VICCNCCTT12108: A Non-Myeloablative Conditioning and Transplantation of Partially HLA-Mismatched and HLA-Matched Bone Marrow for Patients with Sickle Cell Disease and Other Hemoglobinopathies NCT01850108

Amendment Number: 5
Amendment Date: December 04, 2017

Summary of Changes

Revised/Added Section	Revision Location in Tracked Change Protocol (PDF page numbers)	Description of Revision
Entire Protocol	Throughout protocol	Minor formatting and grammatical revisions, headers and page numbers updated
Cover pages	Pages 1-3	• Amendment 4 Summary of changes deleted • Amendment number and date updated • Co-Investigators updated • Table of Contents updated
Treatment Schema	Pages 5-6	• Thiotepa dosing clarified • Gonadal shielding added to TBI • Cyclophosphamide (CTX) dose timing clarified • Day 100 visit added
Secondary Objective 1.2	Page 7	• Day 100 visit added
Section 3.4	Page 13	• Thiotepa dosing clarified
Section 3.9	Page 16	• Information about corticosteroid use added
Section 3.10	Page 17	• Information about Total Body Irradiation added
Section 4.3	Pages 19-20	• Criteria for HLA-haploidentical first-degree relatives added to donor eligibility • Donor exclusion criterion added for donors with clinically significant hemoglobinopathy (sickle trait is acceptable)
Section 5.3	Page 21	• Thiotepa dosing clarified
Section 5.5	Page 22	• Cyclophosphamide (CTX) dose timing clarified
Section 5.10	Pages 25	• Updated from “Anti-seizure prophylaxis” to “Prevention of post-BMT Neurological Events and Posterior Reversible Encephalopathy Syndrome (PRES)” • Hypertension management for the prevention of CNS toxicity guidance provided
Section 5.13	Page 25-26	• Contraception guidance added

Revised/Added Section	Revision Location in Tracked Change Protocol (PDF page numbers)	Description of Revision
Section 5.14	Page 26	• Guidance for the management of slow engraftment and graft failure added

Section 6.0	Pages 27-28	• Schedule of assessments table updated
		  ○ Formatting updated
		  ○ Timing of informed consent
		  ○ Day 100 visit added
		  ○ Day 180 visit added
		  ○ Timing of tests pre-enrollment clarified
		  ○ Window for annual visits added
		• Additional footnotes added to clarify assessments

Section 6.1.2	Page 29	• Echo/MUGA updated to include estimation of TRJ velocity and BNP
Section 6.2	Pages 30-31	• Updated to include Day 100 and Day 180 visits
Section 7.4	Page 34	• Updated to include Day 100 visit
Section 8.3	Page 34-35	• Updated to include Day 100 visit
Section 8.4	Page 35	• Updated to include Day 100 visit
Section 9.1	Pages 37-39	• Updated SAE reporting criteria and procedures • Updated Data Monitoring and Auditing language
Section 13.0	Page 42	• Updated to include Day 100 visit

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TREATMENT SCHEMA

DAY	DRUG	DOSE
-9	Thymoglobulin	0.5 mg/kg IV with pre-meds Start steroid taper Start anti-seizure prophylaxis
-8	Thymoglobulin	2 mg/kg IV daily with pre-meds
-7	Thymoglobulin Thiotepa	2 mg/kg IV daily with pre-meds Thiotepa 5 mg/kg IV q 12h (10 mg/kg total)
-6	Fludarabine Cyclophosphamide (CTX)	30 mg/M^2 IV daily 14.5 mg/kg IV daily*
-5	Fludarabine Cyclophosphamide (CTX)	30 mg/M^2 IV daily 14.5 mg/kg IV daily* Donor starts G-CSF on day -5
-4	Fludarabine	30 mg/M^2 IV daily
-3	Fludarabine	30 mg/M^2 IV daily
-2	Fludarabine	30 mg/M^2 IV daily
-1	TBI	200 cGy with gonadal shielding
0	Infuse G-CSF primed bone marrow (start antimicrobial prophylaxis**)	
+3	Cyclophosphamide (CTX) Mesna (First dose of CTX must be administered 60-72 hour after infusion of marrow)	50 mg/kg IV daily 40 mg/kg IV daily**
+4	Cyclophosphamide (CTX) Mesna	50 mg/kg IV daily 40 mg/kg IV daily**
+5	Begin Sirolimus MMF	(section 5.6) ** and 15 mg/kg PO TID with maximum daily dose 3 gm/day
+30	Assess Chimerism in peripheral blood	

+35	Discontinue MMF
+60	Assess Chimerism in peripheral blood
+100	Assess Chimerism in peripheral blood
+180	Evaluate disease Assess Chimerism in peripheral blood
+365	Discontinue sirolimus Evaluate disease Assess Chimerism in peripheral blood
+1yr, 2 yrs	Evaluate disease Assess Chimerism in peripheral blood

* Refer to Section 5.0 (Treatment plan) for complete dosing instructions.
** Or as per institutional standards.

1.0 Objectives

Primary 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 from first-degree relatives ("mini-haploBMT") as well as HLA-matched donors.

Secondary Objectives

1.2 Characterize donor hematopoietic chimerism in peripheral blood at days ~30, ~60, ~100 and ~180 after mini-haploBMT.

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

1.4 Evaluate the impact of Haplo-identical Bone Marrow Transplant (BMT) in attenuating the progression of stroke, silent infarct or abnormal TCD with progressive vasculopathy in children with sickle cell disease (SCD) performed by MRA.

1.5 Attenuation of progressive disease (adults): including severe and debilitating vaso-occlusive pain (despite hydroxyurea or chronic blood transfusion therapy), stroke and silent infarct, chronic kidney and chronic lung diseases.

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, investigators at Johns Hopkins have developed a non-myeloablative conditioning regimen for transplantation of marrow from partially HLA-mismatched, or haploidentical, bone marrow from first-degree relatives. The regimen's main goal, 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 (MMF) and tacrolimus (FK), 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 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 the 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 leukemia after alloBMT. Relapse will not be an issue with hemoglobinopathies.

Since the toxicities of non-myeloablative haploidentical BMT (haploBMT) 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. Eligibility on the protocol has been expanded to 'standard risk' hematologic malignancies in trial J0457, which is still ongoing.

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 this 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 hemoglobinopathies 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), sirolimus was preferred.

Based on preliminary data, as of July 2012, 19 patients with SCD were referred for BMT (17 adults), using the haploBMT approach. Due to donor specific antibodies, 2 donors were medically ineligible. Thus 17 (89%) received a haploBMT, 3 matched sibling donors and 14 haploidentical donors. Median age was 30 years (range, 15-46). There has been no mortality or GVHD requiring treatment. 11 of 17 (65%) engrafted at levels sufficient to reverse their sickle cell phenotype. 1 patient had primary graft failure (GF) and 5 late GF. Six patients (all haploBMT) had full donor chimeras and off immunosuppressive therapy. 5 mixed chimeras. One patient had acute grade II skin GVHD, 3 patients experienced PRES, none since using sirolimus. There were 3 cases of CMV reactivation, but no CMV disease. Hemolytic anemia due to SCD was completely eliminated in all patients. Thus, haploBMT from related haploidentical donors is relatively safe and feasible for children and adults with SCD. By expanding the donor pool to include haploidentical donors most patients with SCD will be eligible for BMT.

While these results are actually very good considering the fact that most of 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, it was decided to increase 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 filgrastim 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].

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 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 Thiotepa

Antineoplastic and alkylating agent, that reacts with DNA phosphate groups to produce cross-linking of DNA strands leading to inhibition of DNA, RNA, and protein synthesis; mechanism of action has not been explored as thoroughly as the other alkylating agents, it is presumed that the aziridine rings open and react as nitrogen mustard; reactivity is enhanced at a lower pH

No dosage adjustment provided in manufacturer’s labeling. For renal or hepatic impairment, caution and dose adjustment may be required. Extensively hepatic; major metabolite (active): TEPA.

Half-life elimination: Terminal (dose-dependent clearance): ~2 hours;
Excretion: Urine (as metabolites and unchanged drug)

b) Solution Reconstituted, Injection: Generic: 15 mg (1 ea); I.V.: Administer as a rapid injection. Infusion times may be longer for high-dose (unlabeled use) treatment. On Day

c) The most common adverse reactions include: Fertility effects: May be mutagenic and teratogenic; Myelosuppression, secondary malignancies: Potentially carcinogenic; myelodysplastic syndrome and acute myeloid leukemia (AML) have been reported; Use with caution in patients with hepatic and renal impairment; dosage reduction recommended.

Thiotepa will be administered by IV infusion over 2 hours in a dose of Thiotepa 5 mg/kg IV q 12h (10 mg/kg total)on days -7 prior to thymoglobulin infusion.

Thiotepa will be dispensed by the Oncology Pharmacy and is produced by Adienne Pharma & Biotech

3.5 **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) Sirolimus oral solution: 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; it should not be diluted with grapefruit juice. 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 below:

Toxicity Summary Table: Sirolimus			
Timing	**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 sequelae, 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. Due to the long and variable half-life of sirolimus, wait 7 days from initiation to monitor first trough level. Monitor levels twice weekly and wait 7-10 days after dosage adjustments to reassess. For sirolimus without CNI as in this study, a 20-25% reduction of sirolimus dose is recommended for trough levels >15 – 20 ng/mL, and a 20-25% increase is recommended for trough levels < 5 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.

3.6 Mycophenolic Acid Mofetil (Cellcept®)

Mycophenolate Mofetil is an ester prodrug of the active immunosuppressant mycophenolic acid (MPA). This active metabolite is a noncompetitive, reversible inhibitor of inosine monophosphate dehydrogenase (IMPDH). There are no pharmacokinetic interactions with ganciclovir, cotrimoxazole, oral contraceptives and cyclosporine.

Side effect profiles include diarrhea, leukopenia, sepsis, allergic reactions, and vomiting. There is also an increase in certain types of infection mainly from the herpes virus family (CMV, HSV & VZV) and Candida.

3.7 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 Central 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 (or per institutional guidelines). 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. Patient should be given a skin test intradermal 1 hour prior to dose of ATG. If positive skin test contact MD/NP prior to continuing. ATG should not be administered during the weekend.

3.8 Filgrastim

Donors will receive G-CSF (Filgrastim® at 5 µg/kg/d subcutaneously for 5 consecutive days until the day of the harvest (i.e. days -5, -4, -3, -2, and -1).

3.9 Corticosteroids

Corticosteroids may not be used as an anti-emetic agent and should not be administered until 24 hours after the completion of post-transplantation cyclophosphamide, unless used for adrenal support or during a medical emergency (e.g. treatment of anaphylaxis). Corticosteroids may be used as a pre-medication during Thymoglobulin infusion and discontinued immediately thereafter.

3.10 Total Body Irradiation

Total body irradiation will be administered (200 cGy). This dose is a fraction of the irradiation delivered in typical myeloablative conditioning regimens, thus modulated toxicity is anticipated. Nonetheless, irradiation can cause short-term and long-term toxicities, particularly in children. The long-term toxicities include a risk of malignancy secondary to the exposure and a risk of infertility.

- Testicular shielding will be administered to all male patients.
  The testis is one of the most radiosensitive tissues, and even low doses of radiation can cause impairment of function. In adult men, doses as low as 10 cGy can cause damage to spermatogonia [15]. At single fraction doses of 200 to 300 cGy there is overt damage to spermatocytes in adult men and return to pre-irradiation sperm concentrations and germinal cell numbers may take up to 30 months [16]. Fractionated doses are more toxic to spermatogenesis, and complete sterilization may occur if the fractionated dose exceeds 100 – 200 cGy. However, in the pre-pubertal state, the impact of radiotherapy is mitigated. In one study of 12 pre-pubertal boys who received 2400 cGy testicular irradiation to treat ALL, it was observed that testosterone levels were normal in all 12 and basal LH was normal in 9 and elevated in 3 [17]. Nonetheless, to preserve fertility and Leydig cell function in post-pubertal males, we propose to administer testicular shielding to all males during the single fraction of TBI. This shielding will have no impact on the immunosuppressive effect of the radiotherapy.

- There will be no gonadal shielding in female patients.
  The effects of radiotherapy on ovarian function and fertility are dose- and age-dependent. However, ovarian doses of less than 400 cGy do not result in permanent ovarian dysfunction and the calculated LD50 of the human oocyte dose is approximately 400 cGy [18, 19, 20]. Thus, ovarian shielding to preserve ovarian function will not be necessary for the dose of TBI administered in this investigation; moreover, shielding in this setting could reduce the immunosuppressive effects of TBI if intra-abdominal lymph nodes were inadvertently shielded.

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 1-70 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. First degree relative 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 Sβ° thalassemia, Hb Sβ+ thalassemia, Hb SC disease, Hb SE disease, Hb SD disease, Hemoglobin SO- Arab disease HbS with hereditary persistence of fetal hemoglobin. Other significant hemoglobinopathies that also fulfill criterion from 4.1.7.

Plus one of the following:

4.1.7: Attenuation of progressive disease (adults):

a. Severe and debilitating vaso-occlusive pain despite hydroxyurea or regular blood transfusion therapy.
b. Stroke and silent infarct; stroke or central nervous system event lasting more than 24 hours; MRI changes indicative of brain parenchyma damage and MRA evidence of cerebrovascular disease.
c. Recurrent acute chest syndrome requiring exchange hospitalization.
d. Chronic lung disease as defined by progressive restrictive disease irrespective of oxygen requirements.
e. Chronic kidney disease, CKD stage II - IV
f. Transfusion dépendent thalassemia

4.2 Criteria for recipient ineligibility

4.2.1 Poor performance status (ECOG>1).

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

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

4.2.4 Pulmonary hypertension moderate to severe by echocardiographic standards.

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

4.2.6 HIV-positive

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

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

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

4.2.10 Patients who have any debilitating medical or psychiatric illness that would preclude their giving informed consent or their receiving optimal treatment and follow-up. However, patients with history of stroke and significant cognitive deficit,that would preclude giving informed consent or assent will not be excluded, if they have a family member or significant other with Power of Attorney to also consent of their behalf.

4.3 Criteria for donor eligibility

4.3.1 Weight ≥ 20kg and age ≥ 18 years or per institutional guidelines

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 HLA-haploidentical first-degree relatives of the patient.
Participants must be HLA typed at high resolution using DNA based typing at HLA-A, -B, -C and DRB1 and have available:
An HLA haploidentical first degree relative donor (parents, siblings or half siblings, or children) with 2, 3, or 4 (out of 8) HLA-mismatches who is willing and able to donate bone marrow. A unidirectional mismatch in either the graft versus host or host versus graft direction is considered a mismatch. The donor and recipient must be HLA identical for at least one antigen (using high resolution DNA based typing) at the following genetic loci: HLA-A, HLA-B, HLA-C, and HLA-DRB1. Fulfillment of this criterion shall be considered sufficient evidence that the donor and recipient share one HLA haplotype, and typing of additional family members is not required.

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
(iii) Donor age <40 years
(iv) Avoid female donors for male recipients and
(v) Avoid CMV mismatched donor-recipient transplants:

HLA cross-matching (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)
1. Compatible
2. Major incompatibility
3. Minor incompatibility
4. Major and minor incompatibility

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

4.3.5 Donors fulfilling the following criteria are ineligible for registration onto this study:
1. All donors will be screened by hemoglobin electrophoresis; donors with a clinically significant hemoglobinopathy are ineligible. Sickle trait is acceptable.

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.

In those patients who have a Hgb S fraction > 30%, a partial exchange transfusion will be performed to reduce the hemoglobin (Hgb) S to 30% or lower within one week before commencing conditioning regimen (prior to Day -9)

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. Patient should be given a skin test intradermally 1 hour prior to dose of ATG. If positive skin test contact MD/NP prior to continuing. 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.

Thiotepa will be infused prior to thymoglobulin over 2 hours on day -7 only. The dose will be 5 mg/kg IV q 12h (10 mg/kg total).

Fludarabine will be administered by intravenous infusion over 30 min. on D-6 to D-2. The dose will be 30 mg/m2.

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

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

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

Fludarabine dosage should be reduced as follows:

C_{Cr} 46-60 ml/min, fludarabine = 24 mg/m2
C_{Cr} 31-45 ml/min, fludarabine = 22.5 mg/m2
C_{Cr} 21-30 ml/min, fludarabine = 19.5 mg/m2
C_{Cr} <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 25% 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: Hydration and Mesna will be utilized for the Day 3 and Day 4 post BMT cyclophosphamide doses, not for the pre-BMT cyclophosphamide doses.
Total body irradiation: 200 cGy AP/PA with 4MV or 6MV photons at 8-12 cGy/min at the point of prescription (average separation of measurements at mediastinum, abdomen, and 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. All donors will receive 5 µg/kg per day of filgrastim as a single subcutaneous injection for 5 consecutive days (days -5 to -1). Bone marrow harvest will be performed on the sixth day. The BM will be infused the same day of collection.

Donor bone marrow will be harvested with to minimum yield of **2.5×10^8** with a target yield of 4×10^8 nucleated cells per kilogram of recipient’s IBW or actual BW, whichever BW is lower. A harvest yielding less than **2.5×10^8** renders the participant ineligible. Participants ineligible due to insufficient yield are deemed off

study and should be treated in accordance with institutional practice. We recommend taking no more than 10 mL per aspirate. In addition to calculating the total nucleated cell dose /kg, a sample of the product will be sent for flow cytometry to determine the content of $CD34^+$cells. The use of cryopreserved marrow is not permitted.

Given that there is always uncertainty about the feasibility of harvesting certain patients, the harvesting team will have the ability to stop the surgery if the minimum target of 2.5 x 10^8 cells has not been reached and it is believed that will be unsafe or technically unfeasible to reach the mentioned target if the surgery was to continue after a reasonable effort has been made. In this case, the study PI will be notified immediately and efforts should be made to assure at least a cell count of 2.5-4 x 10^8/kg of recipient ideal body weight.

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

5.5 Post-Transplantation cyclophosphamide

Cyclophosphamide [50mg/kg (IBW)] will be given on D+3 post-transplant (within 60-72 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).

Patients will be instructed to increase fluids overnight before cyclophosphamide administration. Hydration with normal saline at 3 cc/kg/hr iv will be started 2 hr prior to cyclophosphamide, then the rate will be reduced to 2 cc/kg/hr for 8 hr post-cyclophosphamide or administered per institutional standards. 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 > 18 years old: A one-time sirolimus loading dose, 6 mg PO, is given on Day 5, at least 24 hours after Cytoxan 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**.** Due to the long and variable half-life of sirolimus, wait 7 days from initiation to monitor

first trough level. Monitor levels twice weekly and wait 7-10 days after dosage adjustments to reassess. There is no planned taper. Sirolimus prophylaxis is discontinued after the last dose on Day 365, or may be continued if there is GVHD.

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/m2 PO with the dose not to exceed 6 mg, is given on Day 5, at least 24 hours after Cytoxan completion. Sirolimus is then continued at a maintenance dose (start 1 mg/m2 PO QD, maximum 2 mg PO QD), with dose adjustments to maintain a trough of **5 – 15 ng/mL** as measured by HPLC or immunoassay**.** Due to the long and variable half-life of sirolimus, wait 7 days from initiation to monitor first trough level. Monitor levels twice weekly and wait 7-10 days after dosage adjustments to reassess. There is no planned taper. Sirolimus prophylaxis is discontinued after the last dose on Day 365, or may be continued if there is GVHD.

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 or institutional preference.

An oral antibiotic for gastrointestinal decontamination 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 immunosuppressant to toxic levels. If the patient on sirolimus 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 (see section 3.5).

Pneumocystis jiroveci pneumonia (PCP) prophylaxis will be administered according to institutional preference, starting around Day 30 (or after engraftment) 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. Patient can also use other antibacterial prophylaxis with pneumococcal coverage in-lieu of Penicillin-V based on institutional preference.

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 (or based on institutional guidelines). 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

In those patients who have a Hgb S fraction > 30%, a partial exchange transfusion will be performed to reduce the hemoglobin (Hgb) S to 30% or lower before commencing the conditioning regimen. The Hgb and Hgb S% should be re-checked 4 hours after the exchange transfusion. All blood products will be irradiated.

The hemoglobin level must be maintained between 9.0 and 11.0 g/dL and platelet count > 50,000/mL after transplantation to minimize the risk of neurological adverse events. Irradiated blood products should be administered universally, and Cytomegalovirus (CMV) negative or leuko-filtered blood products are recommended for CMV sero-negative recipients.

5.10 Prevention of post-BMT Neurological Events and Posterior Reversible Encephalopathy Syndrome (PRES)

Prophylaxis against seizures is mandatory in all recipients and should be commenced at the start of conditioning day -9. Levetiracetam (Keppra) is the suggested agent however anti-seizure prophylaxis may be tailored per institution preference. Keppra will be administered at a dose of 500 mg p.o. b.i.d (peds dosing of 50 mg/kg divided bid to max of 500 bid). 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 immune-suppressive therapy to reduce the risk of seizures.

Hypertension should be strictly controlled to prevent central nervous system (CNS) toxicity. Blood pressure should be monitored closely and both systolic and diastolic hypertension should be treated promptly to maintain blood pressure at the patient's pre-transplant baseline. Explicit orders must be written to intervene if systolic or diastolic blood pressure exceeds 10% over baseline.

5.11 Anti-ovulatory treatment

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

5.12 Hepatic prophylaxis

Prophylactic Ursodiol 300 mg PO 2-3 times daily will be administered to all patients or those with history of hepatitis C, hyperbilirubinemia, or bridging fibrosis or cirrhosis. Patients will begin prior to start of conditioning regimen.

5.13 Contraception Practices

Females of child bearing potential (to include all female participants > 10 years of age, unless postmenopausal for a minimum of 1 year before the screening visit or surgically sterilized), must agree to practice two (2) effective methods of contraception at the same time, or agree to practice true abstinence when this is in line with the preferred and usual lifestyle of the subject, from the time of signing of informed consent through 12 months post-transplant. Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception.

Male subjects (even if surgically sterilized) must agree to one of the following: practice effective barrier contraception, or practice true abstinence when this is in

line with the preferred and usual lifestyle of the subject, from the time of signing of informed consent through 12 months post-transplant. Periodic abstinence and withdrawal are not acceptable methods of contraception.

5.14 Management of Slow Engraftment and Graft Failure

Slow engraftment or graft failure shall be managed according to institutional practices, and may include the administration of colony stimulating factors and prophylactic antibiotics.

Graft failure following BMT in patients with sickle cell disease is usually associated with autologous reconstitution of the bone marrow with host hematopoiesis. It is associated with a steady decline in donor chimerism, increasing representation of hemoglobin S (in the absence of ongoing RBC transfusion therapy) and clinical manifestations of sickle cell disease. A second transplant or donor cellular infusion should not be considered unless the patient has < 5% donor chimerism.

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)

SCHEDULE OF ASSESSMENTS

Assessments	Initial	Allowable time frame prior to enrollment**	Day < 60 post SCT	≈Day 30 ± 7 post SCT	≈Day 60 ± 7 post SCT	≈Day 100 ± 7 post SCT	≈Day 180± 7 post SCT	Suspected GVHD	Annually X 3yrs +/- 2 or 3 months
Informed Consent	X	Within 30 days							
History and Physical	X	within 30 days		X	X	X	X		X
Performance status	X	within 30 days		X	X	X	X		
Disease re-evaluation	X	within 30 days		X	X	X	X		X
CBC & Diff. + reticulocyte count	X	within 30 days	*weekly	X	X	X	X		X
Comprehensive Metabolic Panel; Liver function test	X	within 30 days	weekly	X	X	X	X		X
Hemoglobin electrophoresis (HPLC), (Hgb F); plasma free hemoglobin, LDH, reticulocyte count	X	within 30 days		X	X	X	X		X
CXR- Pa/Lat	X	within 60 days							X
Pregnancy test (women, childbearing age)	X	within 7 days							
Chimerism analysis (Including unsorted and CD3) ¥				X	X	X	X		X
PT, PTT	X	within 30 days							X
EKG	X	within 60 days							X
Cardiac Function by 2D-Echo, TRJ Velocity and BNP	X	within 60 days							X
HepB Ag, HBC Ab, HCV Ab, HSV IgG, CMV IgG, RPR, HIV, VZV IgG (if possible)	X	within 30 days							X
Toxicity assessment	X		X	X	X	X	X		
HLA typing/ lymphocytotoxic screen (PRA testing of donors and patient)		within 60 days							
PFTs (Spirometry and lung volumes; DLCO)		within 60 days							X
GVHD and other co-morbidity assessments€			weekly	X	X	X	X		X
Sinus CT***		within 30 days							
Fasting lipid profile		within 30 days			X				X
Skin Biopsy								X	

Assessments	Initial	Allowable time frame prior to enrollment**	Day< 60 post SCT	≈Day 30 ± 7 post SCT	≈Day 60 ± 7 post SCT	≈Day 100 ± 7 post SCT	≈Day 180± 7 post SCT	Suspected GVHD	Annually X 3yrs +/- 2 or 3 months
Renal Function (eGFR and/or radio-nuclide scan); micro-albuminuria		within 60 days							X
Baseline TCD evaluation ǂ		X							X
BrainΩ: MRI/MRA without contrast***		Within 30 days prior to SCT							X
Neurocognitive Assessment: (Weschler (WISC IV) for children and adolescents which is similar to the WAIS used for adults)		Within 6 months prior to SCT							X
Serum ferritin and assessment of liver (R2*) and/or cardiac (T2*) iron by imaging; liver biopsy is optional		Within 3 months prior to SCT							X
QOL measurement (PROMIS)		Within 7 days prior to SCT							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 institutional BMT standards and practices.
***Optional tests (optional at physician's discretion)
¥ Chimerism frequency may be increased based on institutional standards
ǂ TCD evaluation in eligible children with SCD
€ GVHD assessments to be performed at these time points per institutional practices
Ω Brain MRI/MRA should be performed within 30 days prior to SCT for patients whose indication for transplant is stroke

6.1 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

6.1.1. **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.

6.1.2. **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, LDH
   ii. Routine and microscopic urinalysis with C&S, microalbumin
   iii. Fasting lipid profile.
   iv. G6PD screen of donors will be at institutional discretion.

c) Cardiac
   i. EKG
   ii. Echocardiogram or MUGA scan with Left Ventricular Ejection Fraction (LVEF) + Right Ventricular Systolic Pressure and evaluation for pulmonary hypertension, estimation of TRJ velocity and BNP
   iii. Patients with suspected pulmonary hypertension, if indicated may require Cardiology referral for evaluation and possible RHC for confirmation.

d) Pulmonary
   i. Chest X-ray – Pa/Lat
   ii. Sinus CT scan – optional at physician's discretion
   iii. Pulmonary function tests including at least FEV1, lung-total lung capacity 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- CD33 vs CD14 or CD15) and CD3 chimerism.
- CD36 platelet expression testing of donors at institutional discretion.
- RBC chimerism will be at institutional discretion (flow cytometry vs extended RBC phenotyping for minor red cell antigens).

g) Disease specific studies: The following are minimal recommended:
i. Sickle Cell Disease: Hemoglobin electrophoresis (HPLC), hemoglobin F (fetal), plasma free hemoglobin, LDH, reticulocyte count
ii. CNS: MRI/MRA of the brain and Neurocognitive testing (assessment of the brain- Cognitive evaluation is optional at physician's discretion, and will include Weschler (WISC IV) for children and adolescents which is similar to the WAIS used for adults within 6-12 months prior to, 1 and 2 years post-transplant.
iii. Renal function test (assessment of kidney function): include eGFR and radio-nuclide scan, urine micro-albuminuria
iv. Iron overload: Patients will be evaluated by serum ferritin and assessment of liver MRI (T2* or R2*) and/or cardiac (T2*) MRI imaging; liver biopsy is optional at physician's discretion. A Gastroenterology and/or Hepatology consultation is recommended for patients with severe iron overload with elevated liver function tests.

6.2 Post-transplant Evaluation

6.2.1. Day 0 through Day 100 (± 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 through day 60.
2. Comprehensive metabolic panel once a week through day 60.
3. Patients will have evaluations for infectious complications as clinically indicated. Surveillance cultures according to institutional guidelines or BMT program standards are recommended.
4. Evaluations by history and physical examination for GVHD will be performed as per institutional guidelines or BMT program 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 white blood cell differential, reticulocyte count, comprehensive panel.
5. GVHD evaluation

6.2.3 Evaluations on day ~60 (± 7 days)

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

6.2.4 Evaluations on day ~100 (± 7 days)

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

6.2.5 Evaluations on day ~180 (± 7 days)

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

6.2.6 Evaluations for suspected GVHD

1. Comprehensive panel
2. Biopsies if needed.

6.2.7 Annual evaluations ~ (± 7 days):

1. History and physical examination.
2. Disease evaluation.
3. Studies for donor cell chimerism on peripheral blood.
4. CBC and white blood cell differential, reticulocyte count, comprehensive panel.
5. GVHD evaluation
6. Fasting lipid profile.
7. Organ function studies will be done (per section 6.0)
8. Repeat Neurocognitive testing x2
9. QOL assessment (PROMIS)

In the event that the patient is unable to return to Vanderbilt University for these visits, every attempt will be made to obtain data from the patient and referring physician.

7.0 POST-BMT EVALUATION

Patients will be followed during (i) the initial post-BMT period (ii) in VU Long term transplant clinic (LTTC) and (iii) after discharge to the referring physician as per standard practice.

7.1 Chemotherapy toxicities

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

Since this trial is an 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 (Arrhythmia)
- Cardiovascular (General)
- Coagulation
- Constitutional symptoms
- Dermatology/Skin
- Endocrine
- Hemorrhage
- Hepatic
- Infection/Febrile neutropenia
- Lymphatic's
- 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[9] (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 Vanderbilt University 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, Day 60 and Day 100
- 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 100 will be captured at the patients' six month and annual evaluations per institutional standards.

7.3 Transplant-related mortality (TRM)

Causes of TRM, i.e., death in the absence of recurrent sickle cell disease or hemoglobinopathy, 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 100, ~Day 180, ~Day 365, and then yearly per Vanderbilt University 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 recurrent sickle cell disease or hemoglobinopathy, will be characterized at 100 days and at one year after BMT.

8.2 Hematologic toxicity

A secondary endpoint of this study is time to recovery of circulating neutrophils and platelets (following chemotherapy). Neutrophil recovery is defined as the first day of three consecutive laboratory values on different days, after the conditioning regimen-induced nadir of blood counts, that the absolute neutrophil count is> > 500 x 10^9/L. 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 $\geq$ 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, day 60 and day 100. Patients with >5% donor whole blood or myeloid chimerism (myeloid is preferable) assessed by bone marrow or peripheral blood chimerism assays by day +42 post-transplant 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 ≥95%.

8.3.1 Primary Graft Failure

Primary graft failure is defined as never achieving >5% donor whole blood or myeloid chimerism (myeloid is preferable) assessed by bone marrow or peripheral blood chimerism assays by day +42 post-transplant. Second infusion of stem cells is also considered indicative of primary graft failure by day +42 post-transplant.

8.3.2. Secondary Graft Failure

Secondary graft failure is defined as < 5% donor whole blood or myeloid chimerism (myeloid is preferable) in peripheral blood or bone marrow beyond day +42 post-transplant in patients with prior documentation of hematopoietic recovery with >5% donor cells by day +42 post-transplant. Second infusion of stem cells is also considered indicative of secondary graft failure.

Management of Slow Engraftment and Graft Failure

Slow engraftment or graft failure shall be managed according to institutional practices, and may include the administration of colony stimulating factors and prophylactic antibiotics.

Graft failure following BMT in patients with sickle cell disease is usually associated with autologous reconstitution of the bone marrow with host hematopoiesis. It is associated with a steady decline in donor chimerism, increasing representation of hemoglobin S (in the absence of ongoing RBC transfusion therapy) and clinical manifestations of sickle cell disease. A second transplant or donor cellular infusion should not be considered unless the patient has < 5% donor chimerism.

8.4 Disease Status

Disease status will be evaluated through the following laboratory tests: hemoglobin S, hemoglobin electrophoresis (HPLC), hemoglobin F (fetal), free hemoglobin, reticulocyte count. These disease evaluations will be performed at ~Day 30, ~Day 60, ~Day 100 ~Day 180, ~Day 365, and then yearly per institutional standards. Relapse will be defined as complete loss of donor chimerism in all cell lines.

8.5 GVHD

Patients will be followed for development of acute and chronic GVHD using institutional criteria. Chronic GVHD is assessed according to institutional criteria.

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.

The data gathered will be entered into REDCap data repository with each ID having a unique identifier for each participant. REDCap is a secure, web-based application for building and managing online databases. All data will be de-identified and samples will only have a unique identifier that will link the unique identifier with the patient's name.
This will be accessible to all participating investigators.

The REDCap clinical database will be maintained and coordinated at Vanderbilt University Medical Center under Dr. DeBaun and Dr. Kassim.

The study team members are trained in HIPAA privacy regulations and other applicable site privacy policies. No information will be released, nor will participation in the research be acknowledged, to any party except where compulsory according to law or intuitional policy.

The results of the research study may be published, but volunteers' names or identities will not be revealed. Records will remain confidential. In order that confidentiality can be maintained, the principal investigators will keep records in locked cabinets and results of tests will be coded to prevent association with volunteers' names. Volunteers' records will be available to the study staff and to each site's IRB.

This study is being conducted at sites both inside and outside the U.S. Each site is conducting the study independently with no sharing of PHI;
no one site serves as the coordinating center. Vanderbilt has entered into a Memorandum of Understanding (MOU) with other sites conducting the study. The MOU will allow sites to share and aggregate de-identified data for analysis and publishing purposes. The trial and participating sites will be listed in clinical trials.gov

9.1 DATA and Safety Monitoring

This study must be approved by an appropriate institutional review committee as defined by Federal Regulatory Guidelines (Ref. Federal Register Vol. 46, 17, January 27, 1981, part 56) and the Office for Protection from Research Risks Reports: Protection of Human Subjects (Code of Federal Regulations 45 CFR 46

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 Vanderbilt University Institutional Review Board.

Serious Adverse Event reporting

Study site personnel must report all SAEs by e-mail or fax as soon as possible to the Coordinating Center, but within 24 hours of first becoming aware of the SAE.

The SAE report should be submitted via secure email transfer or fax using the contact information listed below:

Transmission of the SAE report via fax should be confirmed via e-mail by the site personnel submitting the report.

Each serious adverse event must be followed up until resolution or stabilization by submission of updated reports to the designated person. Patients must be monitored for any Adverse Events or Serious Adverse Events and these must be reported from initiation of preparative regimen until 100 days after transplant. For the purpose of the study, only the following SAEs will be considered clinically significant and will be reportable:

- Death on study for reasons other than disease relapse/progression
- Admission to an Intensive Care Unit if related to study participation

- Hospital admissions persisting > 14 days if related to study participation

Investigators must submit written safety reports as required by their Institutional Review Board (IRB) or Institutional Ethics Committee (IEC) within timelines set by regional regulations (European Union common technical document or US FDA). The study site should retain documentation of the submission of expedited safety reports to the IRB/IEC, and their receipt.

9.1.2 Data Monitoring and Auditing

The Vanderbilt-Ingram Cancer Center (VICC) oversees patient safety and data monitoring for its investigator-initiated and NIH-NCI funded clinical trials through its Data and Safety Monitoring Committee (DSMC). The purpose of the DSMC is to ensure the efficient implementation and management of VICC Data and Safety Monitoring Plan (DSMP). The Committee maintains authority to intervene in the conduct of studies as necessary to ensure clinical research performed at VICC achieves the highest quality standards.

The VICC DSMC meets on a quarterly basis and ad hoc to discuss data and safety monitoring of clinical trials and to oversee the VICC DSMP. Internal audits for compliance with adverse event reporting, regulatory and study requirements, and data accuracy and completion are conducted according to the VICC DSMP according to study phase and risk. The committee reviews all serious adverse events (SAE) on Vanderbilt sponsored investigator-initiated studies on a quarterly basis and provides DSMC SAE review reports to the Vanderbilt IRB.

A 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.

9.1.3 Monitoring the Progress of the Trial and the Safety of Participants

The Vanderbilt University Medical Center Principal Investigator 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 trial additionally will be monitored by the VICC Multi-Institutional Coordinating Center. The actual frequency of monitoring will depend on the enrollment rate and performance of the site. Monitoring will be conducted through onsite and remote monitoring, teleconferences with the Investigator and site staff, and appropriate communications by mail, fax, email, or telephone. The purpose of monitoring is to ensure that the study is conducted in compliance with the protocol, standard

operating procedures (SOPs), and other written instructions, and to ensure the quality and integrity of the data.

During scheduled monitoring visits, investigators and the investigational site staff must be available to discuss the progress of the trial, make necessary corrections to case report form entries, respond to data clarification requests, provide required regulatory documents, and respond to any other trial-related inquiries of the monitor.

10.0 STATISTICAL CONSIDERATIONS

The primary objective of this clinical trial is to obtain risk-stratified estimates of two-year progression-free survival with a precision of +/- 20 percentage points (95% confidence bound). To achieve this precision, it will be necessary to accrue at least 25 patients. We expect the accrual to take 7 years based on a recruitment of 6-7 patients per year once the study reaches a steady accrual of patients.

Stopping criteria: As outlined in Section 2.0 (Background), the overall transplant related mortality (TRM) attributable to HLA-identical sibling bone marrow transplantation (BMT) is 20-30%, with approximately half of these deaths occurring in the first 100 days. The incidence of severe GVHD (defined as grade 4 acute GVHD and/or NIH severe chronic GVHD) is approximately 13-17% after HLA-identical sibling BMT. The working hypothesis is that the overall toxicity of mini-haploBMT is not significantly greater than HLA-identical sibling BMT after myeloablative conditioning. Consequently, if we accumulate enough evidence that the probability of graft failure is greater than 20% or the probability of severe GVHD is greater than 10%, it would raise concerns of excessive toxicity and would trigger a referral to the DSMC for evaluation. This trigger would be met, for instance, if graft failures occurred in 3 or more in the first 5 patients, 4 or more in the first 10 patients (see Table 10.1 for details). As for severe GVHD (defined as grade 4 acute GVHD and/or NIH severe chronic GVHD), 3 or more incidences in the first 10 or 4 or more incidences in the first 20 would be considered too toxic (see Table 10.1). As this study is unblinded, we will review the patients' safety data sequentially and halt the study as soon as the required numbers of toxicities are observed. If the stopping criterion is met, accrual to the trial will be halted temporarily, and the DSMB will review the toxicity data and recommend either modification or termination of the trial.

N	Graft failures to initiate safety review	Severe GVHD to initiate safety review
5	X ≥ 3	-
10	X ≥ 4	X ≥ 3
15	X ≥ 5	-
20	X ≥ 6	X ≥ 4
25	X ≥ 7	-
30	X ≥ 9	X ≥ 5
35	X ≥ 10	-
40	X ≥ 11	X ≥ 6
45	X ≥ 12	-
50	X ≥ 13	X ≥ 8

Table 10.1: Stopping rule for safety review for graft failures and severe GVHD. N is the number of accrued patients, and X is the number of patients with graft failure or severe GVHD.

Details of stopping rule calculations.

Graft failure:

We acknowledge uncertainty of the probability of graft failures, and we modeled it using a Bayesian beta-binomial model. We used a very weak prior distribution, Beta (0.2, 0.8), which was derived in a discussion among the investigators. The amount of the prior information is equivalent to that from one patient in the clinical trial. The unacceptable toxicity is defined as the probability of graft failure exceeding 20%. The probability of graft failure will be estimated at each interim look, and if the data indicate that unacceptable toxicity is highly likely (exceeding 80%), , then we will halt the accrual and review safety data. The corresponding critical values (X) are summarized in Table 10.1. For example, if we observe 5 or more graft failures in the first 15 patients, the estimate of unacceptable toxicity will exceed 80%. Operational characteristics of this procedure were evaluated through simulations, and Table 10.2 summarizes the cumulative probability of stopping under the different assumptions for the true probability of graft failure. According to this simulation study, the probability of unnecessary stops is controlled at 3% if the true toxicity probability is 10%. And if the true probability of graft failure is as high as 30%, this procedure will halt for safety review with probability greater than 50% within the first 15 patients and 75% within the first 30 patients.

N	P[Safety review] if p = 10%	P[Safety review] if p = 15%	P[Safety review] if p = 20%	P[Safety review] if p = 25%	P[Safety review] if p = 30%
5	0.01	0.03	0.07	0.10	0.16
10	0.02	0.06	0.14	0.25	0.37
15	0.02	0.09	0.21	0.37	0.53
20	0.02	0.11	0.26	0.46	0.65
25	0.03	0.13	0.31	0.53	0.73
30	0.03	0.13	0.32	0.55	0.75
35	0.03	0.13	0.33	0.58	0.78
40	0.03	0.14	0.35	0.61	0.81
45	0.03	0.14	0.36	0.63	0.84
50	0.03	0.14	0.38	0.67	0.87

Table 10.2. Probabilities of stopping for safety review for different assumed true toxicity probability (p) based on simulation studies.

Severe GVHD:

Similarly, we used the same Bayesian approach based on Beta-Binomial models to develop the stopping criteria and compute/simulate the design characteristics. The prior distribution of the severe GVHD probability is Beta (0.10, 0.90). For severe GVHD, the unacceptable toxicity probability is 10%, and the critical values in Table 10.1 were set so that the accrual will be halted if the data indicate that the

unacceptable toxicity is highly likely (80%). With the decision rule shown in Table 10.1, we computed the operating characteristics (probability of early stopping) shown below in Table 10.3. According to this, if the toxicity probability is acceptable (5%) we have very small probability to conduct unnecessary data review, and if it is 20%, we will have high probabilities, 62%, and 78%, to halt the study reasonably early at 20, and 30 patients, respectively.

N	P[Safety review] if p = 5%	P[Safety review] if p = 10%	P[Safety review] if p = 15%	P[Safety review] if p = 20%
10	0.01	0.06	0.14	0.33
20	0.02	0.15	0.26	0.62
30	0.03	0.22	0.32	0.78
40	0.03	0.28	0.35	0.87
50	0.04	0.29	0.38	0.90

Table 10.3 Probabilities of stopping for safety review for different assumed true toxicity probability (p) based on simulation studies.

10.2 PLANNED PROTOCOL AMENDMENTS

Graft Failure: If the stopping rules for graft failure are met, the protocol will be amended by modifying conditioning regimen, such as addition of immunosuppressive chemotherapy and/or TBI to decrease graft rejection rate after consultation with the group.

Graft versus host disease: If the stopping rules for graft versus host disease are met, the protocol will be amended to reduce incidence of GVHD such as increasing dose of thymoglobulin for in-vivo purging of T-cells, after consultation with the group.

TRM: If the stopping rules for TRM are met, the protocol will be amended to reduce toxicity of regimen after consultation with the group.

11.0 RECORDS

Clinical records will be maintained as confidentially as possible. Collection of CRFs at standard intervals is the primary method of collecting data from collaborating centers. The PI at Vanderbilt will maintain a patient database to allow storage and retrieval of patient data collected from a wide variety of sources. The principal investigator will ensure that data collected conform to all established guidelines for coding collection, key entry and verification. These data are then entered into a secure dedicated database operated by a data manager. Any publication or presentation will refer to patients by a unique patient number and not by name to assure patient confidentiality.

Archiving Records:
Patient Shadow files will be archived once the PI has confirmed individual file is completed and all data needs completed, CRFs pulled and review completed.

Some patient shadow files will be archived before the study is closed with the IRB and some will be archived with IRB study closure.

Regulatory files will be archived when study is closed with the IRB.

12.0 ON-STUDY DATE:

Date of consent signing.

13.0 OFF-STUDY DATE:

Upon completion of “Day 100” evaluations, patients have completed their treatment except for sirolimus, which continues until day 365. Patient follow-up beyond day 100 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:

- Death
- Patient decision (or decision by a parent or guardian on behalf of a minor)
- 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

(1) Walters MC, Patience M, Leisenring W et al. Bone marrow transplantation for sickle cell disease. N Engl J Med. 1996;335:369-376.

(2) Jacobsohn DA, Duerst R, Tse W, Kletzel M. Reduced intensity haemopoietic stem-cell transplantation for treatment of non-malignant diseases in children. Lancet. 2004;364:156-162.

(3) Horan JT, Liesveld JL, Fenton P, Blumberg N, Walters MC. Hematopoietic stem cell transplantation for multiply transfused patients with sickle cell disease and thalassemia after low-dose total body irradiation, fludarabine, and rabbit anti-thymocyte globulin. Bone Marrow Transplant. 2005;35:171-177.

(4) Iannone R, Casella JF, Fuchs EJ et al. Results of minimally toxic nonmyeloablative transplantation in patients with sickle cell anemia and beta-thalassemia. Biol Blood Marrow Transplant. 2003;9:519-528.

(5) Blaise D, Kuentz M, Fortanier C et al. Randomized Trial of Bone Marrow Versus Lenograstim-Primed Blood Cell Allogeneic Transplantation in Patients With Early-Stage Leukemia: A Report From the Societe Francaise de Greffe de Moelle. Journal of Clinical Oncology. 2000;18:537.

(6) Bensinger WI, Martin PJ, Storer B et al. Transplantation of bone marrow as compared with peripheral-blood cells from HLA-identical relatives in patients with hematologic cancers. N Engl J Med. 2001;344:175-181.

(7) Schmitz N, Beksac M, Hasenclever D et al. Transplantation of mobilized peripheral blood cells to HLA-identical siblings with standard-risk leukemia. Blood. 2002;100:761-767.

(8) Couban S, Simpson DR, Barnett MJ et al. A randomized multicenter comparison of bone marrow and peripheral blood in recipients of matched sibling allogeneic transplants for myeloid malignancies. Blood. 2002;100:1525-1531.

(9) Przepiorka D, Weisdorf D, Martin P et al. 1994 Consensus Conference on Acute GVHD Grading. Bone Marrow Transplant. 1995;15:825-828.

(10) Giralt S, Estey E, Albitar M et al. Engraftment of allogeneic hematopoietic progenitor cells with purine analog-containing chemotherapy: harnessing graft-versus- leukemia without myeloablative therapy. Blood. 1997;89:4531-4536.

(11) Slavin S, Nagler A, Naparstek E et al. Allogeneic nonmyeloablative stem cell transplantation (NST) as an emerging new modality for immunotherapy of hematologic malignancies and non-malignant disorders [abstract]. Blood. 1998;92:519a.

(12) Greenberg P, Cox C, LeBeau MM et al. International Scoring System for Evaluating Prognosis in Myelodysplastic Syndromes. Blood. 1997;89:2079-2088.

(13) Greenberg P, Cox C, LeBeau MM et al. International Scoring System for Evaluating Prognosis in Myelodysplastic Syndromes. Blood. 1997;89:2079-2088.

(14) Bernaudin F, Socie G, Kuentz M et al. Long-term results of related myeloablative stem-cell transplantation to cure sickle cell disease. Blood 2007; 110(7):2749-2756.

(15) Shalet SM. Effect of irradiation treatment on gonadal function in men treated for germ cell cancer. Eur Urol 1993:23:148-51 discussion 152.

(16) Howell S, Shalet S. Gonadal damage from chemotherapy and radiotherapy. Endocrinology and Metabolism Clinics of North Amereica. 1998:27:927-43.

(17) Thorner MO, Rogol AD, Blizzard RM et al. Acceleration of growth rate in growth hormone-deficient children treated with human growth hormone-releasing hormone. Hormone Research. 1988:30:111-4.

(18) Van Dorp W, van der Geest I, Laven JSE, van den Heuvel-Eibrink M. Gonadal function recovery in very long-term male survivors of childhood cancer. International Journal of Radiation Oncology, Biology, Physics 1990; 19:873-80

(19) Faddy MJ, Gosden RG, Gougeon A et al. Accelerated disappearance of ovarian follicles in mid-life: implications for forecasting menopause. Human Reproduction 1992; 7:1342-6.

(20) LaRiviere PD. The quality of high-energy X-ray beams. British Journal of Radiology 1989; 62:995-8

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.