Version 0.05: Edit./Elig. Rev. Nov. 8, 2012

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Reduced Intensity Matched Sibling Bone Marrow Transplantation for Sickle Cell Anemia in Patients 2-30 years old

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I. Background/Rationale

Sickle Cell Disease

Sickle cell disease (SCD) affects approximately 72,000 individuals in the United States and causes considerable morbidity and mortality[1]. It is a recessive disorder caused by a point mutation that results in the substitution of valine for glutamic acid at the sixth position in the B-chain of hemoglobin. This leads to sickling of the red blood cells under many conditions, such as hypoxia, dehydration, and hyperthermia. The sickling leads to vaso-occlusion, which causes **irreversible** damage in almost all systems in the body, including the central nervous system (CNS), lungs, heart, bones, eyes, liver, and kidneys. The most devastating complication is cerebral infarction[2], but other serious complications include pulmonary disease due to recurrent acute chest syndrome (ACS), nephropathy, retinopathy, vaso-occlusive pain crises (VOC), and avascular necrosis (AVN). Organ damage begins early in life and worsens over time; therefore, early treatment is a worthy goal, even in patients who have not yet developed signs or symptoms of the disease. Three prospective screening studies using echocardiography have shown that 20% of adults with sickle cell disease have borderline or mild pulmonary hypertension, defined by a pulmonary artery systolic pressure greater than 35 mm Hg; 10% of these adults have moderate to severe pulmonary hypertension, defined by a pressure greater than 45 mm Hg[2,3]. Despite pulmonary artery systolic pressures that are much lower than those in idiopathic or hereditary pulmonary hypertension, in sickle cell disease borderline or mild pulmonary hypertension is associated with an extremely high risk of death[3]. Because of these many complications, the average life expectancy is decreased for sickle cell patients, being 42 years for males and 48 years for females[4].

Overt strokes occur in 9% of sickle cell patients before their 14th birthday[5], and the stroke-free survival decreases to 88.5% by 18 years of age[6]. An additional 18% of children with Hb SS disease will have silent cerebral infarcts by the age of 14[7]. Silent infarcts are associated with an increased risk of overt stroke, low IQ, and poor academic performance[8-11]. Currently, the treatment for stroke is regular transfusions to keep the Hb S% less than 30%. However, in one study, 20% of the patients on a chronic transfusion regimen had a second stroke[12]. Known side effects of chronic transfusion include iron overload and alloimmunization, which can both adversely affect the patient.

Hydroxyurea Use in Sickle Cell Disease

Hydroxyurea has emerged as an encouraging therapeutic agent in SCD. Hb F is known to be protective against clinical severity; a low Hb F% is associated with a higher risk of developing VOC, organ damage, and early death[4,13]. Because hydroxyurea increases the Hb F%, it may be beneficial in treating sickle cell patients. Multiple studies have shown that it reduces the number of painful events along with hospitalizations in both children and adults[14-17]. In addition, there is evidence that it may have clinical efficacy for sickle-related organ damage, including proteinuria[18], glomerular hyperfiltration[19], splenic dysfunction[20-22], hypoxemia[23], pulmonary hypertension[24], neurocognitive delay[25], silent brain infarcts[18], elevated Transcranial Doppler (TCD) velocities[19,26,27], primary stroke prevention[27,28], and secondary stroke prevention[29,30]. Multiple large studies have also showed that hydroxyurea is well tolerated with little short-term or long-term toxicity in patients as young as six months[20,31-34]. However, recent data from the prematurely closed SWiTCH study, examining if patients with a history of CVA could be switched from chronic transfusion therapy to hydroxyurea as treatment, showed an increased incidence in second stroke in the patients receiving hydroxyurea[35]. This further demonstrates the need for other therapy for sickle cell patients.

Hematopoietic Stem Cell Transplantation in Sickle Cell Disease

Hematopoietic stem cell transplantation (HSCT) is the only currently available cure for patients with SCD. The CIBMTR reported on 67 pediatric patients who received HLA-matched sibling transplants after a myeloablative conditioning regimen[36]. They found that the overall survival (OS) and disease-free survival (DFS) were 97% and 85%, respectively. 9 patients had graft failure, and the rates of acute and chronic GVHD were 10% and 22%, respectively. The French group reported similar results in 87 patients who received transplants from siblings who were either fully-matched or mismatched at 1 antigen after myeloablative conditioning[37]. The OS was 93.1% and the event-free survival (EFS) was 86.1%. Graft failure occurred in 7% of the patients; however, after the introduction of ATG as part of the conditioning regimen, this decreased to 2.9%. Acute and chronic GVHD developed in 20% and 12.6%, respectively. Both of these studies demonstrate that matched sibling transplants after myeloablative conditioning have acceptable results for the cure of sickle cell disease.

HSCT regimen: Myeloablative vs. Reduced-Intensity

Another limitation is the risk of mortality or treatment related toxicities associated with myeloablative (MA) regimens. Organ toxicities are more likely to occur and be more severe in sickle cell patients who have impaired organ function or exposure to multiple transfusions before HSCT[38-40]. MA conditioning facilitates durable engraftment of donor cells, but is limited by toxicities of the drugs as well as transplant-related complications[41,42]. In addition to prolonged myelosuppression, acute toxicities include mucositis, anorexia, renal insufficiency, cardiac dysfunction, rash, seizures, GVHD, and veno-occlusive disease (VOD)[42]. Late toxicities include chronic GVHD, pulmonary dysfunction, endocrine insufficiency, gonadal failure, and neurocognitive deficits[43-47].

Because stable mixed chimerism is sufficient to cure sickle cell disease[48], it is possible that reduced intensity conditioning (RIC) might be effective even if it only results in mixed chimerism. Krishnamurti et al reported on stable donor engraftment after RIC with Busulfan, Fludarabine, equine antithymocyte globulin, and total lymphoid irradiation[49]. 6/7 patients demonstrated long term engraftment. 4/6 patients showed only partial donor chimerism, but these patients still had an Hb >10 and alleviation of their clinical sickle cell symptoms.

However, because of the risk of graft rejection, particularly in the heavily transfused patients, a high level of immunoablation is important for successful donor engraftment. The regimen of fludarabine and melphalan in combination with alemtuzumab would provide a reduced intensity regimen while potentially preserving immunoablation. This regimen was used in 44 patients with malignant disorders[50]. Only 2 patients experienced graft failure, the incidence of acute and chronic GVHD were 6.5% and 0%, respectively. In this study, the alemtuzumab was given 4-8 days before HSCT, which may account for the low GVHD risk, as it was likely still present in the recipient's serum early post-transplant. However, in an immunocompetent host such as a sickle cell patient, this could increase the risk of graft rejection. Because of this, another study modified the regimen to give alemtuzumab on days -21 to -19 prior to transplant in non-malignant patients[51,52]. So far, 60 patients between the ages of 2 and 20 have been treated with this regimen, receiving either unrelated donors matched 8-10/10, HLA-identical sibling donors, or UCB matched 4-6/6. Graft failure has occurred in 5% of patients, although 2 of these patients had received a lower dose of melphalan in an attempt to further reduce the intensity. Acute and chronic GVHD occurred in 15% and 11 % of the patients, respectively. There were seven patients with SCD on this study. The OS and DFS are 100% and 71%, respectively. The reduced EFS is due to two patients that experienced graft rejection, but they had received lower doses of Melphalan as discussed above.

Acute and chronic GVHD occurred in 0% and 14%, respectively. This data supports the use of this regimen in pediatric sickle cell patients.

HSCT for Sickle Cell Disease in Adults

All of the studies mentioned previously have mostly included patients <16, with a few studies going up to the age of 27. Because of this, there had been minimal evidence on performing HSCT in young adult sickle cell patients. However, a recently published study examined this in 10 adult patients, ranging in age from 16 to 45[53]. They received alemtuzumab and TBI as a reduced intensity conditioning regimen prior to receiving a fully matched sibling HSCT. The OS and DFS were 100% and 90%, respectively, with only one patient failing to engraft. In addition, no patient experienced either acute or chronic GVHD. This small study demonstrates that young adult sickle cell patients may also be able to tolerate a reduced intensity HSCT with minimal complications.

Organ Recovery Following HSCT

In addition to symptom alleviation, HSCT has also been shown to stabilize or reverse the organ damage due to SCD. In a long term follow-up study of patients who received matched related HSCT, pulmonary function tests were stable in 22 of 26 patients, worse in 2, and not studied in 2[54,55]. Linear growth measured by median height standard deviation score improved from -0.7 before HSCT to -0.2 after HSCT[54,55]. Radiologic improvement of a patient with avascular necrosis of the humeral head has been reported[56], as well as correction of splenic reticuloendothelial dysfunction[57,58].

The effect of HSCT on reversal of cerebral vasculopathy has been variable. Many studies have found that patients who successfully engraft do not experience any sickle-related CNS complications and have evidence of stabilization of CNS disease on MRI[55,59,60]. In addition to stabilization, a few studies have found improvement in areas of previous abnormality; however, this was in an extremely small subset of patients[54,55,61]. One study compared the vessel diameter on MRI of sickle cell patients treated with HSCT vs. those treated with either chronic transfusion or hydroxyurea[62]. They found a 12% increase in the lumen of 22 vessels in patients who underwent HSCT vs. an 8% increase in the lumen of 42 vessels in the transfusion/hydroxyurea patients. However, worsening of cerebral large vessel disease and stroke has been reported after HSCT[59]. Given this conflicting data, this is an area that needs to be studied closer to determine the patient characteristics that may contribute to improvement in the vessels after HSCT.

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Results from previous patients enrolled on this clinical trial

We enrolled 23 patients so far on the original Pro1894 trial, 20 patients who underwent matched sibling HSCT and three patients who underwent unrelated donor HSCT. The first three unrelated HSCT patients experienced only a 33% EFS, as one patient died from GVHD related complications and one patient suffered early graft rejection with autologous recovery. Because of this, this trial was closed for unrelated HSCT, but continued to enroll matched sibling HCST. After 7 total patients had been enrolled on the trial (5 sibling and 2 unrelated), the Alemtuzumab in the conditioning regimen was moved up a week from d-22 to d-16 in an attempt to decrease GVHD while still preserving engraftment. Another 7 patients were enrolled (6 sibling & 1 unrelated), but unfortunately 3 patients suffered graft rejection with autologous recovery without a significant decrease in the GVHD incidence. Because of this, the last 6 matched sibling HSCT patients received the Alemtuzumab starting back at d-22.

In the 20 sibling HSCT patients, the overall survival rate is 94% with an EFS rate of 85%. The one patient who passed away died from a non-transplant related infection at more than 12 months post-BMT. Two of the sibling HSCT recipients suffered secondary rejection, both of whom received the intermediate timed Campath. There has been no graft rejection in patients whom received the distal timed Campath.

While these results are encouraging that matched sibling donor HCST using a reduced intensity regimen is a safe and effective cure for all sickle cell patients regardless of disease severity, the numbers are small. Because of this, I would like to re-open this study in order to continue to study the use of this regimen in any sickle cell patient with sickle cell disease age 2-30 who has a matched sibling donor.

Primary objective:

1) To determine DFS at two years after matched sibling transplant using BM after a conditioning regimen consisting of distal timed Alemtuzumab, Fludarabine, and Melphalan for patients 2-30 y/o

Secondary objectives:

1) Overall survival
2) Rate of neutrophil and platelet engraftment for BM
3) Incidence of graft failure
4) Incidence of grade II-IV and grade III-IV acute GVHD
5) Incidence of chronic GVHD
6) Incidence of other transplant complications, such as veno-occlusive disease, CNS toxicity, and idiopathic pneumonia syndrome
7) Incidence of reactivation of CMV, EBV, adenovirus, BK/JC virus
8) Incidence of invasive fungal disease
9) Time to immune reconstitution via monitoring of lymphocyte subpopulations and immunoglobulin levels

III. Patient Eligibility

1) Matched sibling donors (10/10 marrow; can use stored sibling umbilical cord blood to supplement bone marrow if available)

a. Age 2-30

b. Hb SS, Sβthal^0, Sβthal$^+$, SC

c. Evidence of ongoing hemolysis: Hb<10, retic >5%, LDH > 500, TB>2 (need at least 1 of 4 criteria)

d. Karnofsky/Lansky score ≥50

e. LVSF>26% or LVEF>40%

f. DLCO >40% or O2 sat >85% for those patients that can't perform PFTs

g. GFR >70 and serum creatinine < 1.5 * ULN for age

h. ALT and AST < 5 x ULN, direct bilirubin <3 x ULN

i. If the patient has been on chronic transfusion or has a ferritin >1000, liver biopsy should be done and show no evidence of bridging fibrosis or cirrhosis

2) Exclusion criteria

a. Evidence of uncontrolled bacterial, viral, or fungal infection within one month prior to initiation of the conditioning regimen
b. Pregnant or breastfeeding
c. HIV positive
d. Written informed consent not obtained

IV. Treatment Plan

1) Patients will receive a conditioning regimen composed of Alemtuzumab, Fludarabine, and Melphalan as detailed in the table below.

Day	Treatment
-22	Alemtuzumab 3mg IV (test dose)
-21	Alemtuzumab 10mg IV
-20	Alemtuzumab 15mg IV
-19	Alemtuzumab 20mg IV
-8	Fludarabine 30mg/m^2 IV
-7	Fludarabine 30mg/m^2 IV
-6	Fludarabine 30mg/m^2 IV
-5	Fludarabine 30mg/m^2 IV
-4	Fludarabine 30mg/m^2 IV
-3	Melphalan 140mg/m^2 IV
-2	Rest Day
-1	Rest Day
0	Stem Cell Infusion

For patients > 18 y/o, Fludarabine and Melphalan will be adjusted to the ideal body weight (IBW) in patients weighing >125% IBW based on the following formulas (ht in inches; IBW in kg):

For patients >60inches:

Males: IBW = 50 kg + 2.3 kg for each inch over 5 feet.
Females: IBW = 45.5 kg + 2.3 kg for each inch over 5 feet.

Adjusted Ideal Body Weight Formula: AIBW = IBW + [(0.4) x (ABW – IBW)]

a) Medications

i.) Alemtuzumab

I. Hb S% must be < or = 45% within 7 days prior to initiation of Alemtuzumab
II. **Iron chelation and hydroxyurea must be discontinued >48 hours before initiating therapy**
III. Alemtuzumab will be diluted in 100mL of 0.9% NS and infused at a rate as below
    a. 0 – 30 minutes: 10 mL/hr
    b. 30 – 60 minutes: 20 mL/hr
    c. 60 – 90 minutes: 40 mL/hr
    d. 90 minutes – completion: 80 mL/hr

IV. Premedication

a. Pre-medication with the following medications should be given 30 minutes prior to the start of each infusion of Alemtuzumab
    i. Diphenhydramine 1mg/kg IV or PO (maximum dose 50mg/dose)
    ii. Acetaminophen 10-15mg/kg PO (maximum dose 4g qday)
    iii. Hydrocortisone 1-2mg/kg IV
    iv. Meperidine 0.5g/kg IV q4-6 hr prn rigors (To be kept at the bedside)

V. Test dose
    a. The dose should be administered in 75 mls of 0.9% NS over 2 hours and not less than 24 hours prior to administration to the first dose.
    b. **If not tolerated, please notify the study chair.**

ii) Fludarabine

I. Fludarabine should be diluted in 100 ml 0.9%NS and given over 30 minutes.
II. A daily dose of an antiemetic should be given 30 minutes prior to administration of the Fludarabine

iii) Melphalan

I. Melphalan should be diluted in 0.9%NS to a concentration of 0.1 -0.45 mg/mL and given over 45 minutes. ***Entire dose must be infused within 60 minutes of reconstitution in Pharmacy.**
II. A daily dose of an antiemetic should be given 30 minutes prior to administration of the Melphalan
III. Patients should be encouraged to suck on a popsicle or something similar during the Melphalan infusion.

2) Infusion of Hematopoietic Stem Cells (See SOPs on intranet)

a. Under no circumstance should the stem cell product be irradiated
b. A filter should be used as instructed in SOP.
c. Vital signs should be monitored before beginning the infusion and every 15 minutes until the infusion has been completed.
d. Premedications should be given as below:
   i. Diphenhydramine 1mg/kg IV or po (maximum dose 50mg/dose)
   ii. Acetaminophen 10-15mg/kg po (maximum dose 4g qday)
   iii. Hydrocortisone 1-2mg/kg IV
   iv. Epinephrine 0.01mg/kg IM/IV (maximum dose 0.5mg) at bedside prn anaphylactic reaction
e. Oxygen via nasal canula or mask and ambu bag should be at bedside.

V. GVHD Prophylaxis

1) Patients will be given the regimen as described below:

Day	Regimen
-1	Tacrolimus dosed to maintain appropriate levels. Given through day 100 then taper to day 180 Mycophenolate Mofetil given through day +45 or 7 days after engraftment, whichever is later
0	Stem Cell Infusion

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i. Tacrolimus
1. Administration will begin on day -1 at a dose of 0.015mg/kg/day as a continuous drip, and dosing will be adjusted to maintain a level of 10-20 ng/mL.
2. Once the patient can tolerate oral medications and has a normal gastrointestinal transit time, Tacrolimus will be converted to an oral form, at 3x the current IV dose divided into two daily doses.
3. Patients will receive Tacrolimus until day 100, and then it will be tapered between days 100 to 180 by 10% per week as long as there is no evidence of GVHD.

ii. Mycophenolate Mofetil (MMF)
1. MMF will be given at a dose of 1gram IV q8 hours for patients weighing >50kg or 15mg/kg IV q8 hrs for patients weighing <50kg beginning the morning of day -1.
2. Once the patient can tolerate oral medications and has a normal gastrointestinal transit time, MMF will be converted to oral at the same dose as the IV dose.
3. MMF will be stopped at day 45 or 7 days after engraftment, whichever day is later, if there is no evidence of acute GVHD.
4. If the patient has active acute GVHD requiring systemic therapy, MMF may be stopped at the discretion of the treating physician.

VI. Supportive Care Recommendation

1) Engraftment syndrome
   - **a.** This syndrome is a clinical diagnosis, with the most common manifestations being transient fever, rash, and respiratory symptoms not attributable to infection or GVHD.
   - **b.** Diagnostic criteria include fever >38.5 without an identifiable infectious cause within 4 days of the start of neutrophil recovery **AND** any of the symptoms below
     - **i.** An erythematous rash not attributable to GVHD or medications
     - **ii.** Capillary leak (i.e. weight gain, edema, ascites, effusions)

iii. Respiratory symptoms not attributable to idiopathic pneumonitis syndrome (IPS)

c. Mild symptoms may not require therapy; for progressive symptoms, methylprednisolone at 2mg/kg/day is recommended for 5 days.
d. If symptoms are recurrent or prolonged, investigation for GVHD is recommended.

2) Venous access
   a. All recipients should have appropriate long-term central venous access placed prior to beginning conditioning.
   b. The placement of a triple lumen broviac is recommended.

3) Seizure prophylaxis
   a. This is mandated in all recipients and should begin at the time conditioning with Alemtuzumab starts.
   b. The preferred drug is levetiracetam dosed as below:
      i. For patients <16 years old, give 20mg/kg/day divided into two doses (Maximum dose 500mg po BID)
      ii. For patients 16 and older, give 500mg po BID
   c. Levels do not need to be monitored unless there is a clinical indication (i.e. history of seizure, concern for CNS event)
   d. Levetiracetam should be continued until day 180 or until Tacrolimus is discontinued, whichever is later.
   e. Serum magnesium should be maintained >1.5 mg/dL while the patient is receiving Tacrolimus to reduce the risk of seizures.

4) Hypertension
   a. Hypertension should be strictly controlled to prevent CNS toxicity. Blood pressure should be followed closely and should be treated promptly to maintain blood pressure at the patient's pre-transplant baseline +/- 20%.

5) Growth Factor
   a. GCSF will be given at a dose of 5mcg/kg/day SQ beginning on day 7 after SCT.

b. It should be continued until the absolute neutrophil count (ANC) is >1000/μL for 2 subsequent days post-nadir.

6) Blood products
   - **a.** The hemoglobin should be maintained between 8 and 11 g/dL for at least 100 days after SCT.
   - **b.** Platelets should be maintained >50,000/μL after Alemtuzumab administration and continuing after SCT to decrease the risk of neurological events.
   - **c.** All blood products should be irradiated and leukodepleted. Only recipients who are CMV negative need to receive CMV-negative products.

7) Treatment of fever/infections
   - **a.** Patients should be closely monitored for signs of infection closely and treated as per institution guidelines with broad spectrum antibacterial, antiviral, and antifungal agents.
   - **b.** Since patients will receive Alemtuzumab, they are all at high risk for infections in the early post-transplant period.

8) Infection surveillance and prophylaxis
   - **a.** HSV
     - **i.** Acyclovir prophylaxis is recommended for 6 months post-SCT for any patient who is HSV or VZV sero-positive.
     - **ii.** Acyclovir should be dosed 250mg/m2/dose PO or IV q8 hours (maximum dose 800mg PO/IV q8)
   - **b.** PCP
     - **i.** Recipients should be started on trimetheprim-sulfamethoxazole or an equivalent medication.
       1. PCP prophylaxis should be started when Alemtuzumab is begun.
       2. Prophylaxis should be discontinued after the pm dose on day -3, and not resumed until day 50 post-SCT unless neutrophil recovery has not occurred.
          - **a.** If neutrophil recovery has not occurred by day 50, PCP prophylaxis should be started with Pentamidine 4mg/kg IV q4weeks.
   - **c.** Fungal prophylaxis
     - **i.** Anti-fungal prophylaxis against Aspergillus sp. is recommended with Voriconazole beginning w/ Alemtuzumab conditioning and continuing until day 180 or until immunosuppression is discontinued, whichever is later.

- **ii.** The dose should be 4mg/kg/dose PO/IV q12 (maximum dose 600mg/day for patients >40kg or 300mg/day for patients <40kg)
- **iii.** Because of the possibility of interactions between voriconazole and Tacrolimus, Tacrolimus levels should be monitored at least weekly to avoid toxicity.

d. Bacterial prophylaxis
- **i.** Penicillin prophylaxis should begin when Alemtuzumab is started and continue through day 180.
- **ii.** The dose should be 250mg po BID for all patients.

e. CMV
- **i.** All patients must be screened with CMV PCR weekly until day 100 (If clinically stable and not requiring weekly visit CMV PCR should be done at each visit).
- **ii.** From day 100 to 180, screening with CMV PCR should occur at least once a month.
- **iii.** Antiviral treatment should be started if PCR reveals a viral load >1000. If this occurs before engraftment, foscarnet may be used to prevent marrow suppression.

f. Adenovirus
- **i.** All patients should be screened with weekly adenovirus PCR from serum until day 100 (If clinically stable and not requiring weekly visit ADV PCR should be done at each visit).
- **ii.** From day 100 to 180, screening with adenovirus PCR should occur at least once a month.
- **iii.** If the patients has clinical symptoms of a possible adenoviral infection (i.e. diarrhea, hepatic dysfunction, respiratory distress), adenovirus PCR should be sent from pertinent source (i.e. stool, bronchoalveolar lavage).
- **iv.** If an active systemic infection is diagnosed, cidofovir 5mg/kg q week with Probenecid should be started.

g. EBV
- **i.** All patients should be screened with EBV PCR weekly until day 100 (If clinically stable and not requiring weekly visit EBV PCR should be done at each visit).
- **ii.** From day 100 to 180, screening with EBV PCR should occur monthly.

iii. In the event of persistent EBV viremia or symptoms suggestive of EBV PTLD (i.e. lymphadenopathy, fever, etc), therapy with Rituximab 375mg/m^2 q week for 4 doses is recommended.

VII. Required/Recommended Observations

1) Pre-Transplant

a. **History and physical examination: A complete history and physical examination including accurate assessment and documentation of hepatomegaly, splenomegaly; vital signs to include blood pressure, oxygen saturation, weight, height, BSA.**
b. **Hematology (must be within one week prior to starting therapy): CBC, differential, ABO and Rh typing, antibody screen, PT, PTT, Fibrinogen.**
c. **Microbiology studies on donor must be done within 30 days of day 0. Microbiology studies on recipient must be done within 30 days of starting therapy:**
    i. **Antigen: Hepatitis Surface Antigen**
    ii. **Antibody: Hepatitis A IgG/IgM, Hepatitis B Core Antibody, Hepatitis B Surface Antibody, Hepatitis C Antibody, CMV IgG/IgM, EBV IgG/IgM, VZV IgG/IgM, HSV 1 & 2 IgG/IgM, HIV 1/2 antibody, Toxoplasmosis IgG/IgM, HTLV I/II IgG, West Nile Virus IgG/IgM**
    iii. **Trypanosoma cruzi**
    iv. **RPR**
d. **Chemistry: Electrolytes, serum creatinine, BUN, total and direct bilirubin, SGPT, SGOT, albumin, calcium, phosphorus, uric acid, magnesium, LDH, glucose, alkaline phosphatase, cholesterol, Triglycerides.**
e. **HLA Typing**
f. **STR: on donor and recipient**
g. **Cardiac Evaluation: Echocardiogram or MUGA scan with ejection fraction (or fractional shortening) (within 45 days of starting therapy)**
h. **Pulmonary Evaluation: Chest x-ray (PA and lateral), PFTs (if age appropriate, O2 saturation if unable to perform PFT) (within 45 days of starting therapy)**

i. **Renal Function: urinalysis, 12 or 24-hour creatinine clearance or GFR (within 45 days of starting therapy)**
j. **MRI/A of brain (within 45 days of starting therapy)**
k.
l. **Performance Status: Karnofsky or Lansky score (age appropriate)**

m. **Pregnancy test for females of childbearing age (within 7 days of starting therapy)**
n. **Consults: Dentistry, ophthalmology (within 60 days of starting therapy)**

2) Day +1 to +60 Post Allogeneic Donor Transplant

Required/ Recommended Observations

a. History and Physical: An assessment to include graft-versus host disease assessment, vital signs, and weight. Karnofsky or Lansky (age appropriate) with history/physical at every visit.
b. Hematology:
    i. Inpatient: Daily CBC with manual differential until the absolute neutrophil count (ANC) reaches 500/mm^3 for three consecutive days and the platelet count reaches >50,000/mm^3 untransfused x 7 days, and then as clinically indicated
    ii. Post-discharge: CBC at every visit
c. Chemistry: Electrolytes, BUN, creatinine, glucose, total and direct bilirubin, Ca^{++}, Mg^{++}, PO_4, SGOT, SGPT, Albumin, and Alkaline Phosphatase as clinically indicated. Cholesterol, Triglycerides, Uric Acid, LDH, amylase, lipase, and GGT as clinically indicated
d. **STR Studies and hemoglobin electropheresis at Days +30 and +60 (± 3 days)**
e. Tacrolimus levels at every visit.
f. CMV DNA PCR, EBV PCR, and adenovirus DNA PCR q week

3) **Required/** Recommended Observations Day +60 to +365

a. History and Physical: Assessment of graft vs. host disease, vital signs, weight, with each clinic visit and as clinically indicated.
b. Hematology: CBC with manual differential on Days +100, +180, and +365 and as clinically indicated
c. **Cardiac Evaluation: Echocardiogram or MUGA scan with ejection fraction (or fractional shortening) will be done on Day +365 (± 7 days)**
d. Performance Evaluation: Karnofsky or Lansky Score during clinic visit on day +100 and +180 (± 7 days)
e. **STR Studies and hemoglobin electropheresis at Days +100, +180, and +365 (± 7 days)**
f. Tacrolimus levels as clinically indicated.

g. CMV DNA PCR, EBV DNA PCR, and adenovirus DNA PCR every visit (not more than weekly) until Day+180 (± 7 days), then as clinically indicated.
h. **Immune reconstitution: Lymphocyte subset panel and immunoglobulins at Day+100, Day+180, and Day+365 (± 7 days)**

4**) Required**/Recommended Observations on or after Day +366

a. History and Physical: Assessment to include chronic graft vs. host disease stage and grading), vital signs, weight, performance score on the following post transplant dates: 15 months, 18 months, and 2 years
b. Hematology: CBC with manual differential as clinically indicated.
c. **STR Studies and hemoglobin electropheresis: 2 years (± 10 days) post allogeneic stem cell transplant**
d. Tacrolimus levels as clinically indicated
e. CMV DNA PCR every visit while receiving GVHD immunosuppressants if clinically indicated.
f. **Immune reconstitution: Lymphocyte subset panel and immunoglobulins at 18 months post HSCT (± 10 days)**
g.
h. **MRI/A of brain 2 years post allogeneic stem cell transplant (if initial scan abnormal)**

5) Required Late Effects Evaluation

a. **Development and Endocrine Follow up**
    i. **Yearly growth assessment (height, weight) with appropriate growth work-up if <5th percentile or patient is falling off the growth curve.**
    ii. **Yearly pubertal assessment (if applicable) and appropriate endocrine work-up if indicated.**

VIII. Drug Information

Alemtuzumab

Source and Pharmacology:

Alemtuzumab binds to CD52, an antigen present on the surface of B and T lymphocytes, a majority of monocytes, macrophages, NK cells, and a subpopulation of granulocytes. A proportion of bone marrow cells, including some $CD34^+$ cells, express variable levels of CD52. The proposed mechanism of action is antibody-dependent cellular-mediated lysis following cell surface binding of Campath to the leukemic cells. In adult patients, the mean half-life was 11 hours (range 2 to 32 hours) after the first 30 mg dose and was 6 days (range 1 to 14 days) after the last 30 mg dose. Comparisons of AUC in patients ≥ 65 years (n=6) versus patients < 65 years (n=15) suggested that no dose adjustments are necessary for age. Comparisons of AUC in female patients (n=4) versus male patients (n=17) suggested that no dose adjustments are necessary for gender. However, the pharmacokinetics of Campath in pediatric patients have not been studied. The effects of renal or hepatic impairment on the pharmacokinetics of Campath have not been studied.

Toxicity:

Likely *("Likely" refers to a side effect that is expected to occur in more than 20% of patients.)*	**Less Likely** *("Less likely" refers to a side effect that is expected to occur in 20% or fewer patients.)*	**Rare, but Serious** *(These possible risks have been reported in rare occurrences, typically less than 2% of patients. They may be serious if they occur.)*
● Fever ● Chills ● Anemia due to decreased number of red cells ● Infection due to decreased number of white blood cells ● Bleeding due to decreased numbers of platelets ● Weakened immune system	● Nausea ● Vomiting ● Diarrhea ● Rash ● Headache ● Sweating ● Back pain ● Severe itching ● Allergic reaction of skin and blood vessels ● Tiredness ● Loss of appetite	● Abdominal pain ● Dizziness ● High blood pressure ● Blisters ● Pain in the muscles ● Herpes simplex infection ● Inflammation of the throat

Formulation and Stability:
Alemtuzumab is a sterile, clear, colorless, isotonic solution (pH 6.8-7.4) for injection. Each single use vial contains 30 mg alemtuzumab, 8.0 mg sodium chloride, 1.44 mg dibasic sodium phosphate, 0.2 mg potassium chloride, 0.2 mg monobasic potassium phosphate, 0.1 mg polysorbate 80, and 0.0187 mg disodium edetate dihydrate. No preservatives are added.

Guidelines for Administration:
Administer as an IV infusion over 2 hours. **Do not administer as intravenous push or bolus.**
Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration. If particulate matter is present or the solution is discolored, the vial should not be used. **DO NOT SHAKE VIAL.**
Use aseptic technique during the preparation and administration of Alemtuzumab. Withdraw the necessary amount from the vial into a syringe. Inject syringe contents into 100 mL sterile 0.9% Sodium Chloride USP or 5% Dextrose in Water USP. Gently invert the bag to mix the solution. Discard syringe. The vial contains no preservatives and is intended for single use only. DISCARD VIAL including any unused portion after withdrawal of dose.
Use within 8 hours after dilution. Store diluted Alemtuzumab at room temperature (15-30°C) or refrigerated (2-8°C). Protect from light.

Alemtuzumab is supplied in single-use clear glass vials containing 30 mg in 1 mL of solution. Store Alemtuzumab at 2-8°C (36-46°F). Do not freeze. If accidentally frozen, thaw at 2-8°C before administration. Protect from direct sunlight.

Supplier: Commercially available. See package insert for further information.

Fludarabine

Source and Pharmacology:
Fludarabine phosphate is rapidly dephosphorylated to 2-fluoro-ara-A and then phosphorylated intracellularly by deoxycytidine kinase to the active triphosphate, 2-fluoro-ara-ATP. This metabolite appears to act by inhibiting DNA polymerase alpha, ribonucleotide reductase and DNA primase, thus inhibiting DNA synthesis. The mechanism of action of this antimetabolite is not completely characterized and may be

multi-faceted. Limited pharmacokinetic data for Fludarabine are available from a published study of children (ages 1-21 years) with refractory acute leukemias or solid tumors (Children's Cancer Group Study 097[1]). When Fludarabine was administered as a loading dose over 10 minutes immediately followed by a 5-day continuous infusion, steady-state conditions were reached early.

Toxicity:

Likely *("Likely" refers to a side effect that is expected to occur in more than 20% of patients.)*	**Less Likely** *("Less likely" refers to a side effect that is expected to occur in 20% or fewer patients.)*	**Rare, but Serious** *(These possible risks have been reported in rare occurrences, typically less than 2% of patients. They may be serious if they occur.)*
• Anemia due to decreased number of red cells • Infection due to decreased number of white blood cells • Bleeding due to decreased numbers of platelets • Tiredness • Nausea • Vomiting • Weakened immune system	• Pneumonia • Diarrhea • Mouth sores • Skin rash • Fever • Swelling of hands and feet	• Numbness and tingling in hands and/or feet related to irritation of nerves of the hand and/or feet • Changes in vision • Agitation/nervousness • Confusion • Cough • Difficulty breathing • Weakness • Severe brain injury and death

Formulation and Stability:
Fludarabine is supplied as a white, lyophilized solid cake. Each vial contains 50 mg of fludarabine phosphate, 50 mg of mannitol, and sodium hydroxide to adjust pH to 7.7. The pH range for the final product is 7.2-8.2. Store under refrigeration, between 2º-8ºC (36º-46ºF).

Fludarabine is supplied in a clear glass single dose vial (6mL capacity) and packaged in a single dose vial carton in a shelf pack of five.

Version 0.05: Edit./Elig. Rev. Nov. 8, 2012

Version 6 – PI Changes and Editorial Changes – March 26, 2013

Version 7 – Treatment Schedule Change: September 30, 2013

Version 8 – Editorial Changes: June 30, 2014

Version 9 – Removal of unrelated arm and editorial changes: March 26, 2015

Version 10 - Editorial Changes (Patient Eligibility): May 27, 2015

Version 11 – Campath Dosing Change: November 25, 2015

Version 12: April 11, 2019

Guidelines for Administration:
Administer by IV infusion through a peripheral or a central line.
Fludarabine should be prepared for parenteral use by aseptically adding Sterile Water for Injection USP. When reconstituted with 2mL of Sterile Water for Injection, USP, the solid cake should fully dissolve in 15 seconds or less; each mL of the resulting solution will contain 25 mg of fludarabine phosphate, 25 mg of mannitol, and sodium hydroxide to adjust the pH to 7.7. The pH range for the final product is 7.2-8.2. In clinical studies, the product has been diluted in 100 cc or 125 cc of 5% Dextrose Injection USP or 0.9% Sodium Chloride USP.

Reconstituted Fludarabine contains no antimicrobial preservative and thus should be used within 8 hours of reconstitution. Care must be taken to assure the sterility of prepared solutions. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration. Fludarabine should not be mixed with other drugs.

Supplier: Commercially available. See package insert for further information.
Melphalan

Source and Pharmacology:
Melphalan, a phenylalanine derivative of nitrogen mustard, is a bifunctional alkylating agent. Melphalan forms covalent cross-links with DNA or DNA protein complexes thereby resulting in cytotoxic, mutagenic, and carcinogenic effects. The end result of the alkylation process results in the misreading of the DNA code and the inhibition of DNA, RNA, and protein synthesis in rapidly proliferating tumor cells. It is cell cycle non-specific. After IV administration, melphalan plasma concentrations decline rapidly in a
bi-exponential manner with distribution phase and terminal elimination phase half-lives of approximately 10 and 75 minutes, respectively. Plasma melphalan levels are highly variable after oral dosing, both with respect to the time of the first appearance of melphalan in plasma (range approximately 0 to 6 hours) and to the peak plasma concentration achieved. These results may be due to incomplete intestinal absorption, a variable "first pass" hepatic metabolism, or to rapid hydrolysis. The oral dose averages 61% ± 26% of that following IV administration. The terminal elimination plasma half-life of oral melphalan is 1.5 ± 0.83 hours. The steady-state volume of distribution of melphalan is 0.5 L/kg. The extent of melphalan binding to plasma proteins ranges from 60-90%. Melphalan is eliminated from plasma primarily by chemical hydrolysis to

monohydroxymelphalan and dihydroxymelphalan. The 24-hour urinary excretion of parent drug is approximately 10% suggesting that renal clearance is not a major route of elimination of parent drug. Penetration into CSF is low. Despite the fact that the contribution of renal elimination to melphalan clearance appears to be low, one pharmacokinetic study suggests dosage may need to be reduced in patients with renal impairment.

Toxicities:

Likely *("Likely" refers to a side effect that is expected to occur in more than 20% of patients.)*	**Less Likely** *("Less likely" refers to a side effect that is expected to occur in 20% or fewer patients.)*	**Rare, but Serious** *(These possible risks have been reported in rare occurrences, typically less than 2% of patients. They may be serious if they occur.)*
● Loss of appetite ● Nausea, vomiting ● Skin breakdown if drug leaks from vein ● Anemia due to decreased number of red cells ● Infection due to decreased number of white blood cells ● Bleeding due to decreased numbers of platelets ● Mouth sores ● Temporary hair loss	● Diarrhea ● Inflammation of the lung ● Weakness ● Weight loss	● Low blood pressure ● Excessive perspiration ● Allergic reaction ● Damage/ scarring of lung tissue ● Sterility ● Seizure

Formulation and Stability:
Melphalan is available as a 2 mg scored tablet. Inactive ingredients include colloidal silicon dioxide, crospovidone, hypromellose, macrogol/PEG 400, magnesium stearate, microcrystalline cellulose, and titanium dioxide. Store in a refrigerator, 2°-8°C (36°-46°F). Protect from light.

Melphalan for Injection is supplied as a sterile, nonpyrogenic, freeze-dried powder. Each single-use vial contains melphalan hydrochloride equivalent to 50 mg melphalan and 20 mg povidone. Melphalan for Injection is reconstituted using the sterile diluent provided. Each vial of sterile diluent contains sodium citrate 0.2 g, propylene glycol 6.0 mL, ethanol (96%) 0.52 mL, and Water for Injection to a total of 10 mL. Store at controlled room temperature 15°-30°C (59°-86°F) and protect from light.

1) Reconstitute to a concentration of 5 mg/mL by rapidly injecting 10 mL of the supplied diluent directly into the vial of lyophilized powder using a sterile needle (20-gauge or larger needle diameter) and syringe. Immediately shake vial vigorously until a clear solution is obtained. Rapid addition of the diluent followed by immediate vigorous shaking is important for proper dissolution.
2) **Immediately** dilute the dose to be administered in NS to a final concentration not to exceed 2 mg/mL for IV central line administration or 0.45 mg/mL for peripheral IV administration
3) A precipitate forms if the reconstituted solution is stored at 5°C. Do not refrigerate the reconstituted product.

(*The time between reconstitution/dilution and administration of melphalan should be kept to a minimum because reconstituted and diluted solutions of melphalan are unstable. Over as short a time as 30 minutes, a citrate derivative of melphalan has been detected in reconstituted material from the reaction of melphalan with the sterile diluent for melphalan. Upon further dilution with saline, nearly 1% label strength of melphalan hydrolyzes every 10 minutes.)*

Guidelines for Administration:
IV: Administer by IV infusion through a peripheral or a central line. **Complete infusion within 1 hour of product reconstitution.**

PO: Give on empty stomach. Food decreases bioavailability. Melphalan is unstable in solution and should not be mixed with fluids prior to administration. Tablets should not be split or crushed in advance because humidity may lead to drug degradation.

Supplier: Commercially available. See package insert for further information.

G-CSF (Filgrastim)

Source and Pharmacology:
Filgrastim is a human granulocyte colony-stimulating factor (G-CSF), produced by recombinant DNA technology. Filgrastim is a 175 amino acid protein with a molecular weight of 18,800 daltons manufactured by recombinant DNA technology utilizing E coli bacteria into which has been inserted the human granulocyte colony stimulating factor gene. It differs from the natural protein in that the N- amino acid is methionine and the protein is not glycosylated. G-CSF is a lineage specific colony-stimulating factor which regulates the production of neutrophils within the bone marrow and affects neutrophil progenitor proliferation, differentiation, and selected end-cell functional activation (including enhanced phagocytic ability, priming of the cellular metabolism associated with respiratory burst, antibody dependent killing, and the increased expression of some functions associated with cell surface antigens).The elimination half-life is similar for subcutaneous and intravenous administration, approximately 3.5 hours. The time to peak concentration when administered subcutaneously is 2-8 hours

Toxicity:

Likely *("Likely" refers to a side effect that is expected to occur in more than 20% of patients.)*	**Less Likely** *("Less likely" refers to a side effect that is expected to occur in 20% or fewer patients.)*	**Rare, but Serious** *(These possible risks have been reported in rare occurrences, typically less than 2% of patients. They may be serious if they occur.)*
	● Local irritation (skin) at injection site ● Ache or pain inside the bones. Increased levels of liver enzymes and uric acid in the blood ● Bleeding due to decreased numbers of platelets	● Allergic reaction, low fever ● Enlargement or rupture of the spleen ● Worsening of pre-existing skin rashes ● Temporary hair loss ● Inflammation of a blood vessel in the skin

Formulation and Stability:
Supplied as a clear solution in 300 mcg/mL 1 mL or 1.6 mL vials and prefilled syringes containing 300 mcg/0.5 mL or 480 mcg/0.8 mL. Vials are preservative free single use vials. Discard unused portions of open vials. Store refrigerated at 2º-8ºC (36º-46ºF). Prior to injection, filgrastim may be allowed to reach room temperature for a maximum of 24 hours. Avoid freezing and temperatures > 30ºC.

For IV use, dilute in D5W only to concentrations >15 mcg/mL. At concentrations between 5 and 15 mcg/mL, human serum albumin should be added to make a final albumin concentration of 0.2% (2 mg/mL) in order to minimize the adsorption of filgrastim to infusion containers and equipment.
Dilutions of 5 mcg/mL or less are not recommended. Diluted filgrastim should be stored at 2º-8ºC (36º-46ºF) and used within 24 hours. Do not shake.

Guidelines for Administration:
See Supportive Care section of the protocol. Filgrastim should not be administered within 24 hours of chemotherapy.

Supplier: Commercially available from various manufacturers. See package insert for further information

TACROLIMUS (FK-506, Prograf®) NSC # 717865 (01/17/08)
Source and Pharmacology: Tacrolimus is a macrolide immunosuppressant produced by *Streptomyces tsukubaensis*. Tacrolimus is a potent immunosuppressive agent which prolongs the survival of the host and transplanted grafts in animal transplant models of liver, kidney, heart, bone marrow, small bowel and pancreas, lung and trachea, skin, cornea, and limb. Tacrolimus inhibits T-lymphocyte activation, although the exact mechanism of action is not known. Experimental evidence suggests that tacrolimus binds to an intracellular protein, FKBP-12. A complex of tacrolimus-FKBP-12, calcium, calmodulin, and calcineurin is then formed and the phosphatase activity of calcineurin inhibited. This effect may prevent the dephosphorylation and translocation of nuclear factor of activated T-cells (NF-AT), a nuclear component thought to initiate gene transcription for the formation of lymphokines (such as interleukin-2, gamma interferon). The net result is the inhibition of T-lymphocyte activation (immunosuppression). Additionally, tacrolimus may inhibit cellular activities such as nitric oxide synthetase activation and apoptosis, and may potentiate the action of corticosteroids in these

processes. Tacrolimus activity is primarily due to the parent drug. The plasma protein binding of tacrolimus is approximately 99% and is independent of concentration over a range of 5-50 ng/mL. Tacrolimus is bound mainly to albumin and alpha-1-acid glycoprotein, and has a high level of association with erythrocytes. The t½ in adult patients ranges from 11-19 hours. The pharmacokinetics of tacrolimus have been studied in pediatric liver transplant patients (0.7 to 13.2 years of age). Following the IV administration of a 0.037 mg/kg/day dose to 12 pediatric patients, mean terminal half-life, volume of distribution and clearance were 11.5 ± 3.8 hours, 2.6 ± 2.1 L/kg and 0.138 ± 0.071 L/hr/kg, respectively. Following oral administration to 9 pediatric patients, the absolute bioavailability was 31 ± 21%. Whole blood trough concentrations from 31 patients less than 12 years old showed that pediatric patients needed higher doses than adults to achieve similar tacrolimus trough concentrations. Tacrolimus is extensively metabolized by the mixed-function oxidase system, primarily the cytochrome P-450 system (CYP3A) in the liver and to a lesser extent in the intestinal mucosa. The major metabolite identified in incubations with human liver microsomes is 13-demethyl tacrolimus. The main route of elimination is via the biliary tract and excretion in faeces. The mean clearance in renal dysfunction and mild hepatic dysfunction is the same as normal volunteers. Severe hepatic dysfunction (Pugh score >10) led to a substantially decreased clearance. A retrospective comparison of Black and Caucasian kidney transplant patients indicated that Black patients required higher tacrolimus doses to attain similar trough concentrations; there were no gender-based differences.

The absorption of tacrolimus from the gastrointestinal tract is incomplete and variable exhibiting large intra- and inter-patient variability. Administration with food significantly decreases the rate and extent of absorption Drugs that stimulate or inhibit hepatic p-450 enzymes will alter clearance of tacrolimus and close attention to potential drug interactions is crucial.

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Likely *("Likely" refers to a side effect that is expected to occur in more than 20% of patients.)*	**Less Likely** *("Less likely" refers to a side effect that is expected to occur in 20% or fewer patients.)*	**Rare, but Serious** *(These possible risks have been reported in rare occurrences, typically less than 2% of patients. They may be serious if they occur.)*
•Headache • Nausea and vomiting • High blood pressure • Loss of desire to eat or appetite • Reduced ability of the body to fight infection • Diarrhea or constipation • Fever • Tremor (shakiness usually of the hands) • Increases in the levels of certain chemicals in the blood because the kidney is not working as well as normal which may require lowering the dose • Fewer red blood cells in the blood • Difficulty sleeping or falling asleep • A feeling of weakness and/or tiredness • Pain which may be in the abdomen (belly), back and/or other parts of the body • High levels of sugar in the blood that may require treatment	•Chest pain • Hair loss • Dizziness • Elevation in the blood of certain enzymes or bilirubin found in the liver which may mean the liver is not working as well • Bladder or kidney infection • Fluid retention and build-up in the tissues usually of the lower legs leading to an increase in weight • Rash that may itch • An increase in the levels of lipids (fats) and cholesterol in the blood which, if prolonged, could lead to heart problems later in life • Acid or upset stomach (heartburn) • Difficulty or discomfort on swallowing • Inflammation of the stomach or esophagus • Too much gas produced in the intestines • Shortness of breath and/or a tight feeling in the chest with wheezing and shortness of breath • Increased cough • Flu type symptoms with fever, tiredness, aches and pains	• Severe allergic reaction which can be life threatening with shortness of breath, low blood pressure, rapid heart rate chills and fever (usually only with the IV form) • Allergic reactions • Fluid build-up in the lungs • Convulsions • A fast heartbeat which may cause pain in the chest • Chest pain or discomfort that occurs when heart muscle does not get enough blood • Severe damage to the brain which may lead to difficulty carrying out normal daily tasks and to coma • Abnormal clotting of the blood which could lead to bleeding in the intestines or elsewhere • Inflammation and clotting of blood vessels which can lead to pain and swelling in the area of the clot • Infections including those caused by bacteria, virus, and fungus which could be located in the skin, blood, throat, sinuses, lungs or abdomen

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• Abnormal levels of magnesium in the body which may require that extra magnesium by mouth or vein • Low (*or High*) levels of certain salts in the body like potassium and phosphate which may require treatment • Numbness and tingling in the fingers and toes	• Changes in brain function such that have difficulty in thinking clearly, are sleepy, depressed, anxious, have strange dreams, are nervous, have changes in mood which may include severe depression, feelings of suicide, feelings of aggressiveness and violent behavior or see or hear things that are not there • Fewer white blood cells and platelets in the blood • Greater than normal numbers of white or red blood cells in the blood • Aches and Pains in the joints or muscles • Skin changes including pimples, change in color, increased tendency to sun burn and skin sores • Wounds may be slower to heal • Excessive hair growth such as on the face, eyebrows, arms and legs • Bleeding or tender gums, overgrowth of gum tissue • Changes in your vision or a decrease in vision • Ear pain or ringing in the ears • Gallstones	• Severe rashes which can result in loss of skin and damage to mucous membranes • Erosion (ulceration) of the lining of the intestines which can result in pain and/or bleeding or a hole in the intestines which would cause leakage into the abdomen with pain and infection • Damage to the liver which can lead to inflammation and/or scarring which could lead to a yellow appearing skin, and fluid collection in the abdomen which makes it look larger • Severe kidney damage (which may be permanent) • Diabetes mellitus - a condition where the sugar in the blood is not appropriately controlled and may require treatment with insulin by injection or drugs taken by mouth • Damage to the heart muscle • Excessive growth of white blood cells that may lead to lymphoma a cancer of the white blood cells • Increased chance of skin cancers

Formulation and Stability:
IV formulation: Tacrolimus is available as a sterile solution (tacrolimus injection) containing the equivalent of 5 mg anhydrous tacrolimus in 1 mL Each mL contains polyoxyl 60 hydrogenated castor oil (HCO-60), 200 mg, and dehydrated alcohol, USP, 80% v/v. Store between 5°C and 25°C (41°F and 77°F).

Oral formulations:
Tacrolimus is available for oral administration as capsules containing the equivalent of 0.5 mg, 1 mg or 5 mg of anhydrous tacrolimus. Inactive ingredients include lactose, hydroxypropyl methylcellulose, croscarmellose sodium, and magnesium stearate.
The 0.5 mg capsule shell contains gelatin, titanium dioxide and ferric oxide, the 1 mg capsule shell contains gelatin and titanium dioxide, and the 5 mg capsule shell contains gelatin, titanium dioxide and ferric oxide. Store at 25°C (77°F); excursions permitted to 15°-30°C (59°-86°F).

Guidelines for Administration: See Treatment and Dose Modifications sections of the protocol.

Tacrolimus Injection must be diluted with 0.9% Sodium Chloride Injection or 5% Dextrose Injection before use to a concentration between 0.004 mg/mL and 0.02 mg/mL. Diluted infusion solution should be stored in glass or polyethylene containers and should be discarded after 24 hours. The polyoxyethylated castor oil contained in the concentrate for intravenous infusion can cause phthalate stripping from PVC**. It is strongly recommended that glass bottles and non-PVC tubing be used to minimize patient exposure to DEHP**. Due to the chemical instability of tacrolimus in alkaline media, Tacrolimus Injection should not be mixed or co-infused with solutions of pH 9 or greater (e.g., ganciclovir or acyclovir). Monitor closely for an acute allergic reaction for the first 30 minutes and at frequent intervals thereafter.

Oral: Administer at a consistent time of day and at consistent intervals with regard to meals. Tacrolimus may be given with food as long as it is given the same way each time; however; administration with food significantly decreases the rate and extent of absorption.

Supplier: Commercially available from various manufacturers. See package insert for further information.

Methylprednisolone or prednisone

Methylprednisolone is a corticosteroid and the methyl derivative of prednisolone. Glucocorticoids produce widespread and diverse physiologic effects on carbohydrate, protein, and lipid metabolism, electrolyte and water balance, functions of the cardiovascular system, kidney, skeletal muscle, and the nervous systems. Glucocorticoids reduce the concentration of thymus-dependent lymphocytes (T-lymphocytes), monocytes, and eosinophils. Glucocorticoids selectively bind to the cortisol receptors on human lymphoid cells which are found in larger numbers on leukemic lymphoblasts. They also decrease binding of immunoglobulin to cell surface receptors and inhibit the synthesis and/or release of interleukins, thereby decreasing T-lymphocyte blastogenesis and reducing expansion of the primary immune response. The specific cellular mechanisms that act to halt DNA synthesis are thought to be related to inhibition of glucose transport or phosphorylation, retardation of mitosis, and inhibition of protein synthesis. At the cellular level, corticosteroids appear to act by controlling the rate of protein synthesis. The half-life of methylprednisolone is approximately 2.5 hours; however, the metabolic effects at the tissue level persist for up to 20-30 hours. It is primarily metabolized in the liver and excreted by the kidneys. Methylprednisolone 4 mg has equivalent potency to prednisone 5mg.

Prednisone is a synthetic compound closely related to hydrocortisone. Glucocorticoids produce widespread and diverse physiologic effects on carbohydrate, protein, and lipid metabolism, electrolyte and water balance, functions of the cardiovascular system, kidney, skeletal muscle, and the nervous systems. Glucocorticoids reduce the concentration of thymus-dependent lymphocytes (T-lymphocytes), monocytes, and eosinophils. Glucocorticoids selectively bind to the cortisol receptors on human lymphoid cells which are found in larger numbers on leukemic lymphoblasts. They also decrease binding of immunoglobulin to cell surface receptors and inhibit the synthesis and/or release of interleukins, thereby decreasing T-lymphocyte blastogenesis and reducing expansion of the primary immune response. The specific cellular mechanisms that act to halt DNA synthesis are thought to be related to inhibition of glucose transport or phosphorylation, retardation of mitosis, and inhibition of protein synthesis. Peak blood levels occur within 2 hours of oral intake. Prednisone is approximately 75% protein bound with a plasma t½ of 3.2 to 4 hours. (Biologic half-life is 12-36 hours.)

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● Water retention ● Overeating ● Weakened immune system ● Temporary personality changes ● Abnormal hormone production ● High blood sugar ● Slowed growth ● Decreased bone density	● Headaches ● Poor wound healing ● Stomach swelling or pain ● Tissue swelling ● High blood pressure ● Stomach ulcer ● Muscle weakness ● Cataracts	● Difficulty in falling asleep ● Worsening of diabetes ● Inflammation of pancreas ● Personality disturbances ● Bleeding in the stomach and intestines ● Increased pressure within the eye ● Disturbance of bone calcium which can lead to possible fractures or permanent bone damage

Formulation and Stability:
Methylprednisolone:
Available for oral use in 2, 4, 8, 16, 24 and 32 mg tablets. Inactive ingredients vary depending on manufacturer but tablet formulations may include: calcium stearate, corn starch, erythrosine sodium, lactose, mineral oil, sorbic acid, sucrose, croscarmellose sodium, lactose monohydrate, magnesium stearate, microcrystalline cellulose, polacrilin potassium, sodium starch glycolate, and stearic acid.

Lyophilized Powder for Injection as methylprednisolone sodium succinate equivalent to methylprednisolone is available as: **Dual chamber vials** of 40 mg (1 mL), 125 mg (2 mL), 500 mg (4 mL), 1000 mg (8 mL) also containing mono, dibasic sodium phosphate and benzyl alcohol (diluent); Vials of 500 mg, 1 g, 2 g also containing monobasic and dibasic sodium phosphate with or without separate vials of diluent containing benzyl alcohol. Protect from light.
Store unreconstituted product at controlled room temperature 20°- 25°C (68°- 77°F).

Prednisone:
Available in 1 mg, 2.5 mg, 5 mg, 10 mg, 20 mg, 25 mg, and 50 mg tablets; liquid, 5 mg/5 mL or 5 mg/mL. Inactive ingredients vary depending on manufacturer but tablet formulations may include: calcium or magnesium stearate, corn starch, lactose, erythrosine sodium, mineral oil, sorbic acid, sucrose, talc and various dyes. Liquid formulations may include: 5-30% alcohol, fructose, sucrose, saccharin, and sorbitol.

Guidelines for Administration: See Treatment section of the protocol.

Methylprednisolone:
Dilute lyophilized powder vials for injection with sufficient diluent to obtain a concentration of 62.5 mg/mL. The manufacturer recommends the use of Bacteriostatic sterile water for injection containing benzyl alcohol. **Use sterile water for injection without benzyl alcohol for neonates and infants < 2 years of age or patients with hypersensitivity to benzyl alcohol.**

Mix dual chamber vials as directed. Store solution at controlled room temperature 20°-25°C (68°-77°F).Use solution within 48 hours after mixing.

May be further diluted in Saline or Dextrose containing solutions for intravenous administration.

Supplier: Both commercially available from various sources. See package insert for further information

Mycophenolate Mofetil: This drug will be used as part of GVHD prophylaxis for cord blood recipients.

Source and Pharmacology:
Mycophenolate mofetil is rapidly absorbed following oral administration and hydrolyzed to form MPA, which is the active metabolite. MPA is a potent, selective, uncompetitive, and reversible inhibitor of inosine monophosphate dehydrogenase (IMPDH), and therefore inhibits the de novo pathway of guanosine nucleotide synthesis without incorporation into DNA. Because T- and B-lymphocytes are critically dependent for their proliferation on de novo synthesis of purines, whereas other cell types can utilize salvage pathways, MPA has potent cytostatic effects on lymphocytes. MPA inhibits proliferative responses of T- and B-lymphocytes to both mitogenic and allospecific stimulation.

Addition of guanosine or deoxyguanosine reverses the cytostatic effects of MPA on lymphocytes. MPA also suppresses antibody formation by B-lymphocytes. MPA prevents the glycosylation of lymphocyte and monocyte glycoproteins that are involved in intercellular adhesion to endothelial cells and may inhibit recruitment of leukocytes into sites of inflammation and graft rejection. Mycophenolate mofetil did not inhibit early events in the activation of human peripheral blood mononuclear cells, such as the production of interleukin-1 (IL-1) and interleukin-2 (IL-2), but did block the coupling of these events to DNA synthesis and proliferation.

Following oral and intravenous administration, mycophenolate mofetil undergoes rapid and complete metabolism to MPA, the active metabolite. Oral absorption of the drug is rapid and essentially complete. MPA is metabolized to form the phenolic glucuronide of MPA (MPAG) which is not pharmacologically active. The parent drug, mycophenolate mofetil, can be measured systemically during the intravenous infusion; however, shortly (about 5 minutes) after the infusion is stopped or after oral administration, MMF concentration is below the limit of quantitation (0.4 µg/mL).

Following oral and intravenous dosing, mycophenolate mofetil undergoes complete metabolism to MPA, the active metabolite. Metabolism to MPA occurs presystemically after oral dosing. MPA is metabolized principally by glucuronyl transferase to form the phenolic glucuronide of MPA (MPAG) which is not pharmacologically active. *In vivo*, MPAG is converted to MPA via enterohepatic recirculation. The following metabolites of the 2-hydroxyethyl-morpholino moiety are also recovered in the urine following oral administration of mycophenolate mofetil to healthy subjects:
N-(2-carboxymethyl)-morpholine, N-(2-hydroxyethyl)-morpholine, and the N-oxide of N-(2-hydroxyethyl)-morpholine.

Secondary peaks in the plasma MPA concentration-time profile are usually observed 6 to 12 hours postdose. The coadministration of cholestyramine (4 g tid) resulted in approximately a 40% decrease in the MPA AUC (largely as a consequence of lower concentrations in the terminal portion of the profile). These observations suggest that enterohepatic recirculation contributes to MPA plasma concentrations.
Increased plasma concentrations of mycophenolate mofetil metabolites (MPA 50% increase and MPAG about a 3-fold to 6-fold increase) are observed in patients with renal insufficiency.

Negligible amount of drug is excreted as MPA (< 1% of dose) in the urine. Orally administered radiolabeled mycophenolate mofetil resulted in complete recovery of the administered dose, with 93% of the administered dose recovered in the urine and 6% recovered in feces. Most (about 87%) of the administered dose is excreted in the urine as MPAG. At clinically encountered concentrations, MPA and MPAG are usually not removed by hemodialysis. However, at high MPAG plasma concentrations (> 100 ug/mL), small amounts of MPAG are removed. Mean (±SD) apparent half-life and plasma clearance of MPA are 17.9 (±6.5) hours and 193 (±48) mL/min following oral administration and 16.6 (±5.8) hours and 177 (±31) mL/min following intravenous administration, respectively.

The pharmacokinetic parameters of MPA and MPAG have been evaluated in 55 pediatric patients (ranging from 1 year to 18 years of age) receiving CellCept oral suspension at a dose of 600 mg/m^2 bid (up to a maximum of 1 g bid) after allogeneic renal transplantation.

The CellCept oral suspension dose of 600 mg/m^2 bid (up to a maximum of 1 g bid) achieved mean MPA AUC values in pediatric patients similar to those seen in adult renal transplant patients receiving CellCept capsules at a dose of 1 g bid in the early posttransplant period. There was wide variability in the data. As observed in adults, early posttransplant MPA AUC values were approximately 45% to 53% lower than those observed in the later post transplant period (> 3 months). MPA AUC values were similar in the early and late posttransplant period across the 1 year to 18 year age range.

Toxicity:

Likely *("Likely" refers to a side effect that is expected to occur in more than 20% of patients.)*	Less Likely *("Less likely" refers to a side effect that is expected to occur in 20% or fewer patients.)*	Rare, but Serious *(These possible risks have been reported in rare occurrences, typically less than 2% of patients. They may be serious if they occur.)*
● Diarrhea ● Stomach pain ● Upset stomach ● Vomiting ● Difficulty falling asleep or staying asleep	● Pain, especially in the back, muscles, or joints ● Constipation	● Swelling of the hands, feet, ankles, or lower legs ● Difficulty breathing ● Shaking hands that you cannot control ● Unusual bruising or bleeding ● Headache ● Fast heartbeat ● Excessive tiredness ● Dizziness ● Pale skin ● Weakness ● Blood in stools ● Bloody vomit ● Loose, floppy muscles ● White patches in mouth or throat ● Swelling of gums ● Vision changes ● Rash ● Low blood counts ● Damage to unborn baby ● Limited effectiveness of birth control ● Progressive Multifocal Leukoencephalopathy

Formulation and Stability:
Mycophenolate mofetil is a white to off-white crystalline powder. It is slightly soluble in water (43 µg/mL at pH 7.4); the solubility increases in acidic medium (4.27 mg/mL at pH 3.6). It is freely soluble in acetone, soluble in methanol, and sparingly soluble in ethanol. The apparent partition coefficient in 1-octanol/water (pH 7.4) buffer solution is 238. The pKa values for mycophenolate mofetil are 5.6 for the morpholino group and 8.5 for the phenolic group.

Mycophenolate mofetil hydrochloride has a solubility of 65.8 mg/mL in 5% Dextrose Injection USP (D5W). The pH of the reconstituted solution is 2.4 to 4.1.
CellCept is available for oral administration as capsules containing 250 mg of mycophenolate mofetil, tablets containing 500 mg of mycophenolate mofetil, and as a powder for oral suspension, which when constituted contains 200 mg/mL mycophenolate mofetil.

Inactive ingredients in CellCept 250 mg capsules include croscarmellose sodium, magnesium stearate, povidone (K-90) and pregelatinized starch. The capsule shells contain black iron oxide, FD&C blue #2, gelatin, red iron oxide, silicon dioxide, sodium lauryl sulfate, titanium dioxide, and yellow iron oxide.

Inactive ingredients in CellCept 500 mg tablets include black iron oxide, croscarmellose sodium, FD&C blue #2 aluminum lake, hydroxypropyl cellulose, hydroxypropyl methylcellulose, magnesium stearate, microcrystalline cellulose, polyethylene glycol 400, povidone (K-90), red iron oxide, talc, and titanium dioxide; may also contain ammonium hydroxide, ethyl alcohol, methyl alcohol, n-butyl alcohol, propylene glycol, and shellac. Inactive ingredients in CellCept Oral Suspension include aspartame, citric acid anhydrous, colloidal silicon dioxide, methylparaben, mixed fruit flavor, sodium citrate dihydrate, sorbitol, soybean lecithin, and xanthan gum.

CellCept Intravenous is the hydrochloride salt of mycophenolate mofetil. The chemical name for the hydrochloride salt of mycophenolate mofetil is 2-morpholinoethyl (E)-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate hydrochloride. It has an empirical formula of $C_{23}H_{31}NO_7$ HCl and a molecular weight of 469.96.

CellCept Intravenous is available as a sterile white to off-white lyophilized powder in vials containing mycophenolate mofetil hydrochloride for administration by intravenous infusion only. Each vial of CellCept Intravenous contains the equivalent of 500 mg

mycophenolate mofetil as the hydrochloride salt. The inactive ingredients are polysorbate 80, 25 mg, and citric acid, 5 mg. Sodium hydroxide may have been used in the manufacture of CellCept Intravenous to adjust the pH. Reconstitution and dilution with 5% Dextrose Injection USP yields a slightly yellow solution of mycophenolate mofetil, 6 mg/mL.

Guidelines for Administration: See Treatment section of protocol.

For IM/IV use: Caution should be exercised in the handling and preparation of solutions of mycophenolate Intravenous. Avoid direct contact of the prepared solution of mycophenolate Intravenous with skin or mucous membranes. Mycophenolate Intravenous does not contain an antibacterial preservative; therefore, reconstitution and dilution of the product must be performed under aseptic conditions.
Additionally, this product is sealed under vacuum and should retain a vacuum throughout its shelf life. If a lack of vacuum in the vial is noted while adding diluent, the vial should not be used.

Mycophenolate Intravenous infusion solution must be prepared in two steps: the first step is a reconstitution step with 5% Dextrose Injection USP, and the second step is a dilution step with 5% Dextrose Injection USP. A detailed description of the preparation is given below:

Step 1

a. Two (2) vials of Mycophenolate Intravenous are used for preparing each 1 g dose, whereas three (3) vials are needed for each 1.5 g dose. Reconstitute the contents of each vial by injecting 14 mL of 5% Dextrose Injection USP.
b. Gently shake the vial to dissolve the drug.
c. Inspect the resulting slightly yellow solution for particulate matter and discoloration prior to further dilution. Discard the vials if particulate matter or discoloration is observed.

Step 2

a. To prepare a 1 g dose, further dilute the contents of the two reconstituted vials (approx. 2 x 15 mL) into 140 mL of 5% Dextrose Injection USP. To prepare a 1.5 g dose, further dilute the contents of the three reconstituted vials (approx. 3 x 15 mL) into 210 mL of 5% Dextrose Injection USP. The final concentration of both solutions is 6 mg mycophenolate mofetil per mL.
b. Inspect the infusion solution for particulate matter or discoloration. Discard the infusion solution if particulate matter or discoloration is observed.

If the infusion solution is not prepared immediately prior to administration, the commencement of administration of the infusion solution should be within 4 hours from reconstitution and dilution of the drug product. Keep solutions at 25°C (77°F); excursions permitted to 15° to 30°C (59° to 86°F).

Mycophenolate Intravenous should not be mixed or administered concurrently via the same infusion catheter with other intravenous drugs or infusion admixtures.

For oral use: It is recommended that Mycophenolate oral suspension be constituted by the pharmacist prior to dispensing to the patient.
Mycophenolate oral suspension should not be mixed with any other medication.

Supplier: Commercially available from various manufacturers. See package insert for further information.

References

1. Solomon, L. Treatment and prevention of pain due to vaso-occlusive crises in adults with sickle cell disease: an educational void. *Blood* **111**, 997-1003 (2008).

2. Ashley-coch, A., Yang, Q. & Olney, R. Sickle Hemoglobin (HbS) A Allele and Sickle Cell Disease. *American Journal of Epidemiology* **151**, 839-845 (2000).

3. Gladwin, M. & Vichinsky, E. Pulmonary Complications of Sickle Cell Disease. *New England Journal of Medicine* **359**, 2254-2265 (2008).

4. Platt, O. & et al Mortality in sickle cell disease. Life expectancy and risk factors for early death. *New England Journal of Medicine* **330**, 1639-1644 (1994).

5. Ohene-Frempong, K., Weiner, S. & Sleeper, L. Cerebrovascular accidents in sickle cell disease rates and risk factors. *Blood* **91**, 288-294 (1998).

6. Quinn, C., Rogers, Z. & Buchanan, G. Survival of children with sickle cell disease. *Blood* **103**, 4023-4027 (2004).

7. Kinney, T., Sleeper, L., Wang, W. & et al Silent cerebral infarcts in sickle cell anemia: a risk factor analysis. The Cooperative Study of Sickle Cell Disease. *Pediatrics* **103**, 640-645 (1999).

8. Armstrong, F., Thompson, R., Wang, W. & et al Cognitive functioning and brain magnetic resonance imaging in children with sickle cell disease. Neuropsychology Committee of the Cooperative Study of Sickle Cell Disease. *Pediatrics* **97**, 864-870 (1996).

9. Craft, S., Schatz, J., Glauser, T., Lee, B. & DeBaun, M. Neuropsychologic effects of stroke in children with sickle cell anemia. *Journal of Pediatrics* **123**, 712-717 (1993).

10. DeBaun, M., Schatz, J. & Siegel, M. Cognitive screening examinations for silent cerebral infarcts in sickle cell disease. *Neurology* **50**, 1678-1682 (1998).

11. Miller, S., Macklin, E., Pegelow, C. & et al Silent infarction as a risk factor for overt stroke in children with sickle cell anemia: a report from the cooperative study of sickle cell disease. *Journal of Pediatrics* **139**, 385-390 (2001).

12. Scothorn, D., Price, C., Schwartz, D. & et al Risk of recurrent stroke in children with sickle cell disease receiving blood transfusion therapy for at least five years after initial stroke. *Journal of Pediatrics* **140**, 348-354 (2010).

13. Leikin, S., Gallagher, D., Kinney, T. & et al Mortality in children and adolescents with sickle cell disease. Cooperative Study of Sickle Cell Disease. *Pediatrics* **84**, 500-508 (1989).

14. Charache, S., Dover, G., Moore, R. & et al Effect of hydroxyurea on the frequency of painful crises in sickle cell anemia. *New England Journal of Medicine* **332**, 1317-1322 (1995).

15. Scott, J., Hillery, C., Brown, E., Misiewicz, V. & Labotka, R. Hydroxyurea therapy in children severely affected with sickle cell disease. *Journal of Pediatrics* **128**, 820-828 (1996).

16. Jayabose, S., Tugal, O., Sandoval, C. & et al Clinical and hematologic effects of hydroxyurea in children with sickle cell anemia. *Journal of Pediatrics* **129**, 559-565 (1996).

17. Hoppe, C. *et al.* Use of hydroxyurea in children ages 2 to 5 years with sickle cell disease. *Journal of Pediatric Hematology Oncology* **22**, 330-334 (2000).

18. Fitzhugh, C., Wigfall, D. & Ware, R. Enalapril and hydroxyurea therapy for children with sickle nephropathy. *Pediatric Blood and Cancer* **45**, 982-985 (2005).

19. Thornburg, C., Dixon, N., Burgett, S. & et al A pilot study of hydroxyurea to prevent chronic organ damage in young children with sickle cell anemia. *Pediatric Blood and Cancer* **52**, 609-615 (2008).

20. Wang, W. *et al.* A two-year pilot trial of hydroxyurea in very young children with sickle-cell anemia. *Journal of Pediatrics* **139**, 790-796 (2001).

21. Claster, S. & Vichinsky, E. First report of reversal of organ dysfunction in sickle cell anemia by the use of hydroxyurea: splenic regeneration. *Blood* **88**, 1951-1953 (1996).

22. Hankins, J. *et al.* Preservation of spleen and brain function in children with sickle cell anemia treated with hydroxyurea. *Pediatric Blood and Cancer* **50**, 293-297 (2008).

23. Singh, S., Koumbourlis, A. & Aygun, B. Resolution of chronic hypoxemia in pediatric sickle cell patients after treatment with hydroxyurea. *Pediatric Blood and Cancer* **50**, 1258-1260 (2008).

24. Pashankar, F., Carbonella, J., Bazzy-Asaad, A. & Friedman, A. Longitudinal follow-up of elevated pulmonary artery pressures in children with sickle cell disease. *British Journal of Haematology* **144**, 736-741 (2008).

25. Puffer, E., Schatz, J. & Roberts, C. The association of oral hydroxyurea with improved cognitive functioning in sickle cell disease. *Child Neuropsychology* **13**, 142-154 (2007).

26. Kratovil, T. *et al.* Hydroxyurea therapy lowers TCD velocities in children with sickle cell disease. *Pediatric Blood and Cancer* **47**, 894-900 (2006).

27. Zimmerman, S., Schultz, W., Burgett, S., Mortier, N. & Ware, R. Hydroxyurea therapy lowers transcranial Doppler flow velocities in children with sickle cell anemia. *Blood* **110**, 1043-1047 (2007).

28. Gulbis, B., Haberman, D., Dufour, D. & et al Hydroxyurea for sickle cell disease in children and for prevention of cerebrovascular events: the Belgian experience. *Blood* **105**, 2685-2690 (2005).

29. Ware, R., Zimmerman, S. & Schultz, W. Hydroxyurea as an alternative to blood transfusions for the prevention of recurrent stroke in children with sickle cell disease. *Blood* **94**, 3022-3026 (1999).

30. Ware, R., Zimmerman, S., Sylvestre, P. & et al Prevention of secondary stroke and resolution of transfusional iron overload in children with sickle cell anemia using hydroxyurea and phlebotomy. *Journal of Pediatrics* **145**, 346-352 (2004).

31. Kinney, T., Helms, R., O'Branski, E. & et al Safety of hydroxyurea in children with sickle cell anemia: results of the HUG-KIDS study, a phase I/II trial. *Blood* **94**, 1550-1554 (1999).

32. Wang, W., Helms, R., Lynn, H., Redding-Lallinger, R. & et al Effect of hydroxyurea on growth in children with sickle cell anemia: results of the HUG-KIDS Study. *Journal of Pediatrics* **140**, 225-229 (2002).

33. Ballas, S., McCarthy, W., Guo, N. & et al Exposure to hydroxyurea and pregnancy outcomes in patients with sickle cell anemia. *Journal of the National Medical Association* **101**, 1046-1051 (2009).

34. Hanft, V. *et al.* Acquired DNA mutations associated with in vivo hydroxyurea exposure. *Blood* **95**, 3589-3593 (2000).

35. National Heart, L. a. B. I. Stroke Prevention Study in Children with Sickle Cell Anemia, Iron Overload Stopped Early. NIH News. 2010.
Ref Type: Electronic Citation

36. Panepinto, J. *et al.* Matched-related donor transplantation for sickle cell disease: report from the Center for International Blood and Transplant Research. *British Journal of Haematology* **137**, 479-485 (2007).

37. Bernaudin, F. *et al.* Long-term results of related myeloablative stem-cell transplantation to cure sickle cell disease. *Blood* **110**, 2749-2756 (2007).

38. Hoppe, C. & Walters, M. Bone marrow transplantation in sickle cell anemia. *Current Opinions in Oncology* **13**, 85-90 (2001).

39. Friedman, D. *et al.* Alloimmunization to platelets in heavily transfused patients with sickle cell disease. *Blood* **88**, 3216-3222 (1996).

40. Zimring, J. & et al Immunization to minor histocompatibility antigens on transfused RBCs through crosspriming into recipient MHC class I pathways. *Blood* **107**, 187-189 (2006).

41. Socie, G., Salooja, N., Cohen, A. & et al Nonmalignant late effects after allogeneic stem cell transplantation. *Blood* **101**, 3373-3385 (2003).

42. Vassal, G., Deroussent, A., Hartmann, O. & et al Dose-dependent neurotoxicity of high-dose busulfan in children: a clinical and pharmacological study. *Cancer Research* **50**, 6203-6207 (1990).

43. Giri, N., Davis, E. A. & Vowels, M. R. Long-term complications following bone marrow transplantation in children. *J Paediatr. Child Health* **29**, 201-205 (1993).

44. Locatelli, F., Giorgiani, G., Pession, A. & Bozzola, M. Late effects in children after bone marrow transplantation: a review. *Haematologica* **78**, 319-328 (1993).

45. Beinert, T., Dull, T., Wolf, K. & et al Late Pulmonary Impairment Following Bone marrow Transplantation. *European Journal of Medical Research* **1**, 343-348 (1996).

46. Shalitin, S. & et al Endocrine dysfunction and parameters of the metabolic syndrome after bone marrow transplantation during childhood and adolescence. *Bone Marrow Transplant* **37**, 1109-1117 (2006).

47. Gurney, J. & et al Visual, auditory, sensory, and motor impairments in long-term survivors of hematopoietic stem cell transplantation performed in childhood: results from the Bone Marrow Transplant Survivor Study. *Cancer* **106**, 1402-1408 (2006).

48. Walters, M. & et al Stable mixed hematopoietic chimerism after bone marrow transplantation for sickle cell anemia. *Biology of Blood and Marrow Transplantation* **7**, 665-673 (2001).

49. Krishnamurti, L. *et al.* Stable Long-Term Donor Engraftment following Reduced-Intensity Hematopoietic Cell Transplantation for Sickle Cell Disease. *Biology of Blood and Marrow Transplantation* **14**, 1270-1278 (2008).

50. Chakraverty, R., Peggs, K., Chopra, R., Millegan, D. & Kottaridis, P. Limiting transplantation-related mortality following unrelated donor stem cell transplantation by using a nonmyeloablative conditioning regimen. *Blood* **99**, 1071-1078 (2002).

51. Shenoy, S., Grossman, W., DiPersio, J. & et al A novel reduced-intensity stem cell transplant regimen for non-malignant disorders. *Bone Marrow Transplant* **35**, 345-352 (2005).

52. Rao, A., Kamani, N. & Filipovich, A. Successful bone marrow transplantation for IPEX syndrome following reduced-intensity conditioning. *Blood* **109**, 383-385 (2007).

53. Hsieh, M. *et al.* Allogeneic Hematopoietic Stem-Cell Transplantation for Sickle Cell Disease. *New England Journal of Medicine* **361**, 2309-2317 (2009).

54. Walters, M., Storb, R., Patience, M. & et al Impact of bone marrow transplantation for symptomatic sickle cell disease: an interim report. Multicenter investigation of bone marrow transplantation for sickle cell disease. *Blood* **95**, 1918-1924 (2000).

55. Walters, M., Patience, M., Leisenring, W. & et al Updated results of bone marrow transplantation (BMT) for sickle cell disease (SCD): impact on CNS disease. *Blood* **100**, 160, p45a (2002).

56. Hernigou, P., Bernaudin, F., Reinert, P. & et al Bone-marrow transplantation in sickle cell disease. Effect on osteonecrosis: a case report with a four-year follow-up. *The Journal of Bone and Joint Surgery* **79**, 1726-1730 (1997).

57. Werner, E., Yanovich, S., Rubinstein, P. & et al Unrelated placental cord blood transplantation in a patient with sickle cell disease and acute non-lymphocytic leukemia. *Blood* **90**, 4542, 399b (1997).

58. Ferster, A., Bujan, W., Corazza, F. & et al Bone marrow transplantation corrects the splenic reticuloendothelial dysfunction in sickle cell anemia. *Blood* **81**, 1102-1105 (1993).

59. Kalinyak, K., Morris, C., Ball, W. & et al Bone marrow transplantation in a young child with sickle cell anemia. *American Journal of Hematology* **48**, 256-261 (1995).

60. Steen, R., Helton, K., Horwitz, E. & et al Improved cerebrovascular patency following therapy in patients with sickle cell disease: initial results in 4 patients who received HA-identical hematopoietic stem cell allografts. *Annals of Neurology* **49**, 222-229 (2001).

61. Walters, M. Sickle cell anemia and hematopoietic cell transplantation: When is a pound of cure worth more than an ounce of prevention? *Pediatric Transplantation* **8**, 33-38 (2004).

62. Steen, R., Emudianughe, T., Hankins, G. & et al Brain imaging findings in pediatric patients with sickle cell disease. *Radiology* **228**, 216-225 (2003).

63. Laver, J., Hulsey, T., Jones, J. & et al Assessment of barriers to bone marrow donation by unrelated African-American potential donors. *Biology of Blood and Marrow Transplantation* **7**, 45-48 (2001).

64. Krishnamurti, L., Abel, S., Maiers, M. & Flesch, S. Availability of unrelated donors for hematopoietic stem cell transplantation for hemoglobinopathies. *Bone Marrow Transplant* **31**, 547-550 (2003).

65. Adamkiewicz, T., Boyer, M., Bray, R., Haight, A. & Yeager, A. Identification of Unrelated Cord Blood Units for Hematopoietic Stem Cell Transplantation in Children With Sickle Cell Disease. *Journal of Pediatric Hematology Oncology* **28**, 29-32 (2006).

66. Stevens, C. & et al. An empirical analysis of the probability of finding a well matched cord blood unit: Implications for a National Cord Blood inventory. Blood 106(11). 2005.
Ref Type: Abstract

67. Wagner, J., Barker, J., DeFor, T., Baker, K. & et al Transplantation of unrelated donor umbilical cord blood in 102 patients with malignant and nonmalignant disease: influence of CD34 cell dose and HLA disparity on treatment-related mortality and survival. *Blood* **100**, 1611-1618 (2002).

68. Wall, D., Carter, S., Kernan, N. & et al Busulphan/melphalan/antithymocyte globulin followed by unrelated donor cord blood transplantation for treatment of infant leukemia in young children: the Cord Blood Transplantation study (COBLT) experience. *Biology of Blood and Marrow Transplantation* **11**, 637-646 (2005).

69. Locatelli, F. *et al.* Related umbilical cord blood transplantation in patients with thalassemia and sickle cell disease. *Blood* **101**, 2137-2143 (2003).

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Appendix A: Statistics

Study Design

The single center study is Phase III trial, consisting of one arms: sickle cell disease patients 2-30 years old receiving related transplant using bone marrow after a conditioning regimen consisting of Alemtuzumab, Fludarabine, and Melphalan. The anticipated trial will enroll 20 additional patients.

Accrual
It is anticipated that 7 years will be needed to enroll the additional patients.

Study Duration
Patients will be followed for at least 2 years post transplant.

Randomization
This is a nonrandomized trial; there will be no randomization considerations in this trial.

Primary Objective:
1) To determine DFS at two years after matched sibling transplant using BM after a conditioning regimen consisting of distal timed Alemtuzumab, Fludarabine, and Melphalan for patients 2-30 y/o

The composite endpoint consists of death and graft rejection.

Secondary objectives:

10) Overall survival
11) Rate of neutrophil and platelet engraftment for BM vs. UCB
12) Incidence of graft failure
13) Incidence of grade II-IV and grade III-IV acute GVHD
14) Incidence of chronic GVHD
15) Incidence of other transplant complications, such as veno-occlusive disease, CNS toxicity, and idiopathic pneumonia syndrome
16) Incidence of reactivation of CMV, EBV, adenovirus, BK/JC virus
17) Incidence of invasive fungal disease
18) Time to immune reconstitution via monitoring of lymphocyte subpopulations and immunoglobulin levels

Sample Size and power calculations.

This study will enroll 20 patients. Sample size calculation was not performed as this study is exploratory in nature. Given the small size of the study, we do not expect to be able to obtain appropriate power, which is appropriate as this is a pilot study.

Demographic and Baseline Characteristics

All demographics and characteristics that are continuous will be summarized by means (SD) or median (interquartile range) depending on whether or not the data demonstrate evidence of having come from a normal distribution, as validated by the Shapiro-Wilk test. All categorical data will be presented as a frequency (%). The variables will include age, gender, performance status, disease symptoms, serum bilirubin level, serum creatinine level, pulmonary function test, cardiac function test, donor age, donor gender, cerebral MRI, serum ferritin, and immune reconstitution baseline characteristics.

Analysis of the Primary Endpoint:

The DFS at the 2 year endpoint will be estimated using the Kaplan Meier product limit estimator. The frequencies of the events, i.e. graft failure, will be enumerated and presented.

Analysis of Secondary endpoints:

Overall survival: The distribution of time to death from any cause will be estimated by Kaplan- Meier product limit function and plotted. The overall survival will be measured from the time of transplant to any death and patients will be followed for 2 years.

Rate of neutrophil and platelet engraftment for BM: To assess the incidence and rate of each type of engraftment for bone marrow, cumulative incidences curve will be plotted. Any death occurring before each type of engraftment will be considered a competing risk.

Incidence of graft failure: The incidence of graft failure will be assessed by cumulative incidence function and the corresponding curve will be plotted. Any death occurring before the onset of graft failure will be considered as a competing risk.

Incidence of grade II-IV and grade III-IV acute GVHD: The incidence of grade II-IV or grade III-IV acute GVHD will be assessed from the day of transplant to the first acute GVHD onset using cumulative incidence function and corresponding curve will be plotted. Any death occurring before each type of GVHD will be considered a competing risk.

Incidence of chronic GVHD: The incidence of chronic GVHD from day of transplant will be assessed by a cumulative incidence curved estimated by cumulative incidence function. Any death occurring before the onset of chronic GVHD will be considered as a competing risk.

Incidence of other transplant complications, such as veno-occlusive disease, CNS toxicity, and idiopathic pneumonia syndrome
The incidence of other transplant complications (veno-occlusive disease, CNS toxicity, and idiopathic pneumonia syndrome) from day of transplant will be evaluated by a cumulative incidence curve estimated from the cumulative incidence function. Any death occurring before onset of each of these complications will be considered a competing risk.

Incidence of reactivation of CMV, EBV, adenovirus, BK/JC virus
To assess the incidence of reactivation of CMV, EBV, adenovirus and BK/JC virus, cumulative incidence function will be estimated and plotted. Any death occurring before onset of each of these reactivations will be considered a competing risk.

Incidence of invasive fungal disease: To assess the incidence of reactivation of fungal disease, cumulative incidence function will be estimated and plotted. Any death occurring before onset of each of these reactivations will be considered a competing risk.

Time to immune reconstitution via monitoring of lymphocyte subpopulations and immunoglobulin levels: To assess time to immune reconstitution, lymphocytes subsets B and T cells) and immunoglobulin levels measured at the five time points (Pre-transplantation, Day +100, Day +180, Day +365 and Day 366+) will be presented using boxplots for each treatment group.

Stopping Guidelines and interim analysis

In order to formulate the stopping guidelines, assumptions are based on acceptable rates of the overall mortality by Day 100 and graft rejection by Day 100

2.1 Overall Mortality

The overall mortality at 100 days is anticipated to be ≤10%. When the 100 day an overall mortality rate exceeds 10% based on a truncated SPRT, the stopping rule will be triggered in arm with 10 patients. The stopping rule is summarized in the Table 2.1.2

Table 2.1.2 Stopping Rule for Overall Mortality By Day 100 in Related Donor Matched Patients

Stage *k*	Number of Evaluable[1] Patients Accrued in k^{th} *Stage*	Number of Deaths
1	{1-10}	2

[1] Evaluable patients are those who proceed to transplant.
If the number of deaths out of patients accrued at any stage is equal to the corresponding value in the 3 rd column, then the trial will be halted.

2.2 Graft Rejection

We will describe the stopping guideline for failure to engraft donor cells by Day 100. It is assumed that the rate of occurrence of this toxicity marker is < 20% in both unrelated and related donor matched patients.

When the 100 day graft rejection rate exceeds 20% based on a truncated SPRT, the stopping rule will be triggered in the10 patient arm. The stopping rule is summarized in the Table 2.2.

Table 2.2 Stopping Rule for Graft Rejection By Day 100 in Related BMT Patients

Stage *k*	**Number of Evaluable[1] Patients Accrued in *k*th *Stage***	**Number of Graft Rejections**
1	{1-10}	3

[1] Evaluable patients are those who proceed to transplant.
If the number of deaths out of patients accrued at any stage is equal to the corresponding value in the 3 rd column, then the trial will be halted.

