as outpatients with fludarabine, cyclophosphamide, and total body irradiation, transplanted with non-T cell-depleted marrow, and treated with tacrolimus and mycophenolate mofetil beginning the day after the last dose of cyclophosphamide. The most interesting finding was that compared to patients receiving a single dose of post-transplant cyclophosphamide, those receiving two doses had significantly less grade II-IV aGVHD (43% vs. 78%; p=.01) and grade III-IV aGVHD (20% vs. 53%; p=.006) by day 200 after transplant. Death from GVHD occurred in 5/13 assessable patients receiving one dose versus 2/28 assessable patients receiving 2 doses of cyclophosphamide.

Since the data to date suggest that our treatment regimen may be as safe as HLA-identical sibling BMT after myeloablative conditioning, non-myeloablative haploidentical BMT may be considered a reasonable treatment option for patients who have hemoglobinopathies. Also, the novel use of post-transplant cyclophosphamide on top of the nonmyeloablative conditioning, emerges as an interesting option of immunotherapy to prevent graft rejection. Moreover, as cancer relapse is not a concern in the setting of sickle cell disease, engraftment with NST should be curative.

In clinical transplantation, antithymocyte globulin (ATG) has been used extensively in conventional myeloablative and non-myeloablative conditioning regimens to facilitate engraftment in patients with sickle cell disease[14]. This effect is largely mediated by *in vivo* T-cell depletion produced by the ATG, similar to the effect of monoclonal T-cell antibodies in the murine model.

Given that sirolimus (as opposed to calcineurin inhibitors) has not been commonly associated with the posterior reversible encephalopathy syndrome (PRES), we decided to change the immunosupression, switching sirolimus for tacrolimus. Also, given the very encouraging results obtained after 10 patients, and given that few patients may have a suitable HLA-matched donor, we have decided to expand the protocol to include these patients.

As of June 2011 we have transplanted 11 patients on study and of these, 6 have engrafted (54%) with no transplant related mortality, only 1 case of mild acute GvHD and no cases of chronic GvHD. While these results are actually very good considering the fact that all these patients have been haploidentical, there is a clear need for improvement. Experimental data using high dose cyclophosphamide has clearly shown that in order to increase engraftment efficiency there are 2 clear strategies to follow: increase the intensity of the conditioning (such as increasing the dose of TBI) or increase the cell dose of the graft[9, 10]. Increasing the intensity of the conditioning can translate into increasing toxicities, therefore, we are interested on increasing the cell count in the graft. As with standard bone marrow harvest there may be a limit on the number of cells that can be harvested, “priming the marrow” with filgrastim comes as an attractive option[11-13].Studies using bone marrow priming with filgrastin show that it is possible to double the number of nucleated cells from the marrow, and interestingly, high cell doses have not been associated with increased risk of GvHD or severe toxicities to the donor[12, 13].

Recently published data have shown that on patients at high risk of graft failure receiving non-myeloablative bone marrow transplants, the use of slightly higher doses of total body irradiation improve the probabilities of engraftment[17,18]. Given that we still facing graft failures in the order of 30% in our clinical trial, we decided to increase the dose of TBI to 400cGy after discussions within the protocol team and with Radiation Oncology[19]. The higher dose may be associated with a higher incidence of sterility in men, and the patient has performed sperm banking and will be informed about this increased risk. However, we do not think will increase graft-versus-host disease rates given that our graft-versus-host disease rates using high-dose cyclophosphamide are similar between myeloablative and non-myeloablative transplants[20,21]. Moreover. Published data showed that TBI at 400cGy can be safely done in transplants using low intensity conditioning without increasing toxicities even on patients receiving mis-matched unrelated transplants[22,23].

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 **Sirolimus** (rapamycin, Rapamune®)

Sirolimus is an immunosuppressant that inhibits cytokine-stimulated T-cell activation and proliferation, and also inhibits antibody formation.
The mean bioavailability of sirolimus after administration of the tablet is ~27% higher than the oral solution. Sirolimus oral tablets are not bioequivalent to the oral solution. Clinical equivalence has been demonstrated at the 2-mg dose level; however, it is not known if higher doses are clinically equivalent on a mg to mg basis.
a) <u>Sirolimus oral solution</u>: Sirolimus oral solution (1 mg/mL) should be stored protected from light and refrigerated at 2°C to 8°C (36°F to 46°F). For dilution, the appropriate dose should be measured using an amber oral syringe, then added to a glass or plastic container that holds at least 60 mL. Before taking the dose, it should be diluted with water or orange juice then taken immediately; <u>it should not be diluted with grapefruit juice</u>. The syringe should be discarded after one use. Sirolimus oral solution provided in bottles may develop a slight haze when

refrigerated, which does not affect product quality; allow the product to stand at room temperature and shake gently until the haze disappears.

b) Sirolimus tablets: Sirolimus tablets are available in 1 mg and 2 mg tablets that cannot be crushed or broken. Sirolimus tablets should be stored at 20° to 25° C (68°–77°F), protected from light.

The most common adverse reactions of sirolimus are: peripheral edema, hypertriglyceridemia, hypercholesterolemia, hypertension, increased creatinine, constipation, abdominal pain, nausea, diarrhea, headache, fever, urinary tract infection, anemia, thrombocytopenia, arthralgia, pain. Adverse reactions that have resulted in rates of sirolimus discontinuation >5% were increased creatinine, hypertriglyceridemia, and thrombotic thrombocytopenic purpura (TTP) / thrombotic microangiopathy (TMA). Sirolimus toxicities are summarized:

	Common (>20%)	**Occasional** (5-20%)	Rare (<5%)
Immediate (within 1-2 days)	Headache (L), hypertension (L), immunosuppression (L), fever, nausea, diarrhea, constipation	Chest pain, insomnia, dyspepsia, vomiting, ***dyspnea***	Hypotension, asthma, cough, flu-like syndrome, tachycardia, anorexia, ***hypersensitivity reactions***
Prompt (within 2-3 weeks)	Tremor (L), renal dysfunction, pain (abdominal, back, arthralgias), ***hyperlipidemia*** [c] *(hypercholesterolemia, hypertriglyceridemia),* hyperglycemia, edema including ***peripheral edema*****,** anemia	Elevated LFT's (with elevated sirolimus levels) [a], stomatitis, infections (including UTI, URI), mild ***thrombocytopenia***, ***leukopenia***, electrolyte disturbances (hyper/hypokalemia [L], hypophosphatemia, hypomagnesemia [L]), rash, hives, pruritus, ***delayed wound healing or dehiscence (L), proteinuria, TTP/HUS/TMA*** [b] especially with concurrent CNI	Pleural and pericardial effusions, ***pulmonary toxicity*** (non-infectious pneumonitis, BOOP, pulmonary fibrosis), thombosis, myalgias
Delayed (any time later during therapy, excluding above conditions)	Acne		Kidney disease, CHF, ascites, arthrosis, bone necrosis, osteoporosis
Late (any time after completion of treatment)			Lymphoproliferative disorders, skin malignancies
Unknown frequency and timing	Embryo/fetotoxic; unknown whether excreted in human milk		

(L): Toxicity may also occur later.
[a] Significant transaminitis, generally without sequellae, may occur. Sirolimus has been associated with higher rates of venoocclusive disease after myeloablative conditioning.
[b] Incidence 3% to < 20% in a trial of kidney transplantation. In allogeneic BMT, increase in TMA from 4.2% with tacrolimus or cyclosporine alone, versus 10.8% with tacrolimus/sirolimus combination was noted.[66]
[c] Lipid-lowering agent may be required; consider if fasting serum triglycerides are > 2.5 x ULN, and recommend starting if > 800 mg/dL.

Drug interactions: Sirolimus is known to be a substrate for both cytochrome CYP3A4 and P-glycoprotein. Agents that may increase sirolimus levels include tri-azole drugs (especially voriconazole and posaconazole*), amiodarone, calcium channel blockers, macrolide antibiotics (but not azithromycin), micafungin, gastrointestinal prokinetic agents (cisapride, metoclopramide), cimetidine, cyclosporine, grapefruit juice, and HIV protease inhibitors. Agents that may decrease sirolimus levels include anticonvulsants (carbamezepine, phenobarbital, phenytoin), rifamycins, St. John's Wort.

Dose adjustments: The sirolimus dose is adjusted to maintain a serum trough level of 5-15 ng/mL. Changes in levels due to altered bioavailability should be apparent within 24-48 hours. For sirolimus without CNI as in this study, a 20-25% reduction of sirolimus dose is recommended for trough levels >12 – 18 ng/mL, and a 20-25% increase is recommended for trough levels < 3 ng/mL. Renal failure does not affect the excretion of sirolimus. Excretion is reduced in liver failure; impaired hepatic function should prompt consideration of reduction in sirolimus maintenance doses but no dose adjustment of the loading dose is necessary.

Due to extreme interactions with voriconazole and posaconazole, these drugs are relatively contraindicated during sirolimus therapy. Sirolimus dose is to be reduced by 90% when voriconazole is initiated and should also be significantly reduced with posaconazole.

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. ATG should not be administered during the weekend.

4.0 PATIENT SELECTION

4.1 Criteria for recipient eligibility

4.1.1 Patients who are ineligible for BMT from an HLA-matched, sibling donor can proceed to a haplo-BMT. Patients with an HLA-matched related donor will proceed to a matched BMT.

4.1.2 Age 2-70 years

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. 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 Eligible diagnoses: Patients with sickle cell anemia such as sickle cell anemia (Hb SS), Hb S/ β° thalassemia, Hb S/ β+ thalassemia, Hb SC disease, Hb SE disease, Hb SD disease, Hemoglobn SO-Arab disease Hb S/hereditary persistence of fetal hemoglobin. Other significant hemoglobinopathies that also fulfills a criterion from 4.1.7.

Plus one of the following:
4.1.7:

a. Stroke or central nervous system event lasting more than 24 hours.
b. MRI changes indicative of brain parenchymal damage.
c. MRA evidence of cerebrovascular disease.
d. Acute chest syndrome requiring exchange transfusion or hospitalization.
e. Recurrent vaso-occlusive pain crisis (more than 2/year for the last 2 years).
f. Stage I or II sickle lung disease.
g. Sickle retinopathy.
h. Osteonecrosis.
i. Red cell alloimmunization (>2 antibodies) during long-term transfusion.
j. Constellation of dactylitis in the first year of life and a baseline haemoglobin <7 g/dL and leukocytosis (>13.4 x $10^3/mm^3$) in the absence of infection during the second year of life.
k. History of invasive pneumococcal disease.
l. Pitted RBC count >3.5% during the first year of life.
m. Abnormal transcranial Doppler.
n. Transfusion dependence.
o. β thalassemia major.

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 Poor performance status (ECOG>1).

4.2.3 Poor cardiac function: left ventricular ejection fraction <35%.

4.2.4 Poor pulmonary function: FEV_1 and FVC<40% predicted.

4.2.5 Pulmonary hypertension moderate to severe by echocardiographic standards.

4.2.6 Poor liver function: direct bilirubin $\geq$3.1 mg/dl

4.2.7 HIV-positive

4.2.8 Minor (donor anti-recipient) ABO incompatibility if an ABO compatible donor is available.

4.2.9 Prior transfusions from donor or recipient if caused alloimmunization vs. donor cells.

4.2.10 Women of childbearing potential who currently are pregnant (□-HCG^{+}) or who are not practicing adequate contraception.

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

4.3 Criteria for donor eligibility

4.3.1 Weight ≥ 20kg.. For donors < 18 years, the maximum recipient weight (actual body weight) should not exceed 1.25 times the donor weight (actual body weight).

4.3.2 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.)

4.3.3 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:

HLA crossmatching (in order of priority)

1.Mutually compatible (no cross-matching antibodies)

2.Recipient non-cross-reactive with donor, donor cross-reactive with recipient
3.Mutually cross-reactive

ABO compatibility (in order of priority)

Compatible
Major incompatibility
Minor incompatibility
Major and minor incompatibility

4.3.4 Donors will be selected to minimize HLA mismatch in the host-versus-graft direction.

4.3.5 Donor must have a hemoglobin S =/< ~50%.

5.0 TREATMENT PLAN

5.1 Indwelling central venous catheter

Placement of a double lumen central venous catheter will be required for administration of IV medications and transfusion of blood products.

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: hemoglobin S, hemoglobin electrophoresis, hemoglobin F (fetal), free hemoglobin, reticulocyte count.

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. ATG should not be administered during the weekend. 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: 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 will be administered by intravenous infusion over 30 min. on D-6 to D-2. The dose will be 30 mg/m^2.

For decreased creatinine clearance (< 61 ml/min) determined by the Cockcroft Formula:

$$C_{Cr} = \frac{(140 - \text{age}) \text{ x weight (kg)}}{P_{Cr} \text{ x } 72} \text{ x } 0.85 \text{ (for women)}$$

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

In pediatric patients (<18 years of age) the creatinine clearance must be measured.

For decreased creatinine clearance (CrCl), fludarabine dosage should be reduced as follows:

CrCl ≥ 70 ml/min – fludarabine 30 mg/m2
CrCl 40-69 ml/min - fludarabine 24 mg/m2
CrCl 20-39 ml/min – fludarabine 20 mg/m2
CrCl < 20 ml/min – fludarabine 15 mg/m2

Cyclophosphamide will be administered as an iv infusion over 1- 2 hr, (depending on volume) on D-6 and D-5. The dose of pre-transplantation cyclophosphamide is 14.5 mg/kg/day. Dose is calculated based on the adjusted 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”.

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: 400 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.

5.4 Bone marrow transplantation and graft information

Bone Marrow will be harvested and infused on day 0.

Donor bone marrow will be harvested with a target yield of 4 x 10^8 nucleated cells/kg recipient IBW. 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.

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 (IBW)] will be given on D+3 post-transplant (within 48-72 hr of marrow infusion) 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 immuno suppressive agents are given from the time of the transplant until 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 Sirolimus and Mycophenolic Acid Mofetil (MMF).

Sirolimus for patients $\geq$ 18 years old: A one-time sirolimus loading dose, 6 mg PO, is given on Day 5, at least 24 hours after Cy completion. Sirolimus is then continued at a maintenance dose (start 2 mg PO QD), with dose adjustments to maintain a trough of **5 – 15 ng/mL** as measured by HPLC or immunoassay**.** There is no planned taper. Sirolimus prophylaxis is discontinued after the last dose on Day 365, or may be continued if there is GVHD or other indication such as mixed chimerism. Sirolimus troughs should be checked at minimum weekly. For patients < 18 years old: Sirolimus dosing is based on actual body weight; however an adjusted body weight may be used if the actual weight is > 50% greater than IBW. A one-time sirolimus loading dose, 3 mg/m^2 PO with the dose not to exceed 6 mg, is given on Day 5, <u>at least 24 hours after Cy completion</u>. Sirolimus is then continued at a maintenance dose (start 1 mg/m^2 PO QD, maximum 2 mg PO QD), with dose adjustments to maintain a trough similar to the one described for adults. There is no planned taper. Sirolimus prophylaxis is discontinued after the last dose on Day 365, or may be continued if there is GVHD or other indication such as mixed chimerism. Sirolimus troughs should be checked at minimum weekly.

Mycophenolic acid mofetil will be given at a dose of 15 mg/kg po 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 Sirolimus 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 syncitial virus, influenza A, B and parainfluenza viruses if symptomatic. 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.

An oral antibiotic for gastrointestinal decomination will be administered according to institutional preference until the ANC is >1000 for 3 consecutive days following BMT.

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 sirolimus levels on patients receiving azoles as the combination of both drugs can raise the levels of the immunesuppressant to toxic levels. If the patient on sirolimis is started on azoles, a dose reduction of sirolimus 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 at Day 21 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 either dapsone, atovaquone, or pentamidine as PCP prophylaxis.

On Day 0, all patients should start pneumococcal prophylaxis with Penicillin-V 250 mg PO BID, or if less than 5-years old, 125 mg PO BID and should continue indefinitely. If patient has a Penicillin-V allergy, Bactrim SS every day should be utilized.

Viral prophylaxis for HSV will be administered according to institutional preference.

Although not required, 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 pre-emptively with ganciclovir (5 mg/kg iv q12 hr) for 14 d and then with maintenance ganciclovir (5 mg/kg iv qd) until CMV testing is negative for at least 2 wk. Consideration should be given to administration of CMV hyperimmune globulin (Cytogam), concomitant with ganciclovir at a dose of 150 mg/kg iv qod times 4 doses and then weekly thereafter if the patient continues to be treated with ganciclovir for persistent viremia or antigenemia. If the patient is diagnosed with CMV disease, more frequent Cytogam administration may be considered.

Unless the patient is being treated for CMV infection or CMV disease, ganciclovir should be discontinued for development of neutropenia (ANC<500). If neutropenic, G-CSF may be used if clinically indicated. Ganciclovir should then be restarted, if appropriate. Ganciclovir will be dose-adjusted appropriately for renal failure.

5.8 Growth factor support

Patients will receive G-CSF (Filgrastim®) only if clinically required (i. e. sepsis) but will not routinely be given after transplant to speed recovery given the high incidence of musculoskeletal pain that may aggravate a pain crisis in these patients.

5.9 Transfusion support

Packed red cell transfusions will be given per current institutional recommendations. Platelets will be kept at all times over 50,000/mm^3 (see below). 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.

5.10 Antiseizure prophylaxis

Prophylaxis against seizures is mandatory in all recipients and should be commenced at the start of conditioning with fludarabine (day -6). Levetiracetam (Keppra) will be administered at a dose of 500 mg p.o. b.i.d. Seizure prophylaxis should be continued until sirolimus is discontinued.

Platelets should be kept at all times over 50,000/ml. Serum magnesium level should be maintained > 1.5 mg/dL during the period of treatment with calcineurin inhibitors to reduce the risk of seizures.

5.11 Anti-ovulatory treatment

Menstruating females should be started on an anti-ovulatory agent prior to the initiation of the preparative regimen.

6.0 PATIENT MONITORING

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

	Initial	Allowable time frame from date of consent**	Day < 60	≈Day 30 ± 7	≈Day 60 ± 7	Suspected GVHD
History and Physical	X	within 30 days		X	X	
Performance status	X	within 30 days				
Disease staging	X	within 30 days		X	X	
CBC & Diff.	X	within 7 days	*weekly	X	X	
Comprehensive Metabolic Panel	X	within 7 days	weekly	X	X	

Hemoglobin S, hemoglobin electrophoresis, hemoglobin F (fetal), free hemoglobin, reticulocyte count	X			X	X	
CXR	X	within 30 days				
Pregnancy test (women, childbearing age)	X	within 30 days				
Chimerism analysis Including unsorted and CD3	X			X	X	
PT, PTT	X	within 30 days				
EKG	X	within 60 days				
ECHO	X	within 60 days				
HepB Ag, HBC Ab, HCV Ab, HSV IgG, CMV IgG, RPR, HIV, VZV IgG (if possible)	X	within 30 days				
Toxicity assessment	X			X	X	
HLA typing/lymphocytotoxic screen	X	Must be done at JHH				
PFTs (Spirometry and DLCO)	X	within 30 days				
Sinus CT	X	within 30 days				
Fasting lipid profile	X				X	
Skin Biopsy						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.

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 treatment and response
b) previous transfusions and transfusion reactions
c) previous serious infections
d) allergies
e) current medications
f) assessment of performance status

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
ii. PT, PTT
iii. ABO and Rh typing

b) Chemistries

i. Comprehensive chemistry panel
ii. Routine and microscopic urinalysis with C&S
iii. Fasting lipid profile.

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
ii. Sinus CT scan
iii. Pulmonary function tests including at least FEV1 and FVC (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) RFLP studies will be drawn as a baseline for subsequent engraftment studies including myeloid (unsorted) and CD3 chimerism.

g) Disease specific studies: The following are minimal recommended studies.

i. Sickle Cell Anemia: Hemoglobin S, hemoglobin electrophoresis, hemoglobin F (fetal), free hemoglobin, reticulocyte count

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. RFLP donor chimerism on peripheral blood (including myeloid (unsorted) and CD3 chimerism).
3. Disease evaluation.
4. CBC and differential, comprehensive panel.

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.

6. Fasting lipid profile.

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 3.0 for the first 60 days post BMT.

Since this trial is an out-patient trial, the definition of an adverse event 'interfering with daily function' and 'clinically significant' will be events that require hospitalization. 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.

The following is a list of categories that will not be recorded unless the event becomes a grade 4 or meets the criteria of a SAE, as stated in section 8.1.1.

- Allergy/Immunology
- Auditory/Hearing
- Cardiovascular (Arrhythia)
- Cardiovascular (General)
- Coagulation
- Constitutional symptoms
- Dermatology/Skin
- Endocrine
- Hemorrhage
- Hepatic
- Infection/Febrile neutropenia
- Lymphatics

- Metabolic/Laboratory
- Secondary Malignancy
- Sexual/Reproductive Function

The following categories will be recorded only if the event becomes a grade 3 or grade 4 or meets the criteria of a SAE.

- Gastrointestinal
- Musculoskeletal
- Neurology
- Ocular/Visual
- Pain
- Pulmonary
- Renal/Genitourinary

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 $\geq$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.

The following information shall be collected on all patients with acute GVHD:

Date of onset (defined as the date of first biopsy confirming GVHD)
GVHD evaluation form at the time of onset, weekly until GVHD resolves, and Day 60
Initial overall clinical grade
Maximum overall clinical grade

Date of onset of grade III-IV acute GVHD, if any

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 Transplant-related mortality (TRM)

Causes of TRM, 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, and then yearly per Johns Hopkins standards. Disease evaluations will be performed by collecting the following laboratory tests: hemoglobin S, hemoglobin electrophoresis, hemoglobin F (fetal), free hemoglobin, reticulocyte count.

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 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 $\geq$ 500/uL. 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

Donor chimerism will be measured in the peripheral blood around day 30 and day 60. Patients with any amount of 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 will be defined as >0%, but <95%. Complete donor chimerism will be defined as $\geq$95%.

8.4 Disease Status

Disease status will be evaluated through the following laboratory tests: hemoglobin S, hemoglobin electrophoresis, hemoglobin F (fetal), free hemoglobin, 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.

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. 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

Data will be maintained on case report forms and appropriate Graft Engineering Laboratory spreadsheets. The research team 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. The PI will be responsible for evaluation of chimerism data and weekly overall toxicities.

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 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.

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/mm^3 by day ~60 after transplantation, and any unexpected events as deemed significant by the PI. The PI will be responsible for reporting events to the CRO/IRB and to 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.

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

The primary objective of this phase II clinical trial is to obtain risk-stratified estimates of two-year progression-free survival with a precision of +/- 20% (95% confidence bound). To obtain this precision, it will be necessary to accrue at least 50 patients. We expect the accrual to take 10 years based on a recruitment of 6-7 patients per year once the study reaches a steady accrual of patients.

The hypothesis of this study is that non-myeloablative conditioning with high-dose post-transplant cyclophosphamide will increase the number of sickle cell disease (SCD) patients eligible for allogeneic BMT by allowing the safe and effective use of related haploidentical donors.

The purpose of expanding the sample size in this study is to obtain a better estimate of the proportion of patients who achieve stable mixed donor-host hematopoietic chimerism following transplant. In transplanted patients with these diseases, 10-15% chimerism can constitute cure where patients are symptom-free as long as they continue with immunosuppression treatment to maintain the graft.

Sirolimus immunosuppression replaced tacrolimus by protocol amendment in August 2011 to reduce the incidence of posterior reversible encephalopathy syndrome. A second amendment included G-CSF-primed bone marrow grafts to improve engraftment. Since these two protocol amendments, 16 patients have been treated with Sirolimus and G-CSF-primed marrow. Fourteen patients have follow-up and T cell counts through day 60. Engraftment has been achieved in 10 of 14 patients (71.4%). Of the patients that have engrafted, 6 of 10 patients (60%) have achieved full-donor chimerism and 2 of the 4 patients without full chimerism (50%) have achieved at least 15% chimerism. The follow-up of patients with full chimerism is 2 years for 3 patients, 1 year for 4 patients, 6 months for 2 patients, and 1 month for 1 patient.

Two of the three patients accrued since January of 2014 with follow-up through day 60 have failed to engraft and therefore this protocol amendment will remove GCSF and increase the TBI. With additional follow-up, the 16 patients receiving the Sirolimus plus G-CSF modified treatment plan will allow us to estimate the proportion of patients with mixed chimerism and the proportion of patients with full chimerism with exact 95% binomial confidence intervals of ± 25%.

There are an additional 19 patients to be accrued to the study under the new treatment plan. The proportions of patients with full and mixed chimerism in this group will be estimated with exact 95% confidence intervals of ±25%.

The total sample size of 50 patients will allow us to estimate these proportions with exact 95% binomial confidence intervals of ± 14.5%.

Safety stopping guidelines: The safety stopping criterion for this study is currently based on monitoring the combined incidence of grade III-IV acute GVHD and TRM, and the study design called for stopping if it appeared that this combined incidence was convincingly greater than 20%. This combined incidence will continue to be monitored after every patient in this expansion cohort. Although acute GVHD and TRM are not mutually exclusive, the mortality from acute GVHD is expected to be low. Therefore, a patient who experiences grade III-IV acute GVHD then TRM will be counted once as having the GVHD adverse event. The stopping rule for toxicity will halt enrollment to the study if the posterior probability of risk being larger than 0.2 is 70% or higher. If the original prior for the first patients enrolled on the study was Beta(1,1) and we use a conservative assumption of one acute GVHD event in the cohort of 26 patients treated so far, Beta(1,25), the new prior for this toxicity monitoring rule is Beta(2, 26). This means that after the first 26 patients on trial our posterior estimate of a patient's risk of toxicity is 7.1%, and there is 90% probability that this risk is between 1.3% and 16.4%. As we treat more patients and observe whether or not each experiences an adverse event (i.e., severe acute GVHD or TRM), we update the estimate of the risk and apply the stopping rule. If the posterior probability is at least 70% that the risk of toxicity is 20% or higher, computed using Bayes rule and these assumptions, we will consider stopping the study. The following table shows the stopping boundary. For example, the rule will call for stopping the study if 7 toxicity events are observed before the 10th patient is enrolled in the expansion cohort.

Conclude regimen too toxic*	7	8	9	10	11	12
out of N patients	7-9	10-14	15-18	19-23	24-28	29-30

*Study will stop early if evidence suggests the regimen is too toxic.

The next table shows the simulated operating characteristics of the stopping boundary applied to further enrollment of 30 patients under different hypothetical risks of toxicity. Based on 5,000 simulated studies, if the underlying risk of toxicity is 40%, the stopping rule would call for the study to be stopped 63% of the time with an average sample size of 24.

Hypothetical toxicity	0.10	0.15	0.20	0.25	0.30	0.35	0.40	0.45
% of Time Study Stops	0.0%	0.4%	1.5%	8.5%	22.0 %	43.0 %	63.2 %	82.0 %
Expected Sample Size	30.0	30.0	29.9	29.4	28.3	26.3	23.7	20.8

11.0 RISKS AND BENEFITS

11.1 Risks and toxicity

The major toxicity of using bone marrow from HLA-mismatched, related donors is GVHD. Using nonmyeloablative conditioning regimens and stem cell products from peripheral blood, two different studies with a total of 61 patients have shown an incidence of clinically significant GVHD (>Grade I) of 15-20%[15, 16]. There are no reports of mini-BMT from PMRD to estimate the incidence of GVHD but two small studies presented in abstract form from European and Israeli transplant centers show approximately a doubling of GVHD incidence with mini-BMT from MUDs. Therefore, 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 nonmyeloablative 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, or palliation of disease-related symptoms.

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 sirolimus, 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)
3. Unacceptable toxicity associated with protocol therapy, as determined by the treating physicians in consultation with the investigators.

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

Reference List

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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.