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ALLOGENEIC BONE MARROW TRANSPLANTATION FROM HLA IDENTICAL RELATED DONORS FOR PATIENTS WITH HEMOGLOBINOPATHIES: HEMOGLOBIN SS, HEMOGLOBIN SC, OR HEMOGLOBIN S$\beta^{0/+}$ THALASSEMIA

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ELIGIBILITY CHECKLIST

PATIENT ID PATIENT NAME _____

YES NO VALUE

		The patient must have homozygous hemoglobin S, or hemoglobin SC, or hemoglobin Sβ^0 thalassemia.
_____	_____	Patients with SCD or its variants must have severe hemoglobinopathy defined as: • Previous central nervous system vaso-occlusive episode with or without residual neurologic findings or • Frequent painful vaso-occlusive episodes which significantly interfere with normal life activities and necessitate chronic transfusion therapy or • Recurrent SCD chest syndrome episodes which necessitate chronic transfusion therapy or • Severe anemia which prevents acceptable quality of life and necessitates chronic transfusion therapy
_____	_____	**OR** have the following criteria defined as: • Any of the above symptoms in which the patient is not undergoing chronic transfusion therapy • The patient is undergoing chronic transfusion therapy for symptoms other than that listed above and which significantly interferes with normal life activities • Failed hydroxyurea therapy • Abnormal transcranial Doppler ultrasound • Abnormal MRI/MRA of the brain • Indication of pulmonary hypertension on 2 separate echocardiogram examinations • Patients who plan to return to resource poor areas or countries
_____	_____	The patient must have an HLA genotype identical donor or 5/6 HLA matched related donor
_____	_____	Between the ages of birth and 40 years.
_____	_____	Women of childbearing potential must have a negative pregnancy test.

The patient **must not** have:

_____	_____	Biopsy proven chronic active hepatitis or cirrhosis.
_____	_____	SCD chronic lung disease > stage 3 (see Appendix 1)
_____	_____	Creatinine clearance<40 ml/min/1.73 M_2
_____	_____	Severe cardiac dysfunction defined as shortening fraction <25% or NYHA class III or greater
_____	_____	HIV infection
_____	_____	Severe but unspecified chronic organ toxicity which is likely to diminish the patient's capacity to survive the immediate post BMT phase
_____	_____	Inadequate intellectual capacity to give informed consent (in the case of minors, this criteria must be fulfilled by the legal guardian)
_____	_____	Be pregnant, lactating or unwilling to use appropriate birth control

To check eligibility call Dr. Leung or Dr Krance at 832-824-4219. For patient registration, contact the research coordinator at 832-824-4881.

_____ _____
Signature of M.D.

NOTE: Patients who would be excluded from the protocol strictly for laboratory abnormalities can be included at the investigator's discretion after approval from the CCGT Protocol Review Committee and the FDA Reviewer.

1.0 OBJECTIVES

1.1 To evaluate recovery of organ function known to be compromised in patients with sickle cell disease (SCD) or sickle hemoglobin variants (hemoglobin SC or hemoglobin $S\beta^{0/+}$) after undergoing allogeneic SCT from HLA genotype identical donors. Specifically, to determine whether organ dysfunction (brain, heart, lung, kidney, liver, spleen, etc.) secondary to these severe hemoglobinopathies can be improved or reversed following allogeneic BMT.

1.2 To evaluate the use of PET scan examination in assessing metabolic function of organs known to be compromised in patients with SCD, hemoglobin SC, or hemoglobin $S\beta^{0/+}$ after undergoing allogeneic SCT from HLA genotype identical donors. Specific organs for evaluation include brain, spleen, kidney, and bone.

1.3 To evaluate response to immunization after BMT in patients with SCD, hemoglobin SC, or hemoglobin $S\beta^{0/+}$.

2.0 BACKGROUND

Allogeneic SCT for severe hemoglobinopathies

Data from the Cooperative Study of Sickle Cell Disease (CSSCD) indicates that 85% of children with SCD are likely to survive to their twentieth birthday.[1] This is an improvement over the 50% probability of survival noted during the 1960s.[2] This improvement is largely attributed to better overall medical management of sickle cell disease, patient education, vaccination against Streptococcus pneumoniae and Hemophilus influenza B, and possibly to prophylactic administration of penicillin.[1] Nevertheless, the life expectancy and mortality rate remain unacceptable. Table 1 presents the cause of death among 2,824 children with sickle hemoglobinopathies as reported by the CSSCD.[1]

Table 1

CAUSE OF DEATH	NUMBER OF PATIENTS (%)
Bacterial infection	28 (38.4%)
Cerebrovascular accident	9 (12.3%)
Other	
• Sickle cell disease related	8 (11.0%)
• Non-sickle cell disease related	
- Trauma	5 (6.8%)
- Other	4 (5.5%)
Unknown	
• Died outside CSSCD center	14 (19.2%)
• Died at CSSCD center	
- Autopsy not performed	3 (4.1%)
- Autopsy performed	2 (2.7%)

The high mortality rate is maintained after age 20. As SCD patients age, end organ failure of lung, kidney, and brain results in premature death. At present, the median survival for men with SCD is 42 years and for women 48 years.[3] Compared to the entire Afro-American population, life expectancy for SCD patients is decreased 25-30 years.

Beyond the high mortality rate, SCD patients encounter morbidity that severely demeans their quality of life. Pain crisis, acute chest syndrome, and hand-foot syndrome may require frequent hospitalization. More foreboding sequelae are those that permanently disable, including renal failure, chronic leg ulcers, retinopathy, priapism, and osteonecrosis. Finally, there are those events, such as stroke, that not only permanently disable but also place the patient at imminent risk of death.

Chronic blood transfusion is one form of therapy to prevent sickling. Dilution of the SCD patient's red cells by transfused red cells inhibits the *in vivo* dynamics that promote sickling. Hemoglobin S concentration must be maintained below 30% for this therapy to be effective.[5] However, chronic transfusion is not without risks. Infection, red cell sensitization, and transfusion reaction are potential hazards; and lethal iron accumulation is an inevitable consequence unless chelation is provided. Effective iron chelation requires Deferoxamine administration, intravenous or subcutaneous, 5-7 days per week, up to 10 hours per day. The annual expense of this treatment is estimated to range between $10,000 and $30,000. Even for patients with adequate resources, this therapy is demanding. And for those living in unacceptable social circumstances, the demands may be insurmountable.

Once initiated, there is no recourse; transfusion therapy must be continued indefinitely. The consequences of lifelong treatment are largely unknown. Patient compliance will undoubtedly diminish as the duration of treatment is extended. Red cell sensitization and toxicity from Deferoxamine therapy are also more likely to occur. And if the experience of thalassemic patients is relevant, 10 to 15 years of chronic transfusion and chelation is likely to result in hemosiderosis and diabetes.[26] It is for all these reasons that transfusion therapy is considered only for those patients whose life is clearly endangered. For SCD patients whose survival is not immediately threatened, living with severe morbidity is their only option.

Currently, an accepted alternative therapy is Hydroxyurea (HU), a chemotherapeutic agent that is known to increase fetal hemoglobin production. This agent interrupts DNA synthesis by inhibiting ribonucleoside diphosphate reductase. It is thought that HU produces a toxic effect on late erythroid progenitor cells when it interrupts DNA synthesis in these rapidly dividing cells. This then leads to stress erythropoiesis, which increases the number of "F cells," resulting in fetal hemoglobin production.[64] Increasing fetal hemoglobin also leads to inhibition of HbS polymerization with the increased in solubility of c_2f3^S hybrid molecules.[65]

Multiple reports looking at HU therapy have shown a significant decrease in hospitalization due to recurrent vasoocclusive crisis (VOC). In addition Ferster et al showed a significant decrease in the number of transfusion per patient-year from 1.83 before HU therapy to 0.2 after HU therapy. However, to date, there is no evidence for HU therapy to be beneficial to patients with a history of stroke(s). It is also not known what the long-term side effects would be in a population who will require life long therapy. Of concern is the leukemogenic property of this chemotherapeutic agent. Other side effects that are common include dose dependent transient myelosuppression, nausea, vomiting, and skin rash. These side effects tend to occur in the acute phase of treatment.[65]

Other medical treatments are available, but are currently in ongoing studies with little data. Theses studies include nitric oxide, magnesium supplementation, and short chain fatty acid such as butyrate therapy. However, none of the above mentioned therapies offer a potential cure for patients with SCD.

Given the current dilemma, allogeneic BMT is now being utilized as an alternative therapy for patients with severe SCD.[6] The interest has been prompted by the successful use of BMT for patients with homozygous $j3^{+/0}$ thalassemia.[7] These patients encounter problems similar to those of SCD patients, including disturbances in growth and sexual maturation, episodic bacteremia, and end organ failure. Likewise, management with chronic transfusion and chelation therapy has been used to ameliorate the disease process.[8] With alloBMT, the defect that underlies the pathophysiologic basis for the disease, namely absence of j3 chain synthesis, is corrected once donor hematopoiesis is established. A recent report details the outcome for 222 patients with thalassemia following alloBMT.[9] Among patients with adequate iron chelation and without hepatomegaly or portal fibrosis, the probability of survival and event-free survival are 97% and 94% respectively. This rivals if not surpasses the survival of patients maintained on chronic transfusion and chelation.[26] Even for patients with some degree of hepatomegaly, less optimal iron chelation, or portal fibrosis, disease free survival is 85%. Only for patients with abnormal findings for all these does disease free survival fall to 64%[57,58]. At present, hematopoietic stem cell transplantation must be considered the optimal therapy for patients with severe j3 thalassemia who have an HLA identical related donor. Such patients are eligible for treatment on this study as in keeping with best clinical practice.

The first BMT for a patient with SCD was performed at St. Jude Children's Research Hospital (SJCRH). [10] The indication for transplantation was to treat this patient's acute myeloid leukemia and not to cure sickle cell anemia. The donor was an HLA genotype identical brother with sickle cell trait. The BMT preparatory regimen included single fraction total body irradiation (TBI) and cyclophosphamide; methotrexate and prednisone were used to prevent graft-versus-host disease (GVHD). White cell engraftment occurred at day 12, and platelet engraftment at day 20. Acute GVHD grade III involving the skin and

gastrointestinal tract developed 6 weeks post transplant and evolved into chronic

Currently, very little has been reported about organ function after BMT in patients with sickle cell anemia. Evaluation of splenic function with nuclear scintigraphy

skin GVHD. Although the patient endured a complicated post transplant course, hematologic remission with full chimerism persists to this date.

Subsequently a number of centers here and in Europe undertook allogeneic SCT for patients with SCD. European investigators advocated SCT for any SCD patient for whom an HLA matched related donor was identified; their SCD patients were temporary resident African children destined to return to medically poorly served areas.[10-12] More recently indications for SCT follow the eligibility criteria as proposed by the consensus conference.[52] Vermylen reported the results of SCT for 50 patients, 36 of whom met the consensus criteria.[53] All patients received myeloablative conditioning and cyclosporine combinations for GHVD prevention. Event-free survival remained at 82% overall and 76% for patients transplanted for significant sickle cell disease-related morbidity. Overall survival stood at 93% but 88% for the more severely affected cohort. Three patients never engrafted, and 2 patients developed delayed graft rejection. Two patients died--one from complications of GVHD and the other from sudden death 6 years post transplant. Only 1 patient developed acute GVHD grade III.

Additionally, Walters et al looked at 50 children from the US and Europe who were less than 16 years of age with symptomatic disease and who had an HLA matched related allogeneic donor. Indications for SCT included a history of stroke, recurrent acute chest syndrome or sickle pulmonary disease, and recurrent vaso-occlusive crises. Forty-seven of the 50 patients survived with a median follow-up of 38.6 months. Forty-five patients demonstrated stable engraftment of donor hematopoietic cells. Graft rejection or recurrence of sickle cell disease occurred in five patients, and three patients died of intracranial hemorrhage or GVHD.[62] Overall survival was 94% and EFS 84%.

Our experience includes 7 patients with SCD (meeting the consensus criteria for eligibility) with median follow-up of 24.5 months (range 1yr-6yr). All patients received stem cells from sibling donors of which five were fully matched and 1 was 5/6 HLA matched donor; all engrafted. Two patients had stable mixed chimerism, but without evidence of disease. GVHD either did not develop or was grade I, and all patients are alive. To date, overall survival is 100% with EFS 100%.

In summary the experience of SCT in SCD and thalassemia affirms the feasibility of this undertaking. With alloSCT patient hematopoiesis can be ablated, and donor hematopoiesis established. Data also indicate that SCT can be accomplished without adversely impacting survival and without causing lasting procedure- related morbidity. What remains uncertain is whether SCT will prevent progressive organ damage and improve the quality of life. The next goal is to assess organ functions in SCD patients after SCT. We plan to evaluate post SCT organ functions by using Positron Emission Tomography (PET) technology, a novel approach in the SCD patient population.

Organ function evaluation in patients with sickle cell disease

has shown recovery of function after BMT in 3 patients when splenic function was investigated.[67] Walters et al showed pulmonary function to be stable or improved in a series of 26 patients with sickle cell anemia after BMT. Additionally, CNS abnormalities have been shown to be stabilized after BMT using follow up MRI studies. In one case report osteonecrosis was seen to be improved after allogeneic BMT in a teenage boy with sickle cell disease.[68]

Currently, data that have been reported on organ evaluation in patients with SCD post BMT involve use of conventional radiographic techniques. A powerful and promising scanning technique, positron emission tomography (PET) has been used to look at CNS abnormalities in patients with sickle cell anemia. Information about vascular blood flow using [^{15} O]-H_2O and tissue metabolism using [^{18}F]-fluoro-D-deoxy-glucose (FDG) can be deduced from PET scan. An advantage of PET is its ability to objectively quantify FDG uptake using standardized ratios for various organs. In SCD patients multiple reports have shown larger areas of abnormalities in the brain with regards to vascular flow and metabolic uptake on PET evaluation when compared to MRI findings in patients with sickle cell anemia.[69-70] Additionally, there have been abnormal findings on PET evaluation of the brain despite normal MRI results in patients with sickle cell.

PET scan has also been useful in evaluating other organs beside the CNS under various medical conditions. These medical conditions include malignancies, infections, and areas of inflammation that involve the bone, kidneys, and lungs. These organs are known to be compromised in patients with sickle cell anemia.

Selection of sickle cell patients eligible for SCT

Increased blood flow velocity in the distal internal carotid artery and middle cerebral artery on transcranial doppler evaluation has been shown to be a predictor of future cerbrovascular event (CVA) in patients with SCD.[71] This has been further confirmed by the recent STOP trial looking at prevention of stroke using chronic transfusion therapy in patients who are high risk for CVA based on an abnormal transcranial doppler. Following a cerebrovascular event, SCD patients are at excessive risk of subsequent stroke. In one report, two-thirds of the patients experienced a second event, usually within 12 to 24 months.[5]

As detailed above, chronic transfusion therapy with chelation is the only acceptable therapy. Unfortunately, transfusion therapy once initiated must be continued lifelong. Discontinuing transfusion therapy even after 12 years has precipitated a subsequent stroke[19], but a lifelong transfusion and chelation program is unlikely to be successful for all the reasons mentioned. For patients in this quandary, BMT may be an acceptable alternative.

Sickle cell chronic lung disease (SCCLD) is an unremitting, progressive, and invariably fatal complication of SCD.[20] Typically, symptoms and signs become evident in the second decade. Recurrent episodes of acute chest syndrome often herald its onset. Diagnosis and staging of SCCLD utilizes a constellation of

clinical symptoms, chest x-ray changes, and alteration in pulmonary function

tests.[20] Although sudden death from myocardial hypoxia can be a terminal event, most patients die from pulmonary failure. On average, patients with stage I SCCLD survive 7.1 years.[20] There is no treatment, and the palliating role of transfusion and chelation is not established. The implacable threat of SCCLD justifies the risk of BMT as an alternative to transfusion.

Frequent pain crisis can have mortal consequences for SCD patients and BMT may be justified therapy for such patients. Data from the CSSCD unequivocally establish that after age 20 years, patients having 3 or more pain crises per year die prematurely. Median survival is reached 10 years earlier for these patients compared to that for patients having fewer crises.[21] Until the CSSCD data became available, pain crisis was generally considered a universal feature of SCD. In reality, individual patients tend to maintain a characteristic pain rate (the number of pain crises per year) that is constant over time. This pain rate may be quite low; according to the CSSCD data; over a 5-year period 40% of patients did not seek medical attention for pain. Yet for other patients, the frequency of pain crises is devastatingly high; in this study of over 3000 patients, 5% of patients accounted for one-third of these events. Not clear from the CSSCD data is whether there is a significant correlation between pain rate and mortality for adolescent patients. The fact that few deaths occurred among this age group may have precluded making the association. However, if adolescents with high pain rate maintain this rate as they age, then presumably these patients will become the group that suffers premature mortality. Data from the CSSCD and elsewhere support the constancy of pain rates over time.[22] Further observation of the CSSCD population will clarify the association of adolescent pain rate and premature mortality. For now, patients with pain crises frequent enough to require chronic transfusion will be eligible for BMT.

The risk/benefit relationship for patients treated on this protocol is encouraging; BMT for younger patients with SCD has not been associated with an unacceptable risk of death or severe morbidity. Several series have shown that eradication of patient hematopoiesis (i.e. engraftment) has been followed by excellent quality of life and relief from some manifestations of chronic SCD.[60,61]

3.0 DRUG INFORMATION

3.1 **BUSULFAN**

Therapeutic Classification: Bifunctional alkylating agent

Pharmaceutical Data: Busulfan (Busulfex Injection® Orphan Medical) is supplied as a sterile solution in single-use ampules containing 60 mg at a concentration of 6mg/ml. It is provided as a mixture of demethylacetamide (DMA) and polyethylene glycol 400 (PEG400).

Solution Preparation: Busulfan solution for injection must be diluted with either 0.9% Sodium Chloride Injection (NS) or 5% Dextrose Injection (D5W)

prior to administration. The diluent quantity must be 10 times the *volume* of busulfan, ensuring that the final concentration is > 0.5 mg/ml. Sample calculation

Mechanism of Action: Busulfan is a bifunctional alkylating agent in which two labile methanesulfonate groups are attached to opposite ends of a four-carbon

for a 50 kg patient: (50 kg) x (0.8 mg/kg of busulfan) = 40 mg = 6.7 ml. 6.7 ml of busulfan + 67 ml of NS = 74 ml total volume. Final concentration: 0.54 mg/ml.

Stability and Storage Requirements: After dilution with NS or D5W, busulfan is stable at room temperature (25 degrees Celsius) for 8 hours. The infusion must be completed within that time. Prior to mixing: Store under refrigeration (2 to 8 degrees Celsius). Busulfan for injection is stable at 4 ^{O} for at least 12 months.

Route of Administration: Busulfan should be administered intravenously via a central venous catheter as a two-hour infusion.

Usual Dosage Range: 0.8-1 mg/kg/dose given every 6 hours for a total of 16 doses. For patients less than 4 years of age a dose of 1 mg/kg/dose will be used and for patients > 4 years the starting dose will be 0.8 mg/kg/dose. Doses are based on actual body weight, unless the patient's weight is greater than 30% of ideal body weight, then dosing will be based on adjusted weight of ideal plus 25%. Busulfan pharmacokinetics will be performed on all patients with dose adjustment as appropriate.

Pharmacokinetics: Doses will be adjusted to achieve the desired plasma area under the curve (AUC) of 900 – 1200 µmol-min/L. Doses will be adjusted as necessary pending the results of the first dose pharmacokinetics. For patients whose AUC values are greater than 5% outside the acceptable AUC range, the dose will be adjusted to achieve a target AUC of 1125 µmol-min/L (midpoint of acceptable range) not to exceed a maximum dose of 1.6 mg/kg per dose of busulfan.

Side effects: Myelosuppression, neurotoxicity (manifesting as seizures), mild to moderate nausea and vomiting, mild to moderate tachycardia, skin hyperpigmentation, sterility, and rarely hepatotoxicity (hepatic veno-occlusive disease) and pulmonary toxicity (interstitial fibrosis).

Special Precautions: Increased toxicity in obese patients unless dose is adjusted appropriately. Generalized seizures have been reported after use of high dose busulfan. All patients will be treated with phenytoin 5 mg/kg/dose (IV or PO) q 6 hr beginning on day -10 for 24 hr until completion of busulfan. A trough phenytoin level should be obtained 6 hours after the fourth dose. The therapeutic range for this drug is 10-20 µg/ml. If the level is below 10 µg/ml, two additional doses of 5 mg/kg/dose should be administered q 6 hours. If the level is below 5 µg/ml, an additional four doses of phenytoin should be administered q 6 hours over the next 24 hours. If the phenytoin level is within the therapeutic range, maintenance therapy should begin at a dose of 5 mg/kg/day (IV or p.o.) in two divided doses until day -4. Patients experiencing seizure activity should be evaluated neurologically and treated as clinically indicated.

alkyl chain. In aqueous media, busulfan hydrolyzes to release the methanesulfonate groups. This produces reactive carbonium ions that can alkylate DNA. DNA damage is thought to be responsible for much of the cytotoxicity of busulfan.

Human Pharmacology: Busulfan can be administered orally or intravenously. Busulfan achieves levels in cerebrospinal fluid similar to plasma levels. Busulfan is predominantly metabolized by conjugation with glutathione, both spontaneously and by glutathione S-transferase (GST) catalysis. This conjugate undergoes further extensive oxidative metabolism in the liver. Approximately 30% of busulfan and metabolites can be recovered in the urine within 48 hours after administration.

3.2 **CYCLOPHOSPHAMIDE** (CYTOXAN, CTX) NSC# 26271

Source and Pharmacology: An alkylating agent, related to nitrogen mustard, biochemically inert but metabolized by liver phosphamidases to active components, e.g. 4-hydroxycyclophosphamide, which are excreted exclusively by the kidney. CTX is non-phase-specific. The plasma half-life ranges from 4 to 6.5 hours. When taken orally, 25% may be passed in the stool unchanged.

Formulation and Stability: Injectable form is available as white crystals with sodium chloride added, in vials containing 100 mg, 200 mg and 500 mg. All preparations are stable at room temperature (not to exceed 30^{o}C). Reconstitute with 5 ml sterile water for 100 mg vials, 10 ml for 200 mg vials and 25 ml for 500 mg vials. Also available in 1 g and 2 g vials; reconstitute with 50 ml and 100 ml sterile water, respectively. Discard solution after 24 hours at room temperature; stable up to 6 days if refrigerated (2^{o}-8^{o}C). CTX is also available in 25 and 50 mg tablets.

Supplier: Commercially available.

Toxicity:
Acute dose-limiting toxicity - myelosuppression, primarily leukopenia, with a nadir at 8-14 days. Therapy must be withheld if acute hemorrhagic cystitis occurs. Therapy may be started once hematuria clears and there is no evidence of a contracted bladder. Very high doses over a short period of time may be associated with myocardial necrosis, transiently blurred vision, and cardiac toxicity with arrhythmias. Other adverse reactions include anorexia, nausea and vomiting, alopecia, SIADH, immunosuppression, and gonadal suppression with associated sterility. Pulmonary fibrosis is rare. Hemorrhagic cystitis: Hematuria is not uncommon at this dose level, but is usually not symptomatic or severe. Adequate urine flow is essential to avoid cystitis. All patients will be treated with MESNA coincident with each dose of CTX (see below). Pyridium will be given for symptomatic relief. Alopecia: Usually reversible.

Skin rash: Ten to 20% of patients develop a diffuse maculopapular rash 24-72

hours following CTX. The rash usually clears in 24-48 hours. Diarrhea: treated symptomatically. Fluid retention: CTX has an anti-diuretic effect usually

counteracted by furosemide administration. Careful physical examination should be made and daily weights and electrolytes determined to detect fluid overload early. Anemia: Hemoglobin drops can occur at this dose, presumably due to hemolysis.

Chronic toxicity - gonadal dysfunction is dose-related. The prepubertal gonad is less susceptible. Hormonal function is generally preserved, especially in the male; fertility is mainly affected (spermatogenesis in the male, follicle formation in the female). Sterility may be partially reversible. Gonadal effects of administration during puberty are uncertain; the risk of sterility may be increased.
Secondary neoplasms have occurred in a small number of patients receiving this drug. Cardiomyopathy: At doses greater than 200 mg/kg, CTX can cause fatal cardiac necrosis with heart failure. Patients are monitored by daily EKGs to detect decrease in voltage. Non-specific ST changes are not unusual but a decrease in voltage is significant and ominous. CTX is contra-indicated in patients with existing cardiac disease. The EKG must be checked before each dose of CTX; patients developing significant reduction in cardiac voltage will have all subsequent doses discontinued. Reproduction: The long term effects are unknown but sterility is probable.

Dose adjustments: CTX in the study will be given at a dose of 50 mg/kg IV ideal body weight or actual body weight, whichever is lowest on each of four successive days. CTX is dissolved in 100-250 cc of D5W and administered over a 30-60 min period followed by IV hydration for 12 hr with 2.4 L/m^2.

Diuresis and hydration: The urine flow during and for 12 hours after CTX administration is to be kept at a minimum of 2.5 cc/kg/hr. If the urine output decreases below these numbers, Furosemide 10-20 mg/m^2 IV may be given. IV fluids at 2.4 L/m^2/12 hr post CTX will be routinely employed. A Foley catheter may be placed in young children if difficulty is measuring urine output occurs.

Route of Administration: Intravenous

33 **MESNA (Sodium 2-Mercaptoethane Sulfonate, Mesnex, Urimetexan)**

Source and Pharmacology: MESNA is a thiol compound with the capacity of inhibiting the urotoxicity of the oxazaphosphorines, ifosfamide and cyclophosphamide. Within 1 hour of administration, MESNA is completely oxidized to DiMESNA, a totally inert compound. There is little or no tissue penetration. Following glomerular filtration DiMESNA is rapidly reduced in the renal tubules back to MESNA which inactivates the oxazaphosphamides, thus preventing bladder toxicity. After 3 hours, negligible amounts of MESNA were present in the urine of rats given 100 mg/kg by IV push.
Formulation and Stability: Available in ampules containing 100 mg/ml of solution containing disodium edentate 0.25 mg and sodium hydroxide to adjust pH 6.5 to 8.5 in water for injection. Supplied on 200, 400 and 1000 mg ampules. Store intact ampules at controlled room temperature (15□-30□C). MESNA is not

light-sensitive, and intact ampules are stable for five years from manufacture. MESNA can be mixed in the following intravenous infusion solutions with less than 5% decomposition over 24 hours: 5% dextrose, 5% dextrose in 0.45 sodium chloride, 0.9% sodium chloride and lactated Ringer's. MESNA may be mixed with ifosfamide, but is incompatible with cisplatin.

Supplier: Commercially available.

Toxicity: May cause nausea and vomiting, and a bitter taste during IV administration. At 60-70 mg/kg IV daily x 4, abdominal pain, headache, limb and joint pain, lethargy, diarrhea, and transient hypotension have been encountered.

Route of administration: (To be supplied by PI)

3.4 **CYCLOSPORIN: (SANDIMMUNE)**

Source and Pharmacology: Cyclosporin is an immunosuppressive agent produced by Tolypocladium inflatum Gams or Cylindrocarpon lucidum Booth. Cyclosporin is a nonpolar, cyclic polypeptide antibiotic consisting of 11 amino acids. It is thought that cytotoxic T-lymphocytes (CTLs) together with helper T-cells, natural killer cells and the release of lymphokines, including interleukin-2 (IL-2), play a major role in graft rejection and graft versus host disease. The exact mechanism(s) of the immunosuppressive action of CsA has not been fully elucidated. Experimental studies suggest that the primary actions of CsA in preventing graft rejection and in establishing immunologic unresponsiveness to allografts are (1) the prevention of precursor CTL from acquiring responsiveness to IL-2, and thereby inhibiting the activation of CTLs; (2) the inhibition of production of IL-2 and other cytokines, the signals that lead to proliferation and activation of effector cells. In contrast, CsA apparently leaves B cells and macrophage function essentially unimpaired.

The selective effect of CsA on the immune system is largely unexplained. Studies seem to indicate that at the intracellular level CsA interferes with the calcium-dependent activation of various enzymes systems including the enzymatic cascades accounting for IL-2 production and IL-2 receptor formation.

Formulation and Stability: Cyclosporine (CsA) oral solution is supplied in 50 ml bottles. It has a clear, yellow, oily appearance. Each ml contains 100 mg of CsA and ethanol 12.5% by volume dissolved in an olive oil compound, Labrafil M 1944CS (polyoxethylated oleic glycerides) as the vehicle. Prior to oral administration, the appropriate dose of the CsA solution must be further diluted with milk, chocolate milk, or orange juice, stirred well, and taken by the patient at once. To ensure adequate dose administration, the glass should be rinsed with more milk or orange juice and given to the patient. It should not be allowed to stand for any length of time.
Cyclosporin is also supplied as soft gelatin capsules in a 25 mg and 100 mg strength. Both capsules contain a maximum of 12.7% ethanol, USP dehydrated.

Intravenous CsA is supplied in a 5 ml sterile ampule for intravenous administration. Each ml contains 50 mg of CsA, 650 Cremophor IL

(polyoxethylated castor oil) and ethanol 32.9% by volume. Cyclosporin concentrate for injection diluted to a final volume of 2 mg/ml is stable for 24 hours in 5% dextrose or 0.9% sodium chloride. Dilutions of the drug in dextrose or sodium chloride should not require protection from light. The solution should be checked for particulate matter and discoloration. The solution may be administered as a continuous infusion over 24 hours, or divided as short-term infusions (2-6 hours) every 12 hours.

Supplier: Commercially available (Sandoz).

Toxicity: The most frequent and clinically important adverse effect of cyclosporin is nephrotoxicity. Elevation of BUN and serum creatinine concentrations resulting from cyclosporin therapy appear to be dose-related, may be associated with high trough concentrations of the drug, and are usually reversible with discontinuation of the drug. Clinical manifestations may include fluid retention, edema, and in some cases, hyperchloremic, hyperkalemic metabolic acidosis. The risk of renal toxicity increases with administration of other potentially nephrotoxic agents. Hypertension may develop, generally within the first few weeks of therapy and affects both systolic and diastolic blood pressure. Some evidence suggests that hypertension results from the renal vasoconstrictive effects of the drug. Hypertension may respond to dosage reduction and/or antihypertensive therapy. Adverse nervous system effects occur frequently, with tremor occurring in a large percentage of patients. Tremor may be manifested as fine hand tremor, usually is mild in severity, may improve despite continued therapy, and/or may be alleviated with dosage reduction. Seizures, headache, paresthesia, hyperasthesia, flushing and confusion have also been reported occasionally. Other adverse effects include hirsutism, gingival hyperplasia, and hepatotoxicity. Adverse GI effects occur frequently during cyclosporin therapy, including diarrhea, nausea, vomiting, anorexia and abdominal discomfort. Infectious complications have occurred frequently. Adverse hematologic effects have occurred occasionally during cyclosporin therapy.

Route of Administration: Intravenous and by mouth.

3.5 **METHOTREXATE: (MTX, AMETHOPTERIN)**

Source and Pharmacology: A folate analogue which inhibits the enzyme dihydrofolate reductase, which halts DNA, RNA and protein synthesis. Initial IV half-life is about 1.2 hours with a second phase of 10.4 hours. Orally administered MTX undergoes dose-dependent absorption (average of 35% at doses of 15-75 mg/m^2), with the average time of peak concentration at 2 hours. MTX is excreted unchanged in the urine, and at high doses given over 24 hours, it is partially metabolized (40%) to hydroxy MTX and excreted. After IT administration, the drug is rapidly distributed in the CSF with initial half-life of 4.5 hours and a second phase of 14 hours. <u>High-dose methotrexate should not be given to patients with severe renal dysfunction, significant pleural effusions or ileus.</u> Leucovorin rescue should be started within 48 hours of starting high-dose methotrexate; it may be necessary after IT administration, particularly if IT therapy is given to patients with renal dysfunction.

Formulation and Stability: Commercially available as 12, 25, 50, 100, 200 mg vials as solution and 500 and 1000 mg vials of powder. Tablets are available at 2.5 mg. Intact vials and bottles of tablets bear an expiration date.

Supplier: Commercially available.

Toxicity: Acute dose-limiting toxicity consists of marrow suppression with the nadir for anemia at 6-13 days, leukopenia at 4-7 days and thrombocytopenia at 5-12 days. Other factors which may limit or delay therapy include ulcerative stomatitis, severe diarrhea or acute nephrotoxicity. Most patients experience a transient decrease in glomerular filtration rate during high-dose methotrexate. Other adverse effects include nausea, vomiting, anorexia, gingivitis, glossitis, stomatitis, immunosuppression, hepatic toxicity, rash, pleuritis, pruritus, vasculitis, photosensitivity, depigmentation or hyperpigmentation, alopecia, dizziness, malaise, blurred vision, and increased skin sensitivity to sunlight. Patients with Down Syndrome have a tendency to have delayed MTX clearance and greater risk of toxicity, despite increased leucovorin rescue, so that caution is advised.

NOTE: Renal dysfunction will enhance toxicity.

IT administration has been associated with increased CSF pressure, headache, pleocytosis, fever, vomiting, nuchal rigidity, convulsions, paresis, Guillain-Barre syndrome, leukoencephalopathy and death.

Route of Administration: Intravenous.

3.6 CAMPATH-1H

Source and Pharmacology: CAMPATH-1H is a humanized antilymphocyte monoclonal antibody. The Campath-1 antigen in humans (CD52) is predominantly expressed on peripheral blood lymphocytes, monocytes, and macrophages. CAMPATH-1H causes lysis of lymphocytes by fixing to CD52, a highly expressed, non-modulating antigen on the surface of lymphocytes. It mediates the lysis of lymphocytes via complement and antibody dependent cell mediated cytotoxicity mechanisms. CAMPATH-1H rapidly reaches a peak concentration after IV administration. The half-life has been thought to be approximately 60 hours, although recent data suggest a more prolonged persistence. The route of elimination is not known, but is probably via uptake and metabolism by the reticulo-endothelial system of the liver and spleen.

Formulation and Stability: CAMPATH-1H is supplied as a purified preparation diluted in phosphate buffered saline (PBS) with 0.05 mmol EDTA. Tween 80 is added to a concentration of 0.01%. The final product is a clear, colorless isotonic solution free of visible particulate matter. The glass ampules will contain 30mg of antibody in 3 mL of sterile PBS at a concentration of 10 mg/ml. . It should be stored, protected from light, in a refrigerator at between 2° and 8° C. .
After dilution in D5W or NS, the resulting solution is stable for 24 hours at 2-8^{0}C (36-46^{0}F). However, the product contains no preservative and must therefore be

used within 8 hours. Intravenous CAMPATH-1H will be diluted in 100cc of 0.9% normal saline or 5% Dextrose and administered intravenously over 2 hours. CAMPATH-1H must be filtered with a sterile, low-protein binding, 5 m filter prior to dilution.

Dose: A dose of 10 mg/patient has been chosen based on the plasma concentrations required for activity, and on the previous extensive published human experience using the dose for lymphocyte depletion.

Supplier: Investigational. L&I Partners, LP

Toxicity: CAMPATH-1H is a potent lymphocyte-depleting agent. WHO Grade 3 and 4 neutropenia and thrombocytopenia emerge on treatment in approximately 10-20% of patients. Infections resulting from CAMPATH-1H induced immunosuppression is the major type of adverse event occurring outside the CAMPATH-1H administration period, with the most common infections being mucocutaneous herpes simplex and candidiasis.
The majority of other adverse events seen in CAMPATH-1H trials can be categorized as being administration-related and of short duration. There is usually a first dose effect consisting of cytokine release type phenomena including hypotension, rigors, fever, shortness of breath, chills, rashes, etc. These side effects can be significantly ameliorated or avoided by premedicating the patients with methylprednisolone or hydrocortisone.

Route of Administration: Intravenous.

4.0 PATIENT ELIGIBILITY

4.1 Patients with an HLA genotype identical sibling/related donor and hemoglobin SS, hemoglobin SC, or hemoglobin S$\beta^{0/+}$ and at least one of the following conditions:

4.1.1 previous central nervous system vaso-occlusive episode with or without residual neurologic findings, or has an abnormal transcranial doppler exam without neurologic findings, or abnormal MRI/MRA of the brain with or without neurologic findings;

4.1.2 frequent painful vaso-occlusive episodes which significantly interfere with normal life activities and which necessitate chronic transfusion therapy;

4.1.3 recurrent SCD chest syndrome events, which necessitate chronic transfusion therapy;

4.1.4 severe anemia which prevents acceptable quality of life and necessitates chronic transfusion therapy;

4.1.5 Any of the above symptoms in which the patient is not undergoing chronic transfusion therapy;

4.1.6 The patient is undergoing chronic transfusion therapy for symptoms other than those listed and which significantly interferes with normal life activities;

4.1.7 Failed hydroxyurea therapy;

4.1.8 Indication of pulmonary hypertension on 2 separate echocardiogram examinations;

4.1.9 Patients who plan to return to resource poor areas/countries.

4.2 Between the ages of birth and 40 years.

4.3 Women of childbearing potential must have a negative pregnancy test.

4.4 The following patients who otherwise satisfy eligibility requirements will be ineligible:

4.4.1 Patient with biopsy proven chronic active hepatitis or fibrosis with portal bridging.

4.4.2 Patient with stage 4 SCD chronic lung disease (see Appendix 1).

4.4.3 Patient with severe renal dysfunction defined as creatinine clearance < 40 ml/min/1.73M^2.

4.4.4 Patient with severe cardiac dysfunction defined as echocardiogram shortening fraction < 25% or NYHA class III or IV.

4.4.5 Patient with HIV infection.

4.4.6 Patient with unspecified chronic toxicity serious enough to detrimentally affect the patient's capacity to tolerate bone marrow transplantation.

4.4.7 Patient or patient's guardian(s) unable to understand the nature and risks inherent in the BMT process.

4.4.8 Pregnant/lactating women and those unwilling to use acceptable contraception will be excluded.

4.4.9 Patient or patient's guardian who have not signed an informed consent.

NOTE: Patients who would be excluded from the protocol strictly for laboratory abnormalities can be included at the investigator's discretion

after approval by the CCGT Protocol Review Committee and the FDA reviewer.

5.0 STUDY DESIGN

This is a nonrandomized study looking at organ function improvement after BMT in SCD patients. We plan to enroll 15 patients over a period of 3-4 years. (See section 1 for objectives of this study).

6.0 TREATMENT PLAN

6.1 BONE MARROW PREPARATORY REGIMEN

6.1.1 Prior to initiating the BMT preparatory regimen patients will

- have placement of a double lumen Hickman catheter
- have a percutaneous liver biopsy.

6.1.2 Prior to initiating the BMT preparatory regimen, the patient's hemoglobin SS concentration must be < 30%. This may necessitate the patient undergoing partial exchange transfusion.

6.1.3 Treatment schedule

Protocol day	Treatment
-10	Begin Dilantin or Lorazepan
-9	Busulfan 4.0 mg/kg/day IV divided into four doses daily for four days; total dose = 16 mg/kg
-8	BU X 4 (Based on AUC < or = 1200 tMol-min/L)
-7	BU X 4 (Based on AUC < or = 1200 tMol-min/L)
-6	BU X 4 (Based on AUC < or = 1200 tMol-min/L)
-5	Campath 1H 10mg/IV CTX 50mg/kg +MESNA
-4	Campath 1H 10mg/IV CTX 50mg/kg+MESNA
-3	Campath 1H 10mg/IV + CTX 50 mg/kg + MESNA
-2	CTX 50mg/kg +MESNA + Campath 1H 10mg/IV
-1	REST
0	bone marrow infusion with premeds as per CAGT SOPs.

BU (busulfan) = 4.0 mg/kg/day (total 16 mg/kg), dosage adjusted to age (see section 3.1). Daily dose divided into every 6 hour IV. The dose of busulfan and cyclophosphamide is based upon the ideal body weight. The dose of BU will be further adjusted to achieve the desired plasma area under the curve (AUC) of 1100 - 1200 - ĩmol-min/L. Doses will be adjusted as necessary pending the results of the first dose pharmacokinetics. For patients whose AUC values are greater than 5% outside the acceptable AUC range, the dose will be adjusted to achieve a target AUC of 1125 - ĩmol-min/L (midpoint of acceptable range) not to exceed a maximum dose of 1.6 mg/kg per dose of busulfan.

Anticonvulsant therapy: All patients require anticonvulsant therapy while receiving busulfan. Dilantin or lorazepam are the two agents most frequently used in this regard. **The clinical pharmacist must review orders for anticonvulsants**. Dosing should be dictated by blood anticonvulsant levels whenever possible. Anticonvulsant therapy will be continued through day +100 at a minimum.

CTX (cyclophosphamide) = 50 mg/kg IV on each of four successive days. Hematuria is not uncommon at this dose level of cyclophosphamide. Adequate urine flow is essential and the exact hydration and MESNA doses should be followed. Please see agent information section 3.0 and preprinted order sheets.

Campath 1H on four successive days. Prior to Campath patients will receive a premedication as appropriate to prevent allergic reaction. The dosage is as follows:

Children	Adolescents & Adults
5-15 kg: 3 mg IV in 30 ml NS 15.1 kg: 5 mg IV in 50 ml NS >30 kg: 10 mg IV in 100 ml NS	10 mg IV in 100 ml NS

6.1.4 Bone marrow dose: To ensure the probability for bone marrow engraftment, 4 x 10^8 nucleated cells/kg patient weight will be the target at donor bone marrow harvest.

6.1.5 After transplantation, G-CSF will be administered as medically indicated.

6.2 GRAFT VERSUS HOST DISEASE PROPHYLAXIS

6.2.1 Cyclosporin will be administered beginning day -2. Initial dose will 5 mg/kg infused over 24 hours. Modifications in cyclosporin dose will be determined by the measured blood concentration

(cyclosporin levels will be maintained within the acceptable therapeutic range). For patients without GVHD, cyclosporin will be tapered over 6 months.

6.2.2 Experience in performing stem cell transplantation for patients with SCD indicates that hypertension may precipitate seizures. It is important to measure and maintain blood magnesium levels in the normal range and to provide effective antihypertensive therapy to patients developing hypertension.

6.2.3 Because Campath IH infusions will provide a persisting level of antibody over the transplant period, it will contribute to anti-GvHD activity. Additional Graft versus Host disease prophylaxis will consist of Methotrexate, administered on day +1, at a dose of 15 mg/m^2 IV , and on day +3, +6, and +11 at 10 mg/m^2 IV.

6.2.4 For patients who develop GVHD, methylprednisolone will be added at an initial dose of 1 mg/kg. Dose will be increased for persistent or progressive GVHD.

6.3 CONCURRENT TREATMENT AND SUPPORTIVE CARE (Note these treatment and supportive care measures may be altered or supplemented by any current IRB approved treatment protocol or by substitution of Standard Operating Procedure guidelines)

6.3.1 Irradiate all blood products (except the bone marrow) with 1500-3500 cGy.

6.3.2 Leukocyte filtered RBCs and platelet components will be used to minimize transmission of CMV. Patients who are CMV negative with CMV negative donors will receive CMV negative blood products.

6.3.3 Platelets: Irradiated leukocyte poor platelet concentrates are transfused to prevent bleeding, especially CNS bleeding, and the circulating platelet count should be maintained at or greater than 50,000/mm^3 for the first 5 weeks post-BMT. Thereafter transfusion will be guided by patient's clinical state.

6.3.4 Red cells: PRBCs (irradiated, leukocyte poor) 10-15 cc/kg/dose will be administered to keep the hemoglobin level $>$10 g/dl. Higher hemoglobin concentrations might be considered for patients with pulmonary insufficiency.

6.3.5 Hyperalimentation: Adequate alimentation appears to be an essential for successful transplantation. Parenteral hyperalimen-

tation is maintained until normal enteral alimentation can be resumed as judged by the clinical situation.

6.3.6 Prophylaxis and management of infections:

6.3.6.1 Prophylaxis and management of infections according to CAGT Clinical SOPs for infectious prophylaxis.

6.3.7 Monitoring of Post Transplant Sickle Cell Symptoms: Patients will be requested to report all symptoms of sickle cell disease, and these will be assessed formally at each clinic visit. In particular, they will be asked to report episodes of pain. Acute chest syndrome, CNS disturbances and priapism.

7.0 LABORATORY EVALUATION

7.1 Prestudy Evaluation

7.1.1 History

A complete history will be obtained to document clinical course of symptoms related to the patient's hemoglobinopathy

7.1.2 Physical Exam:

A complete physical exam

7.1.3 Laboratory Evaluation: Pre-BMT

CBC, reticulocyte count

Hemoglobin profile or electrophoresis, quantitate F, A^2, S for both patient and sibling donor

STRs (Serial Tandem Repeats) for patient and donor

Electrolytes, calcium, magnesium, phosphorus, uric acid, BUN, creatinine, albumin, prealbumin, total bilirubin, SGOT, SGPT, alkaline phosphatase, LDH, triglycerides

Ferritin, iron if not done in the last year

FSH, LH, testosterone or estrogen, thyroid battery

Echocardiogram; EKG; CPK; 24 hour Holter monitor if clinically indicated

Pulmonary function tests (FVC, FEV^1, arterial blood gas)

Chest x-ray

Brain MRI & MRA (contrast injection optional)

PET scan of head and body

Ophthalmology consultation as clinically indicated

Dental consultation as clinically indicated

Gulf Coast Donor Panel

Patient and donor immunization with Prevnar 7 at least 2 weeks before BMT

Neuropsychiatric evaluation

24 hr urine protein and creatinine clearance; $Tc^{99}m$ DPTA plasma clearance (GFR)

Liver biopsy

7.2 Laboratory evaluation: During in-patient and up to day +120 and thereafter

7.2.1 CBC with WBC differential and platelet count at a **minimum** weekly or as clinically indicated beginning with the BMT preparatory regimen continuing until the patient is no longer red cell and platelet transfusion dependent.

7.2.2 Electrolytes, BUN, creatinine, magnesium, calcium, phosphorus, glucose at a **minimum** weekly during bone marrow transplant preparatory regimen; thereafter daily until discharge or reestablishment of enteral nutrition.

7.2.3 Albumin, SGOT, SGPT, GGT, alkaline phosphatase, and total bilirubin, and uric acid at a minimum two times/week beginning with BMT preparatory regimen continuing until discharge.

7.2.4 Urinalysis during hospitalization as clinically indicated.

7.2.5 Weekly CMV antigen or as clinically indicated until day +120; thereafter at the discretion of MD.

7.2.6 Bone marrow aspirate at approximately day +28 and at day +120.

7.2.7 Bone marrow for STRs at approximately day +28 and day +120. Peripheral blood for STRs at approximately day of engraftment and at D+100.

7.2.8 Hemoglobin profile or electrophoresis with hemoglobin F quantification if STR studies show mixed chimerism.

7.2.9 Ferritin and iron every three months as clinically indicated

7.2.10 PET scan of brain and body at 6 months and 1 year post SCT

7.2.11 ECHO and EKG as clinically indicated

7.2.12 Pulmonary function studies as clinically indicated

8.0 EVALUATION CRITERIA

The major goal of this study is to determine the benefits of allogeneic BMT in relation to organ function recovery post transplant in patients with severe SCD, its variants, and homozygous β thalassemia. Improvement in FDG uptake on PET showing reversal of organ dysfunction and increase in antibody titers post pneumococcal immunization will be primary endpoints. BMT-related morbidity/mortality, specifically GVHD and infections will be closely monitored and their impact on survival will be monitored in the statistical design. In addition, the effect of BMT on the course of SCD following BMT will be assessed by laboratory tests and diagnostic imaging as outlined in the previous section. Survival and disease free survival will be determined.

81 Engraftment: hematologic recovery post BMT will be scored based on the Dartmouth Transplant Criteria:

Score

0 Polys never <500/μl, platelets never <10,000/μl
1 Polys >500/μl, platelets >10,000/μl within 4 weeks of BMT
2 Polys >500/μl, platelets >10,000/μl 4-8 weeks post BMT
3 Polys <500/μl, platelets <10,000/μl beyond 8 weeks
4 Death due to bacterial or fungal infections, or hemorrhage associated with polys <500/μl, platelets <10,000/μl more than 8 weeks post BMT

82 Graft versus host disease (Seattle criterion)

For skin: Stage	Skin Involvement
0	0

1	greater than 0, less than 25%
2	greater than or equal to 25%, less than or equal to 50%
3	greater than 50%
4	greater than 50% with blisters

For gut:

Stage	Stool Volume
0	less than 7 cc/kg
1	greater than or equal to 7 cc/kg, less than 14 cc/kg
2	greater than or equal to 14 cc/kg, less than 21 cc/kg
3	greater than or equal to 21 cc/kg, less than 28 cc/kg
4	greater than or equal to 28 cc/kg

For liver:

Stage	Bilirubin (mg/dL)
0	less than 2
1	greater than or equal to 2, less than 3
2	greater than or equal to 3, less than 6
3	greater than or equal to 6, less than 15
4	greater than or equal to 15

Overall:

Grade	Organ Stage
0	0
1	skin = 1 or 2
2	skin = 3, or skin less than or equal to 3 and gut or liver equal to 1
3	skin greater than or equal to 3 and gut or liver equal to 2 or 3
4	skin, gut or liver equal to 4

83 Common toxicity criteria (see Appendix 2)

9.0 CRITERIA FOR TAKING PATIENT OFF STUDY

Death and patient withdrawal will be the only reasons a patient can be withdrawn from study.

10.0 REPORTING REQUIREMENTS

10.1 To register patients contact the research coordinator at 832-824-4881. A copy of the consent form should be sent to her.

10.2 The data to be collected will be all data relevant to the objectives of the protocol.

10.3 Forms to be completed:

- Eligibility checklist
- On study form

- Adverse event record
- Response
- Follow up forms
- Off study forms

10.4 Drug toxicity and/or adverse reactions

Adverse events will be collected according to SOP J02.05.XX, J02.06.XX and J02.78.XX.

10.5 Relapse and survival data will be collected until 2 years after transplant.

11.0 STATISTICAL CONSIDERATIONS

This is a nonrandomized investigational study looking at potential organ function recovery after allogeneic BMT using a preparatory regimen of busulfan, cyclophosphamide, and MESNA and Campath IH, in patients with SCD. We expect to enroll 15 patients over a period of 3 to 4 years. Safety monitoring will be conducted using Wald's sequential probability ratio test (SPRT)[25] to monitor the rate of engraftment. The primary endpoint, organ function recovery, will be assessed for each patient at 3 months, 6 months, 1 year, and 2 years post transplant using MRI and PET scans. Also, immune response to pneumococcal vaccine will be evaluated using pneumococcal antibody titers.

We expect abnormalities in organ function to be stabilized or improved after BMT and antibody titers to rise after immunization.

Also, we expect 90% of the patients to engraft with an incidence of AGVDH of 15%-20% stage I and 5%-10% stage II or greater. No data for CGVHD exists for high-risk SCD patients.

The table, below, lists the total number of engraftment failures (including deaths or lost grafts prior to day +120) observed on day +120 necessary to trigger a suspension of accrual until a thorough reevaluation of the protocol has been completed. Additionally, if at any time two deaths prior to day +120 are observed accrual will be suspended and a thorough analysis conducted. These estimates provide a 10% chance of erroneously concluding that the true rate of engraftment is 90% or greater when in fact it is less than 80%. They also allow for a 10% chance of mistakenly concluding that the true engraftment rate is less than 80% when in fact it is at least 90%.

Safety Monitoring Guidelines

Engraftment Failure

Patients Entered (n)	Engraftment Failures
1	-

Patients Entered (n)	Engraftment Failures
2	-
3	-
4	4
5	4
6	4
7	4
8	4
9	5
10	5
11	5
12	5
13	5
14	5
15	5

Kaplan-Meier estimates and associated confidence intervals of disease free survival, time to engraftment failure, survival, and organ function recovery will be developed for all patients registered on the study.

Risk/benefit of this therapy (5.3) can be made only by subjective comparison to the risk/benefit of other available therapy for SCD. No statistical analyses will accompany this objective.

12.0 INVESTIGATOR'S BROCHURE WAIVER

12.1 An investigator's brochure is not required as Houston Methodist Hospital and Texas Children's Hospital Stem Cell Transplant Units function as one single and fully integrated administrative unit. The physicians involved in the Campath studies will be identical at both sites and will be closely involved in the drug administration process and patient evaluation.

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15.0 APPENDICES

APPENDIX 1
Sickle Chronic Lung Disease: Staging Criteria

Clinical Markers	Stage 1	Stage 2	Stage 3	Stage 4
Chest pain	Recurrent substernal pain and chronic cough	Increased pain over Stage 1	Severe midline crushing chest pain	Severe and prolonged pain with dyspnea at rest
Blood gases	Normal oxygen saturation	Normal oxygen saturation	Hypoxia with partial pressure oxygen (70 mm Hg) during stable periods	Partial pressure oxygen (60 mm Hg) during stable periods
X-ray	Decreased distal pulmonary vascularity, hyperexpansion, evidence suggestive of increased interstitial markings	Diffuse, fine interstitial fibrosis involving all lobes of the lung	Pulmonary fibrosis	Severe pulmonary fibrosis
Pulmonary function tests*	Decreased FVC, TLC, FEV_1, and FEV_1/FVC ratio (mild, 80% of predicted normal, or 1 S.D. below normal)	Decreased FVC, FEV^1, TLC, DCD and FEV_1/FVC ratio (monderat 60% of predicted, or 2 S.D. below normal)	Decreased FVC, FEV_1, TLC, DCO, and FEV_1/FVC ratio (severe, 40% of predicted, or 3 S.D. below normal)	size
ECG and ECHO	Left ventricular preponderance persists	Balanced ventricular hypertrophy	Right ventricular hypertrophy and right atrial enlargement. Progressive increase in heart	
Pulmonary artery pressure	Normal	Normal	Borderline elevation or normal	

Patient frequently unable to complete testing due to degree of hypoxia

Severe right ventricular and right atrial hypertrophy. Ischemic T waves in V1 and V2

and p pulmonale

Markedly elevated with pulmonary hypertension

The stage of sickle cell chronic lung disease will be determined by the clinical feature representing the highest (i.e. the most severe) stage. In practice, clinical features tend to move in tandem. Marked disparity between these features as related to stage of lung disease must be satisfactorily addressed by the pulmonologist.

APPENDIX 2 **COMMON TOXICITY CRITERIA**

		GRADE				
	TOXICITY	0	1	2	3	4
Blood/Bone Marrow	WBC	4.0	3.0-3.9	2.0-2.9	1.0-1.9	<1.0
	PLT	WNL	75.0-normal	50.0-74.9	25.0-49.9	<25.0
	Hgb	WNL	10.0-normal	8.0-10.0	6.5-7.9	<6.5
	Granulocytes/ Bands	2.0	1.5-1.9	1.0-1.4	0.5-0.9	<0.5
	Lymphocytes	2.0	1.5-1.9	1.0-1.4	0.5-0.9	<0.5
	Hemorrhage (clinical)	none	mild, no transfusion	gross, 1-2 units transfusion per episode	gross, 3-4 units transfusion per episode	massive, >4 units transfusion per episode
	Infection	none	mild	moderate	severe	life-threatening
Gastrointestinal	Nausea	none	able to eat reasonable intake	intake significantly decreased but can eat	no significant intake	--
	Vomiting	none	1 episode in 24 hrs	2-5 episodes in 24 hrs	6-10 episodes in 24 hrs	>10 episodes in 24 hrs, or requiring parenteral support
	Diarrhea	none	increase of 2-3 stools/day over pre-Rx	increase of 4-6 stools/day, or nocturnal stools, or moderate cramping	increase of 7-9 stools/day, or incontinence, or severe cramping	increase of 10 stools/day or grossly bloody diarrhea, or need for parenteral support
	Stomatitis	none	painlesss ulcers, erythema, or mild soreness	painful erythema, edema, or ulcers, but can eat	painful erythema, edema, or ulcers, and cannot eat	requires parenteral or enteral support
Liver	Bilirubin	WNL	--	<1.5 × N	1.5-3.0 × N	>3.0 × N
	Transaminase (SGOT, SGPT)	WNL	2.5 × N	2.6-5.0 × N	5.1-20.0 x N	>20.0 × N

	Alk Phos or 5'nucleotidase	WNL	2.5 × N	2.6-5.0 × N	5.1-20.0 × N	>20.0 × N
	Liver (clinical)	no change from baseline	--	--	precoma	hepatic coma
Kidney, Bladder	Creatinine	WNL	<1.5 × N	1.5-3.0 × N	3.1-6.0 × N	>6.0 × N
	Proteinuria	no change	1+ or <0.3 g% or <3 g/L	2-3+ or 0.3-1.0 g% or 3-10 g/L	4+ or >1.0 g% or >10 g/L	Nephrotic syndrome
	Hematuria	neg	micro only	gross, no clots	gross + clots	requires transfusion
	Alopecia	no loss	mild hair loss	pronounced or total hair loss	--	--
	Pulmonary	none or no change	asymptomatic, with abnormality in PFTs	dyspnea on significant exertion	dyspnea at normal level of activity	dyspnea at rest
Heart	Cardiac dysrhythmias	none	asymptomatic, transient, requiring no therapy	recurrent or persistent, no therapy required	requires treatment	requires monitoring; or hypotension, or ventricular tachycardia, or fibrillation
	Cardiac function	none	asymptomatic, decline of resting ejection fraction by less than 20% of baseline value	asymptomatic, decline of resting ejection fraction by more than 20% of baseline value	mild CHF, responsive to therapy	severe or refractory CHF
	Cardiac - ischemia	none	nonspecific T-wave flattening	asymptomatic, ST and T wave changes suggesting ischemia	angina without evidence for infarction	acute myocardial infarction
	Cardiac - pericardial	none	asymptomatic effusion, no intervention required	pericarditis (rub, chest pain, ECG changes)	symptomatic effusion; drainage required	tamponade; drainage urgently required
Blood Pressure	Hypertension	none or no change	asymptomatic transient increase by greater than 20 mmHg (Ø) or to >150/100 if previously WNL; no treatment required	recurrent or persistent increase by greater than 20 mmHg (Ø) or to >150/100 if previously WNL; no treatment required	requires therapy	hypertensive crisis
	Hypotension	none or no change	changes requiring no therapy (including transient orthostatic hypotension)	requires fluid replacement or other therapy but not hospitalization	requires therapy and hospitalization; resolves within 48 hrs of stopping the agent	requires therapy and hospitalization for >48hrs after stopping the agent

Neu	Neuro - sensory	none or no change	mild paresthesias, loss of deep tendon reflexes	mild or moderate objective sensory loss; moderate paresthesias	severe objective sensory loss or paresthesias that interfere with function	- -

	Neuro - headache	none	mild	moderate or severe but transient	unrelenting and severe	--
	Neuro - constipation	none or no change	mild	moderate	severe	ileus >96 hrs
	Neuro - hearing	none or no change	asymptomatic hearing loss on audiometry only	tinnitus	hearing loss interfering with function but correctable with hearing aid	deafness not correctable
	Neuro - vision	none or no change	--	--	Symptomatic subtotal loss of vision	blindness
	Skin	none or no change	scattered macular or papular eruption or erythema that is asymptomatic	scattered macular or papular eruption or erythema with pruritus or other associated symptoms	generalized symptomatic macular, papular, or vesicular eruption	exfoliative dermatitis or ulcerating dermatitis
	Allergy	none	transient rash, drug fever <38 C, 100.4 F	urticaria, drug fever >38 C, 100 F, mild bronchospasm	serum sickness, bronchospasm, req parenteral meds	anaphylaxis
	Fever in absence of infection	none	37.1-38.0 C 98.7-100.4 F	38.1-40.0 C 100.5-104.0 F	>40.0 C, >104.0 F for less than 24 hours	>40.0 C (104.0 F) for more than 24 hrs or fever accompanied by hypotension
	Local	none	pain	pain and swelling, with inflammation or phlebitis	ulceration	plastic surgery indicated
	Weight gain/loss	<5.0%	5.0-9.9%	10.0-19.9%	20.0%	--
	Hyperglycemia	<116	116-160	161-250	251-500	>500 or keto-acidosis
	Hypoglycemia	>64	55-64	40-54	30-39	<30
Metabolic	Amylase	WNL	<1.5 × N	1.5-2.0 × N	2.1-5.0 × N	>5.1 × N
	Hypercalcemia	<10.6	10.6-11.5	11.6-12.5	12.6-13.5	13.5
	Hypocalcemia	>8.4	8.4-7.8	7.7-7.0	6.9-6.1	6.0

	Hypomagnesemia	>1.4	1.4-1.2	1.1-0.9	0.8-0.6	0.5
	Fibrinogen	WNL	0.99-0.75 x N	0.74-0.50 x N	0.49-0.25 x N	0.24 x N
	Prothrombin time	WNL	1.01-1.25 x N	1.26-1.50 x N	1.51-2.00 x N	>2.00 x N
Coagulation	Partial thromboplastin time	WNL	1.01-1.66 x N	1.67-2.33 x N	2.34-3.00 x N	>3.00 x N