



## Unrelated Donor Reduced Intensity Bone Marrow Transplant for Children with Severe Sickle Cell Disease

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### BMT CTN PROTOCOL 0601 Version 11.0

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**PROTOCOL SYNOPSIS – BMT CTN PROTOCOL 0601****Unrelated Donor Reduced Intensity Bone Marrow Transplant for Children  
with Severe Sickle Cell Disease**

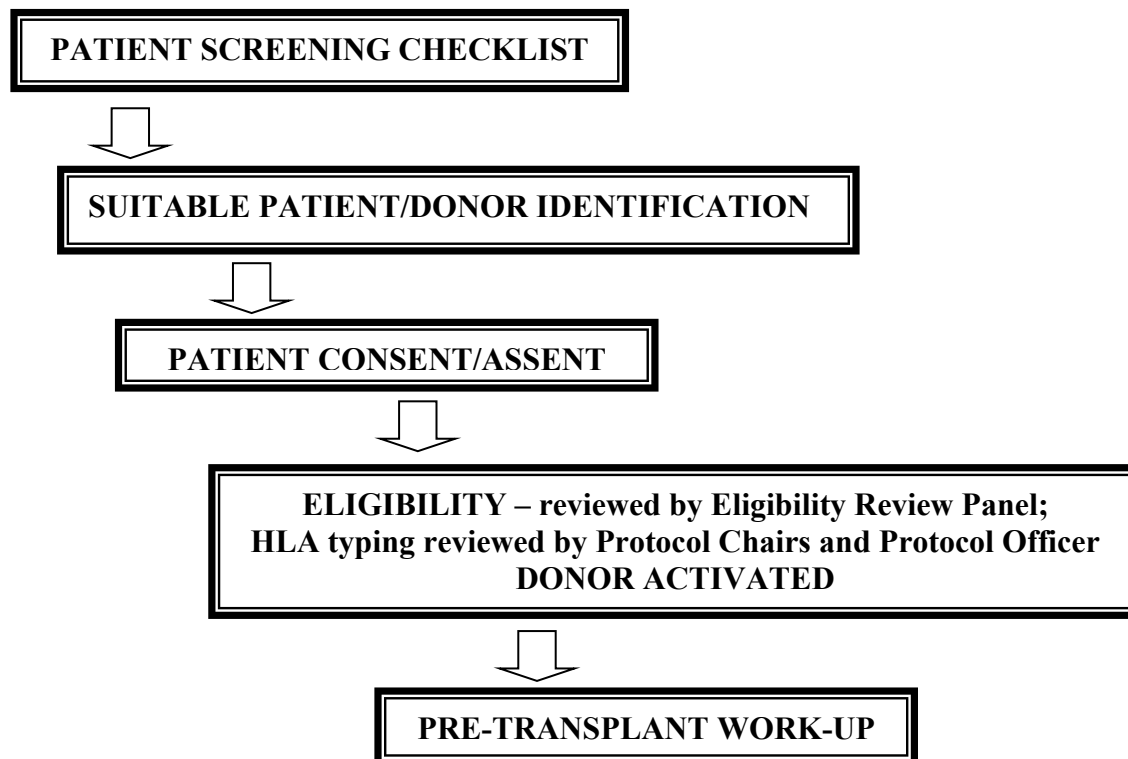
<b>Study Chairpersons:</b>	Shalini Shenoy, M.D., Naynesh Kamani, M.D.
<b>Primary Objective:</b>	The primary objective is to determine event-free survival (EFS) at 1 year after unrelated donor (URD) hematopoietic stem cell transplantation (HCT) using bone marrow (BM) in patients with sickle cell disease (SCD). Death, disease recurrence or graft rejection by 1 year will be considered events for this endpoint.
<b>Secondary Objectives:</b>	Secondary objectives include determining the effect of HCT on clinical and laboratory manifestations of severe sickle cell disease including stroke and determining the incidence of other transplant-related outcomes. The latter include: overall survival; neutrophil, and platelet recovery; grades II-IV and grade III-IV acute graft-versus-host disease (GVHD); chronic GVHD; hepatic veno-occlusive disease (VOD); idiopathic pneumonia syndrome (IPS); central nervous system (CNS) toxicity (reversible posterior leukoencephalopathy syndrome [RPLS], hemorrhage, and seizures); neurocognitive dysfunction; cytomegalovirus (CMV) infection; adenovirus infection; Epstein Barr virus infection; invasive fungal infection; immune reconstitution; and health-related quality of life (QOL).
<b>Study Design:</b>	The study is a Phase II, single arm, multi-center trial. It is designed to estimate the efficacy and toxicity of unrelated donor HCT using a reduced-intensity conditioning regimen in patients with SCD and high risk features who are between 3.0 and 19.75 years of age.
<b>Accrual Objective:</b>	The target sample size is 30 BM recipients.
<b>Accrual Period:</b>	The estimated accrual period is 4 years.
<b>Eligibility Criteria:</b>	Patients 3.0-19.75 years old with symptomatic SCD AND one or more of the following complications: (a)-(i) a clinically significant neurologic event (stroke) or any neurologic defect lasting > 24 hours and accompanied by an infarct on cerebral magnetic resonance imaging (MRI); OR, (a)-(ii) patients who have a TCD velocity that exceeds 200 cm/sec by the non-imaging technique (or TCD measurement of >185 cm/sec by the imaging technique) measured at a minimum of 2 separate occasions one month or more apart; OR, (b) Minimum of two episodes of acute chest syndrome within the preceding 2-year period defined as new pulmonary alveolar consolidation involving at least one complete lung segment (associated with acute symptoms including fever, chest pain,

tachypnea, wheezing, rales, or cough that is not attributed to asthma or bronchiolitis) despite adequate supportive care measures; OR, (c) History of 3 or more severe pain events (defined as new onset of pain that lasts for at least 2 hours for which there is no other explanation) per year in the 2 years prior to enrollment despite adequate supportive care measures (if patients are receiving hydroxyurea and compliant with therapy, being symptomatic is an indication for transplantation; however, if patients decline hydroxyurea or non-compliant with this therapy, they would still remain eligible for study if pain criteria as described above are met). Lansky/Karnofsky performance score must be  $\geq 40$ . Hb S must be  $\leq 45\%$ . Patients must have an unrelated adult bone marrow donor who is HLA-matched at 8 of 8 HLA-A, -B, -C and -DRB1 at high resolution using DNA-based typing. Patients with bridging fibrosis or cirrhosis of the liver, with uncontrolled bacterial, viral, or fungal infection in the past month, or seropositivity for HIV are excluded. Patients with HLA-matched family donors, or who have received prior HCT, and females who are pregnant or breast feeding are excluded.

- Treatment Description:** The HCT preparative regimen will consist of the following:
- Alemtuzumab: Children weighing 10 kg or more will receive 10 mg, 15 mg, 20 mg intravenously (IV) on Days -21, -20, and -19, respectively
  - Fludarabine: 30 mg/m<sup>2</sup>/day IV on Days -8 through -4
  - Melphalan: 140 mg/m<sup>2</sup> IV on Day -3
  - Rest on Day -2, -1
  - Day 0 is the day of transplant
  - GVHD prophylaxis: Tacrolimus or cyclosporine beginning Day -3, methotrexate (7.5 mg/m<sup>2</sup>/day) Day 1, 3, and 6 and methylprednisolone/ prednisone on Day +7 to +28 followed by a taper if there is no GVHD
- Study Duration:** Patients will be followed for two years post-transplant for evaluation.

## TREATMENT SCHEMA

(see Section 4.0 for enrollment procedures)



Day	Treatment <sup>1</sup>
24 hours prior to 1 <sup>st</sup> dose of Alemtuzumab	Alemtuzumab test dose 3 mg IV once
-22	Alemtuzumab 10 mg IV <sup>1</sup> Alemtuzumab 15 mg IV <sup>1</sup> Alemtuzumab 20 mg IV <sup>1</sup>
-21	
-20	
-19	
-18	Fludarabine 30mg/m <sup>2</sup> IV
-8	
-7	
-6	
-5	
-4	Fludarabine 30mg/m <sup>2</sup> IV
-3	Melphalan 140 mg/m <sup>2</sup> IV
-2	Rest
-1	Rest
0	Stem cell infusion
+7	G-CSF 5 µg/kg/day continue until neutrophil engraftment

<sup>1</sup> Alemtuzumab doses may be administered between Days -22 and -18 but are required to be on 3 consecutive days.

**TREATMENT SCHEMA (cont'd)****GVHD Prophylaxis Regimens**

<b>Day</b>	<b>Regimen</b>
-3	Tacrolimus or cyclosporine dosed to maintain appropriate levels. Given through Day 100 then taper to Day 180
0	Stem cell infusion
+1	Methotrexate 7.5 mg/m <sup>2</sup> IV
+3	Methotrexate 7.5 mg/m <sup>2</sup> IV
+6	Methotrexate 7.5 mg/m <sup>2</sup> IV
+7	Methylprednisolone 1.0 mg/kg/day IV or Prednisone 1.2 mg/kg/day PO in divided doses Continued through Day +28, then taper

**TWO-YEAR FOLLOW-UP**

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## CHAPTER 1

### 1. BACKGROUND AND RATIONALE

Sickle cell disease (SCD) is a recessive genetic disorder caused by a point mutation that results in the substitution of valine for glutamic acid at the sixth position in the  $\beta$ -chain of hemoglobin. The homozygous gene disorder affects approximately 1 in every 400 African American newborns and an estimated 70,000 persons in the United States. A major cause of morbidity and mortality in severe SCD is vaso-occlusion, often causing irreversible damage in target organs such as the central nervous system (CNS), lung, bone, and joints. Sequelae of this genetic defect can occur in any organ. The most devastating result is cerebral infarction.<sup>1</sup> Other serious complications of SCD include restrictive pulmonary disease secondary to recurrent acute chest syndrome (ACS), pulmonary hypertension, and severe vaso-occlusive painful crises. These complications predict for early mortality, recurrent sickle-related complications such as ACS, and poor quality of life.<sup>2, 3, 4, 5</sup> The average life expectancy for male patients with SCD is 42 years, and 48 years for females.<sup>6</sup> Among patients with Hb SS and S- $\beta$  thalassemia, the overall rates of death and stroke were 0.59 and 0.85/100 patient-years. The cumulative overall and stroke-free survival decreases to 85.6% and 88.5% by 18 years of age.<sup>7</sup>

Children with SCD may have impaired motor and/or cognitive function because of cerebral infarction manifesting as overt or silent strokes. Overt strokes occur in approximately 9% of patients with sickle cell disease before their 14<sup>th</sup> birthday.<sup>8</sup> Silent cerebral infarctions, defined by abnormal and increased signal intensity in T2 weighted images but with no history or physical findings of focal neurologic deficits lasting longer than 24 hours, are another common form of neurologic injury in children with SCD. By 14 years of age, 18% of children with sickle cell anemia (Hb SS) have silent cerebral infarcts.<sup>9</sup> Silent cerebral infarcts are associated with an increased risk of overt stroke, lower IQ, and poor academic achievement.<sup>10, 11, 12, 13</sup>

Regular blood transfusion is less than optimal for prevention of recurrent strokes. Scothorn et al. identified 137 children with a stroke that were followed for a mean and median of 10 years.<sup>14</sup> Participants were included if they had continuous blood transfusion therapy throughout the observation period with a maximum transfusion interval of less than six weeks. Despite regular blood transfusion therapy, approximately 20% (1 in 5) developed a second stroke, and of that group, 30% (1 in 3) had a third stroke. In the subgroup group of patients that had second or third strokes, the hemoglobin S levels were documented to be less than 30%. Given the high rate of second and third strokes, even with optimal transfusion therapy, stem cell transplant for this high-risk population is a therapeutic option that may provide benefit over transfusions. Improved cerebrovascular patency following HCT has been previously described in SCD.<sup>15</sup>

Other complications of sickle cell disease associated with increased morbidity and/or mortality and/or poor quality of life include severe, persistent, and recurrent painful episodes, recurrent pulmonary vaso-occlusion resulting in progressive pulmonary changes, and the development of pulmonary hypertension. For these co-morbid conditions, the natural history is not well defined but there is a significant risk of progression and early mortality, especially if they remain

unresponsive to hydroxyurea therapy and supportive care measures such as therapy for asthma.<sup>16</sup> This is another subset of patients that could benefit from successful HCT.

The majority of patients with severe disease manifestations and organ damage have Hb SS or Hb S- $\beta^0$  thalassemia. However, clinical severity of the disease is dependent on several factors such as leukocyte and platelet counts, non-globin gene modifiers, Hb S polymer fractions, and the level of reduction of beta chain synthesis.<sup>17, 18, 19, 20</sup> This results in a severe disease phenotype in some patients with sickle cell disease variants such as Hb S- $\beta^+$  thalassemia, Hb SC, etc. Since the pathology and progression of disease in these patients is comparable with those with severe Hb SS disease, it is logical to consider the curative option of stem cell transplantation in all patients with Hb S variant disease that manifest a severe phenotype.

### **1.1. Role of Hematopoietic Cell Transplantation**

Hematopoietic Cell Transplantation (HCT) is the only curative treatment currently available for patients with SCD and can benefit patients who are likely to have significant morbidity and early mortality from the disease.<sup>21, 22</sup> Limitations of HCT include the lack of HLA-identical family donors for most patients, the risk of mortality or treatment related toxicities especially in the unrelated donor setting, and increased complications and mortality following HCT in young adult SCD recipients.<sup>23, 24, 25</sup> Another problem is the absence of a reliable method of defining high-risk patients, for whom the benefits of HCT outweigh the risks associated with the procedure before they develop serious irreversible SCD complications. This is important because in patients on chronic therapy and supportive care for SCD manifestations, the success of HCT is compromised by organ damage and exposure to multiple transfusions.<sup>26, 27, 28</sup> A study of alternative donor HCT in patients with SCD should hence involve careful patient selection and a safe transplant conditioning regimen leading to reliable donor cell engraftment while minimizing treatment-related toxicity and mortality. In addition, avoiding late effects of HCT, such as infertility, needs to be considered when proposing HCT for disorders that may not be immediately life-threatening.

### **1.2. Toxicities of Myeloablative Conditioning Regimen**

Myeloablative therapy facilitates durable engraftment of donor cells after HCT but is limited by toxicities of conditioning drugs and transplant-related complications. These include early and late organ toxicities of individual agents and depend on the age of the recipient and the combination of chemotherapy and/or radiotherapy applied.<sup>29</sup> Since graft failure is a major barrier especially in immunocompetent and extensively transfused recipients, transplantation in this setting has relied upon myeloablative conditioning to achieve donor cell engraftment.

Commonly used myeloablative conditioning regimens contain high doses of total body irradiation (TBI) or busulfan and another alkylating agent. In addition to prolonged myelosuppression, acute toxicities include gastrointestinal (mucositis, diarrhea, nausea, vomiting, anorexia), genitourinary (renal, hemorrhagic cystitis), cardiac, cutaneous (erythema, desquamation, hyperpigmentation), CNS (seizures, hemorrhage, encephalopathy), pulmonary, and hepatic (VOD) manifestations.<sup>30</sup> Organ toxicities are more likely and more severe in

patients who have impaired organ function before HCT. Late toxicities include chronic pulmonary dysfunction, endocrine insufficiency, gonadal failure and sterility, learning disorders, neurocognitive deficits, and chronic alopecia.<sup>31, 32, 33, 34, 35</sup>

Patients receiving non-transplant therapy for SCD are at risk for disease- or therapy-related organ complications such as hepatotoxicity or cardiotoxicity from transfusional iron overload and inadequate chelation, pulmonary fibrosis or pulmonary hypertension, renal damage, and neurotoxicity/hypertension/seizure disorder as a consequence of sickle neurovascular disease. These toxicities render many patients who might benefit from HCT ineligible or more susceptible to transplant related toxicities. The risk of conditioning regimen-related toxicity in these patients must be balanced with the risk of graft rejection due to inadequate host immunosuppression when transplant conditioning options are considered.<sup>36</sup>

### **1.3. Bone Marrow as a Source of Hematopoietic Stem Cells**

Only 14% of patients with SCD are likely to have a HLA-identical sibling donor.<sup>24</sup> Use of HLA compatible unrelated donors is necessary to pursue HCT as a therapeutic option in most patients with SCD. Donors who are matched at 6 (HLA-A, B and DRB1 loci) to 10 HLA-antigens (including HLA-C and DQB1 loci) can be identified via the National Marrow Donor Program in approximately 80% of Caucasian recipients; however, the likelihood of identifying a similarly HLA-matched URD is less likely in other ethnic groups due to under-representation of these groups in the volunteer donor pool and greater genetic diversity of some groups, particularly African-Americans.<sup>37</sup> Identifying a suitable donor is a difficult problem in HCT for SCD and can limit the application of HCT for those who might benefit.<sup>38</sup>

Treatment related mortality (TRM) and the incidence and severity of acute and chronic GVHD depend on the level of HLA matching especially in the unrelated donor setting. The incidence of grade II-IV acute GVHD (within the initial 100 days after HCT) ranges from 10-50% after HLA-identical sibling bone marrow transplantation.<sup>39</sup> The incidence of chronic GVHD ranges between 60-80% in long-term survivors of unrelated HCT. As expected, GVHD rates are higher after unrelated donor HCT, dependent on the degree of HLA mismatch.<sup>40, 41</sup> GVHD rates also vary with age, conditioning therapy, stem cell source, prior transfusions, GVHD prophylaxis, infections, etc. Since HCT outcomes appear to be optimal when bone marrow donors are HLA-matched (at the allele level) at 8 loci (A, B, C and DRB1), this level of matching will be adopted for this study.<sup>42</sup> The recent Health Resources and Services Administration (HRSA) report from 2006 suggests that 36.1% of African American patients will be able to locate a donor matched for 8/8 HLA alleles in the unrelated donor registry (personal communication – D. Confer).

### **1.4. HCT for Severe Sickle Cell Disease**

Allogeneic HCT for SCD following myeloablative conditioning ensures donor cell engraftment and can ameliorate SCD symptoms, improve quality of life, and stabilize vasculopathy. In a multicenter trial of BM transplantation from HLA-identical sibling donors, 59 children with symptomatic SCD received a myeloablative preparative regimen of busulfan, cyclophosphamide, and ATG or Campath-1G before transplantation.<sup>43</sup> Ninety-two percent of children had stable

engraftment. Thirteen of these had stable mixed chimerism (11-90% donor cells); all had a normal hemoglobin levels (11.2 to 14.2 g/dl). Hb S levels ranged from 0-7% when donor chimerism ranged between 11-74% (normal donors) and 36-37% when donor chimerism ranged between 25-60% in donors with SS trait. Stable donor engraftment (whether associated with complete or mixed chimerism) resulted in resolution of symptoms of vaso-occlusive crisis (VOC), stroke and acute chest syndrome. Thus, though 1 in 5 patients developed stable mixed donor-host hematopoietic chimerism, the majority of these patients continue to survive free of SCD symptomatology. The overall survival was 94% and the event-free survival was 85%. A similar study by a French group demonstrated the need for immune ablation to achieve successful donor cell engraftment.<sup>44, 45</sup> Graft failure rates were significantly lower after the addition of rabbit anti-thymocyte globulin (rATG) to a myeloablative combination of busulfan and cyclophosphamide. TRM was 10% and DFS was 85%. Thus, HLA identical sibling HCT has acceptable results and ameliorates disease symptoms, especially in children. Of significance, 5 of 7 female recipients in the first study developed ovarian failure after exposure to myeloablative BU dosing.<sup>46</sup> Sterility and other late effects of myeloablative conditioning regimen remain of concern in transplants for children with non-malignant disorders where the preparative regimen is used solely to achieve stable donor engraftment.

Related donor UCB transplantation for hemoglobinopathies has also been successful. Locatelli et al reported outcomes in 44 children with sickle cell disease (N=11) or thalassemia major (N=33) who received myeloablative doses of busulfan, cytoxan +/- ATG, or busulfan, cytoxan/fludarabine and thiotepea.<sup>47</sup> The median number of TNC infused was  $4.0 \times 10^7/\text{kg}$  (range  $1.2\text{-}10 \times 10^7/\text{kg}$ ). No patient died of transplant-related complications. Thirty-six of 44 recipients remained disease-free after HCT, with a median follow-up of 2 years. Eight patients had graft failure, mainly in the thalassemia cohort and 3 successfully underwent a second HCT. The two-year EFS rates were 79% for thalassemia and 90% for SCD. Graft failure rates were lower after intensifying the conditioning regimen with the addition of thiotepea and eliminating methotrexate from the GVHD prophylaxis.

Although sibling donor HCT for SCD and thalassemia major has demonstrated the effectiveness of this treatment, the approach is only feasible if a suitable donor is available. Consequently, unrelated donor transplant strategies must be designed to explore methods of safely treating patients with hemoglobinopathies by HCT utilizing alternative donors/graft types with acceptable rates of early and late toxicities.

### **1.5. Non-myeloablative or Reduced-intensity Conditioning Regimens: Rationale and Clinical Experience**

Myeloablative conditioning ensures engraftment of donor cells in most HCT recipients. However, reduced intensity conditioning is attractive due to its potential for reduced toxicity. Since stable mixed chimerism is sufficient to ameliorate symptoms of the underlying disease in disorders such as SCD and thalassemia, it is possible that reduced intensity conditioning might be effective even if it results in stable chimerism.

Murine and canine transplantation models demonstrate that myeloablation is not necessary to achieve donor engraftment; suppression of host lymphocytes is adequate to permit this process. This approach could have the following potential advantages: 1) lower regimen-related toxicity, both early and late; 2) a shorter period of neutropenia; 3) shorter hospitalizations; and 4) consideration of transplantation in some recipients with pre-existing disease-related end organ dysfunction.<sup>48</sup>

The experience of HCT utilizing ‘minimally ablative’ conditioning regimens is largely limited to malignant disorders, especially in elderly or high-risk patients, and has been successful in achieving stable engraftment of donor cells in that setting.<sup>49</sup> Several groups have observed graft rejection in most hemoglobinopathy patients after administration of a minimal toxicity regimen of fludarabine, low dose busulfan or TBI, and ATG before HCT, presumably due to applying this approach in immunocompetent, transfusion-sensitized recipients.<sup>50, 51</sup> In one study, 6 of 7 recipients experienced graft rejection when post-grafting immunosuppression was discontinued after a preparative regimen of 200 cGy TBI and fludarabine, and none of the 7 had stable engraftment of donor cells.<sup>52</sup> Exposure to minor histocompatibility antigens after RBC transfusions has also been implicated as a cause of graft failure.<sup>28</sup> Further, two patients in another report died of GVHD and infection. A successful cord blood transplant has been reported recently with an intensely immunosuppressive approach.<sup>53</sup> Reduced intensity transplants with busulfan or melphalan with fludarabine have also demonstrated a high incidence of GVHD similar to that predicted in myeloablative regimens.<sup>54, 55, 56</sup>

Reduced intensity regimens vary substantially in the levels of myeloablation and immune suppression achieved. It is clear that if a reduced intensity regimen is to be successful, a high level of immunoablation is necessary for successful donor engraftment following transplantation for non-malignant disorders such as hemoglobinopathies. Further, GVHD offers no benefit in this group (in contrast to patients with malignancies where GVHD-associated anti-cancer effects mitigate the recurrence risk), and strategies to minimize the incidence of GVHD while ensuring engraftment would optimize outcomes after HCT.

## **1.6. Objectives and Experience with the Conditioning Regimen Proposed**

A reduced intensity conditioning regimen consisting of alemtuzumab, fludarabine, and melphalan was developed in 2001 as a multi-institutional study for non-malignant disorders. The primary goal of this study was to determine if intensive host immunosuppression was sufficient for donor cell engraftment while minimizing conditioning-related toxicities and TRM in pediatric non-malignant disorders. Reducing GVHD was a secondary end point of the study. Hematopoietic stem cell sources included marrow, peripheral blood, and UCB.

There is previous experience with the use of fludarabine and melphalan combinations in reduced intensity transplant regimens.<sup>55, 57</sup> Alemtuzumab, a humanized monoclonal antibody against CD52, targets an antigen expressed predominantly on the surface of lymphocytes and macrophages.<sup>58</sup> The purpose of alemtuzumab administration in the trial was to deplete recipient lymphocytes that cause graft rejection. In addition, macrophage depletion by alemtuzumab might also modulate GVHD by decreasing donor antigen presentation. This conditioning

regimen was based in part on the observations of Chakraverty, et al where the combination of alemtuzumab (following a contracted time course of administration nearer the stem cell infusion) with fludarabine and melphalan was administered in 44 patients with malignant disorders immediately before HCT and resulted in a low rate (2 of 44 patients) of graft failure and a very low frequency of acute (3/44) and chronic GVHD (0/44).<sup>59</sup> Alemtuzumab was commenced 4-8 days before HCT in that study. Because it has a serum half-life of 42-57 hours, its presence in the recipients' serum early post-transplant was probably available to bind donor cells and reduce the GVHD risk. In this group, early TRM was 14.9%, and graft failure was 4.5%.

There are two disadvantages to the approach described above. Elimination of donor T cells by exposure to high levels of alemtuzumab potentially favors graft failure in an immunocompetent host. Pharmacokinetic studies show that a concentration of up to 0.5µg/mL of alemtuzumab can be detected by indirect immunofluorescence through 56 days after HCT when 100 mg alemtuzumab is given between Days -8 and -4.<sup>60</sup> Persistence of the antibody, with accompanying donor immunodepletion, also results in delayed immune reconstitution and increased risk of late infections and infection-related mortality.<sup>61, 62, 63</sup> Hence, we modified the alemtuzumab dosing and administration schedule, moving it to Days -21, -20, and -19, well before infusion of donor cells, to reduce the level and duration of exposure of the infused donor cells to the antibody.

Initial experience with this modified conditioning regimen has been published.<sup>64 65</sup> To date, 60 pediatric patients between the ages of 2 months and 20 years were treated by HCT with this regimen. Four recipients received second allografts after experiencing a graft rejection following an initial myeloablative or reduced intensity regimen. The children were treated by unrelated donor (33 bone marrow; 12 cord) and HLA-identical sibling donor HCT (15). One unrelated donor was matched at 9/10 HLA alleles but mismatched for a single class I antigen; another was matched at 8/10 HLA-alleles but mismatched for 2 class I antigens. Cord blood donors (all unrelated) were mismatched at 1-2 HLA antigens. The median period of follow-up is 15 months (range 1-52 months). The regimen caused little toxicity with the exception of reactions during alemtuzumab infusion. Most had only urticaria and fever that responded to pre-medication. One patient developed superficial blisters on the trunk and extremities and the last dose of alemtuzumab could not be administered. Another patient did not receive the final 5 mg because of generalized urticaria. Myeloid (ANC > 0.5x10<sup>9</sup>/L) and platelet (> 50x10<sup>9</sup>/L) engraftment occurred at a median of 13 (10-36) and 26 (12-82) days, respectively. Among 52 patients with follow-up the overall survival was 87% and event free survival was 77%.

Graft failure (defined as less than 20% donor cells in lymphoid, myeloid, or bone marrow compartments) occurred in 5% of the patients. All graft failures occurred within 9 months of transplantation. Withdrawal of immunosuppression tended to stabilize or increase donor chimerism in recipients with mixed chimerism in contrast to observations of graft rejection that occurred in patients with mixed donor chimerism treated by reduced intensity conditioning regimens. No late graft failure has occurred to date. Two patients with graft rejection had SCD and received a de-escalated dose of melphalan at 70 mg/m<sup>2</sup> as part of an effort to reduce the intensity of conditioning. Consequently, dose de-escalation of melphalan was discontinued. A

third patient had Hurler syndrome, received 140 mg/m<sup>2</sup> of melphalan, and rejected peripheral blood stem cells from a HLA-identical sibling donor.

TRM at 100 days post-HCT was 10%. Causes of TRM were: CMV disease (N=1); *Pseudomonas* sepsis (N=1); GVHD and multi-organ failure (N=1); interstitial pneumonitis (N=1); and intracranial hemorrhage (N=1). The following changes to the protocol were instituted after consideration of these events: 1) exclusion of patients with invasive infections within a month prior to commencing treatment; 2) weekly monitoring and therapy for CMV infection; 3) maintaining higher platelet count after HCT; and 4) the addition of methotrexate to the GVHD prophylaxis for UCB transplants. Acute GVHD (grades II-IV) developed in 15% and chronic GVHD in 11% of patients. The onset of chronic GVHD was 6-9 months post-transplant. Post-transplant complications were predominantly bacterial and viral infections, most frequent in the first 3 months following HCT. No major infections were observed beyond 6 months post-transplant unlike other alemtuzumab-based transplant protocols; this is perhaps due to early immune reconstitution following early administration of alemtuzumab.<sup>66, 67</sup>

Ten patients with hemoglobinopathies (3 with thalassemia and 7 with SCD) were treated by HCT with this regimen. There was no significant regimen-related toxicity and no treatment related mortality. Indications for HCT among SCD patients were stroke and severe VOC, and all patients were receiving chronic RBC transfusions prior to HCT. Two of the 10 patients received a reduced dose of melphalan (70 mg/m<sup>2</sup>) and experienced graft failure as noted above. The remaining 8 patients received a melphalan dose 140 mg/m<sup>2</sup>. Five received bone marrow from HLA-identical sibling donors, and 2 received URD BM grafts. One URD marrow donor was matched at 8/8 HLA loci, and the other was matched at 6 of 8 HLA loci. The remaining patient received an unrelated UCBT that was matched at 5/6 HLA loci. With median follow-up of 9.5 months (range 1.5 to 44 months), all 8 who received full-dose melphalan have engraftment of donor cells. No patient developed acute GVHD; one patient developed chronic extensive GVHD of the skin.

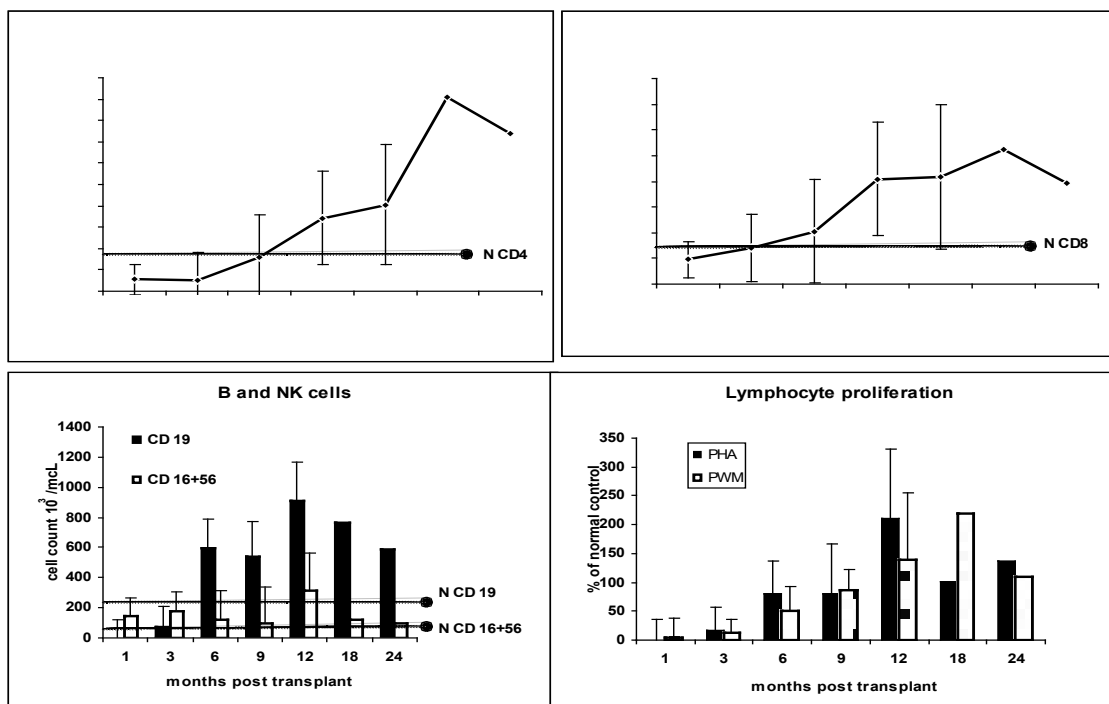
Based upon the preliminary experience of HCT for hemoglobinopathies in this study, it appears that the conditioning regimen is safe in most patients, and there were no deaths related to transplantation in this small series. Several additional patients who were treated with this regimen also had received chronic transfusion therapy (for Evan syndrome, congenital dyserythropoietic anemia type I, and aplastic anemia) before HCT and all had stable engraftment of donor cells. Of interest, an adolescent female had an uneventful pregnancy 9 months after HCT, and delivered healthy twins, which suggests that it is possible to preserve fertility after transplantation with this reduced intensity regimen. Together, these preliminary data support the development of a larger investigation to determine the risks and benefits of unrelated donor HCT for sickle cell disease, adapting this approach in selected subjects with severe disease.

### **1.7. Immune Reconstitution**

Lymphocyte and NK cellular recovery was monitored after HCT. NK cells recovered 3 months after HCT, temporally followed by CD8<sup>+</sup> T cells, B cells, and finally CD4<sup>+</sup> T cells between 9

and 12 months. Lymphocyte proliferation, immunoglobulin levels, and tetanus toxoid antibody titers mirrored lymphocyte recovery.<sup>64</sup> Immunizations were resumed at 1 year.

**TABLE 1.7: IMMUNE RECONSTITUTION KINETICS POST-TRANSPLANT**



### 1.8. Alemtuzumab Pharmacokinetics

In 10 patients, serum alemtuzumab levels were measured serially by an ELISA test that is currently the most sensitive method of analyzing serum levels (BioAnaLab, UK). Peak levels were determined on the last day of administration of alemtuzumab (Day -19), and repeated on Days 0, 30, 100, and 120. Peak levels ranged between 89.68 and 698.95 ng/mL on Day -19. The weights of the patients ranged from 9 kg to 34.5 kg. It should be noted that alemtuzumab levels were undetectable on Day 0 irrespective of weight in 8 patients who had received no previous immunosuppressive therapy. One patient with IPEX syndrome, who was receiving extensive immune suppressive therapy before HCT, had levels of 206 and 220 ng/mL on Day 0 and 30 respectively. Levels were undetectable (< 31.25 ng/mL) on Day 100. Another patient with Wiskott-Aldrich syndrome had levels of 623.54 and 226.40 ng/mL on Days 0 and 30 respectively. Levels were undetectable on Day 100. Both these recipients have stable mixed donor chimerism (50 and 51%) and completed immunosuppressive therapy > 6 months ago. Thus, despite dosing alemtuzumab similarly for all patients (with a different dosing regimen for those less than and  $\geq$  10 kg), the drug was eliminated by Day 30 in the absence of previous immune suppression or immune deficiency. This is due to uniform binding of the drug to CD52+ cells irrespective of age and weight and subsequent elimination. In view of these findings, we propose to use a similar alemtuzumab dosing regimen in this study.

### **1.9. Risk of HCT Related Complications**

Treatment-related mortality after HCT is influenced by the conditioning regimen, the hematopoietic cell source, development of post-transplant infections, and development of GVHD. The risk of TRM is minimized after HLA-identical sibling HCT, especially in non-malignant hematologic disorders. Delayed engraftment after unrelated and HLA-mismatched UCB transplantation is associated with higher TRM within the first 100 days (34-36%).<sup>45</sup> TRM following unrelated HCT with HLA-matched BM as the stem cell source is comparable to that after HLA-matched or 1-HLA antigen mismatched UCBT. In an attempt to minimize the risk of TRM, the proposed study incorporates a plan for optimal donor selection, careful post-HCT safety monitoring, prophylaxis against infections, early therapy for infectious complications, and SCD-specific supportive care. Nevertheless, there will be a period of profound immune suppression until engraftment and immune reconstitution occurs, during which time the risk of opportunistic infections will be increased. Stopping rules are in place to suspend the trial and evaluate results in the event that there is an unacceptable rate of graft rejection, severe acute GVHD, and treatment-related mortality.

The conditioning regimen is designed to minimize the risk of organ toxicity. If successful, this approach will provide a much needed curative option for patients with SCD who currently receive lifelong supportive care and transfusion therapy, and have a risk of recurrent complications (stroke, acute chest syndrome, and pain crisis), poor quality of life, and a shortened life span. This conditioning approach may also preserve fertility.

### **1.10. Health-related Quality of Life**

Health-related quality of life (QOL) is a measure of a patient's well being from a physical, emotional, and social perspective. It is a valid patient reported outcome that measures the impact of disease, morbidity from disease, and treatment of disease on the well being and functioning of the patient and family. Health-related QOL is traditionally measured at multiple intervals to track impact of treatment and the natural history of the disease. It will be used in this setting to determine the impact of HCT on the well being of the child.

The Child Health Questionnaire (CHQ) is a widely used instrument to measure health-related QOL that has been validated in children with sickle cell disease and in children with other chronic diseases.<sup>68</sup> More importantly, it has also been shown to be sensitive to change in disease status over time.<sup>69</sup> It is designed to be self-completed by both parents and children 10 years of age and older. See Appendix F for details on QOL measurement.

### **1.11. Neurocognitive Testing**

Neurocognitive testing measures higher neurologic functions such as those listed below. Patients with sickle cell disease can have damage to brain blood vessels that can result in brain parenchymal damage in the form of an overt or silent stroke, and that this damage is progressive.<sup>70</sup> A major manifestation of silent stroke is deterioration of neurocognitive function, and there is evidence too that the decline in neurocognitive function is also progressive over time.<sup>71</sup> Reduced intensity conditioning as is proposed in this study is designed to minimize

further neurologic toxicity from high dose chemotherapy. The aim of HCT is to stabilize disease related neurologic toxicity after donor cell engraftment, and assess for stabilization and even improvement in neurocognitive function over time. We propose to do this by testing neurocognitive function before and two years after transplantation.

Basic measures of neurocognitive testing are proposed to monitor performance before and after transplant. The simple battery of tests described provide screening of neurocognitive abilities before and after transplant and are appropriate for administration to children and adolescents with SCD, reflecting the recipient population on this study. The age range for testing is 2.5 to young adults, with test batteries divided between 3 groups (2.5 to 3 years, 4-5 years, and 6 years and over). The testing instruments are listed below and address specific domains that are impaired in patients with SCD. Similar testing is previously reported and also underway in an international trial of chronic transfusion therapy for SCD and silent stroke and correlate with MRI changes.<sup>13, 72</sup>

1. WPPSI and Wechsler Abbreviated Scale of Intelligence (WASI): Intelligence scale.
2. BRIEF (completed by parent): Executive function.
3. VMI: Visual Motor skills.
4. CPT: Attention.
5. Children's Memory Scale (CMS): Learning, Memory (verbal/visual, recall/recognition/working, short-delay/long-delay).
6. California Verbal Learning Test (CVLTC): Verbal learning.

Each of the tests will be applied in an age dependent manner. Testing will occur prior to HCT (and before initiation of conditioning therapy) and at 2 years after HCT. This is intended to provide data on the natural history of neurocognitive function following HCT. Additional details are provided in Appendix G.

**Rationale for Choosing Neuropsychologic Tests:** The selected measures were chosen to provide optimal screening of neurocognitive abilities that may be impaired in children with SCD, especially those who have had prior stroke. Measures assessing specific cognitive domains were selected for the following characteristics: 1) appropriate for administration to children and young adults in the age ranges to be studied; 2) widely used in clinical practice; and 3) having good psychometric properties. For example, the WASI was selected because it can be administered to individuals from 6 years of age to adults, which permits assessment across school-age children of all ages without compromising study design due to changes in measures as children age. The WASI also is widely used for the assessment of intelligence, and the reliability and validity of the scale is well documented. Other tests were selected for similar reasons.

## CHAPTER 2

### 2. STUDY DESIGN

#### 2.1. Study Overview

The primary goal of this Phase II study is to determine whether a reduced intensity conditioning regimen in the setting of unrelated donor (URD) HCT is successful in achieving donor engraftment, inhibiting sickle erythropoiesis, and limiting disease-related organ toxicity in patients at high risk for morbidity and mortality associated with SCD. The donor of choice is an unrelated adult bone marrow (BM) donor who is HLA-matched at 8 of 8 at HLA-A, -B -C and -DRB1 at high resolution using DNA-based typing. The transplant conditioning regimen will include alemtuzumab, fludarabine, and melphalan. GVHD prophylaxis will consist of tacrolimus or cyclosporine in combination with short course methotrexate (MTX) and methylprednisolone/prednisone. Post-transplant supportive care will include infection surveillance and prophylaxis, and disease-specific supportive care.

#### 2.2. Hypothesis and Study Objectives

##### 2.2.1. Primary Hypothesis

A preparative regimen that provides intense host immunosuppression without myeloablation will promote stable engraftment of unrelated donor hematopoietic cells, inhibit sickle erythropoiesis, and result in an event free survival of  $\geq 75\%$  of children with SCD.

##### 2.2.2. Secondary Hypotheses

SCD patients who undergo URD HCT following the reduced intensity conditioning regimen described in this study will have stable donor-derived hematopoiesis and immune reconstitution with acceptable rates of acute and chronic GVHD and TRM. QOL following URD HCT will be superior to QOL prior to HCT. Neurocognitive function will not be adversely affected by HCT

##### 2.2.3. Primary Objective

To determine event-free survival at 1 year after URD HCT using BM grafts and a pre-transplant conditioning regimen of alemtuzumab, fludarabine, and melphalan in patients with SCD who are 3.0-19.75 years of age at time of enrollment.

##### 2.2.4. Secondary Objectives

Secondary objectives are to determine the effects of HCT on clinical and laboratory manifestations of SCD and to determine the incidence of HCT complications. Secondary outcome variables include:

1. Overall survival
2. Neutrophil and platelet engraftment
3. Graft failure
4. Grade II-IV and III-IV acute GVHD
5. Chronic GVHD
6. Transplant related complications – hepatic veno-occlusive disease (VOD), idiopathic pneumonia syndrome (IPS), and CNS toxicity (reversible posterior leukoencephalopathy syndrome [RPLS], hemorrhage, and seizures)
7. Reactivation of CMV, adenovirus, invasive fungal infection, and EBV
8. SCD related complication of recurrent stroke
9. QOL measurements
10. Immune reconstitution – lymphocyte subpopulations (absolute number of CD3, CD4, CD8, CD 16+56, and CD19 cells), immunoglobulin levels (Ig G, A, and M) and splenic function
11. Neurocognitive function and neuroimaging

## **2.3. Patient Eligibility**

### **2.3.1. Inclusion Criteria**

1. Patient is 3.0-19.75 years of age at time of enrollment.
2. Patients must have symptomatic SCD AND have 1 or more of the following clinical complications.
  - a. (i) Clinically significant neurologic event (stroke) or any neurologic deficit lasting > 24 hours that is accompanied by an infarct on cerebral MRI; OR (ii) patients who have a TCD velocity that exceeds 200 cm/sec by the non-imaging technique (or TCD measurement of > 185 cm/sec by the imaging technique) measured at a minimum of 2 separate occasions one month or more apart; OR,
  - b. Minimum of two episodes of acute chest syndrome in the preceding two-year period prior to enrollment (defined as new pulmonary alveolar consolidation involving at least one complete lung segment associated with acute symptoms including fever  $\geq 38.5^{\circ}\text{C}$ , chest pain, tachypnea per age adjusted normal, intercostal retractions/nasal flaring/use of accessory muscles of respiration, wheezing, rales, or cough that is not attributed to asthma or bronchiolitis) despite adequate supportive care measures (i.e. despite the use of supportive care and interventions including asthma therapy and/or hydroxyurea; patients who decline hydroxyurea or non-compliant with this therapy are eligible if they meet the other pulmonary criteria defined above for inclusion);<sup>73</sup>,<sup>74</sup> OR,
  - c. History of 3 or more severe pain events (defined as new onset of pain that lasts for at least 2 hours for which there is no other explanation) per year in the 2 years prior to

enrollment despite adequate supportive care measures (if patients are receiving hydroxyurea and compliant with therapy, being symptomatic is an indication for transplantation; however, if patients decline hydroxyurea or are non-compliant with this therapy, they would be considered eligible for study if pain criteria are otherwise met). Pain may occur in typical sites associated with vaso-occlusive painful events and cannot be explained by causes other than vaso-occlusion mediated by sickle cell disease.

3. A Lansky/Karnofsky performance status scale of  $\geq 40$ .
4. Patients must have an unrelated adult bone marrow donor who is HLA-matched at 8 of 8 HLA-A, -B, -C, and -DRB1 at high resolution using DNA-based typing.
5. Patients with adequate physical function as measured by
  - a. Cardiac: Left ventricular ejection fraction (LVEF)  $> 40\%$ ; or LV shortening fraction  $> 26\%$ .
  - b. Pulmonary: Pulse oxymetry with a baseline  $O_2$  saturation of  $\geq 85\%$  is required for all patients, DLCO  $> 40\%$  (corrected for hemoglobin) for patients in whom pulmonary function testing can be performed.
  - c. Renal: Serum creatinine  $\leq 1.5 \times$  upper limit of normal for age and GFR  $> 100$  mL/min/1.73 m. For patients  $\geq 16$  years of age, GFR should be  $> 70$  mL/min/1.73 m<sup>2</sup>.
  - d. Hepatic: Serum conjugated (direct) bilirubin  $< 2 \times$  upper limit of normal for age as per local laboratory; ALT and AST  $< 5$  times upper limit of normal as per local laboratory.
6. If the patient has been receiving chronic transfusion therapy for  $\geq 1$  year AND has clinical evidence of iron overload (serum ferritin level of  $> 1000$  ng/ml), a liver biopsy shall be obtained within 90 days of starting conditioning therapy (alemtuzumab). Histologic exam of the liver must document absence of bridging fibrosis or cirrhosis of the liver. In other cases, a liver biopsy is optional.
7. Hb S should be  $\leq 45\%$  7 days prior to initiation of alemtuzumab.
8. Signed informed consent.

### 2.3.2. Exclusion Criteria

1. Evidence of uncontrolled bacterial, viral, or fungal infections (currently taking medication and progression of clinical symptoms) within 1 month prior to starting the conditioning regimen. Patients with fever or suspected minor infection should await resolution of symptoms before starting the conditioning regimen.
2. Pregnant ( $\beta$ HCG +) or breastfeeding.
3. Patients with 8/8 HLA-matched related family donors able to donate.
4. Seropositive for the human immunodeficiency virus (HIV).

5. Prior allogeneic marrow or stem cell transplantation.
6. Iron chelation must be discontinued  $\geq 48$  hours before initiating the conditioning regimen.
7. Hydroxyurea (if receiving this therapy) must be discontinued  $\geq 48$  hours before initiating the conditioning regimen.

### 2.3.3. Donor Selection Criteria

Donors will be identified through the National Marrow Donor Program (NMDP) and must fulfill all of the NMDP's criteria for donation. Donors will sign an informed consent acknowledging that their donation will be used by a patient participating on this study; consent document available upon request. The target total nucleated cell count (TNC) is  $3.0\text{--}8.0 \times 10^8/\text{kg}$  of recipient weight.

## 2.4. Treatment Plan

All patients will receive the treatment regimen as shown in Table 2.4

**TABLE 2.4: SCHEMA OF CONDITIONING REGIMEN TRANSPLANTS**

Day	Treatment
24 hours prior to 1 <sup>st</sup> dose of Alemtuzumab	Alemtuzumab test dose 3 mg IV once
–22	Alemtuzumab 10 mg IV <sup>1</sup> Alemtuzumab 15 mg IV <sup>1</sup> Alemtuzumab 20 mg IV <sup>1</sup>
–21	
–20	
–19	
–18	
–8	Fludarabine 30mg/m <sup>2</sup> IV
–7	Fludarabine 30mg/m <sup>2</sup> IV
–6	Fludarabine 30mg/m <sup>2</sup> IV
–5	Fludarabine 30mg/m <sup>2</sup> IV
–4	Fludarabine 30mg/m <sup>2</sup> IV
–3	Melphalan 140 mg/m <sup>2</sup> IV
–2	Rest
–1	Rest
0	Stem cell infusion
+7	G-CSF 5 µg/kg/day continue until neutrophil engraftment

<sup>1</sup>Alemtuzumab doses may be administered between Days –22 and –18 but are required to be on three consecutive days.

The cytoreductive medications fludarabine and melphalan will be adjusted to the ideal body weight (IBW) in children weighing > 125% IBW.

The following are dose adjustment formulas:

**Ideal Body Weight Formulas for patients 1 to 18 years of age:** (ht = in, IBW = lb)

Less than 60 inches:  $IBW = (ht^2 \times 1.65)/1000$

More than 60 inches: Males  $IBW = 39.0 + [2.27 \times (ht - 60)]$

Females  $IBW = 42.2 + [2.27 \times (ht - 60)]$

Adjusted Ideal Body Weight Formula:  $AIBW = IBW + [(0.25) \times (ABW - IBW)]$   
(ABW = actual body weight)

2.4.1. Hb S Level Prior to Initiation of Alemtuzumab

Hb S level must be  $\leq 45\%$  within 7 days prior to the initiation of alemtuzumab.

2.4.2. Patients Receiving Iron Chelation Therapy Prior to HCT

Iron chelation therapy will be discontinued no later than 48 hours prior to commencement of alemtuzumab. Iron chelation therapy or a program of phlebotomy may be resumed after neutrophil and red cell engraftment at the discretion of the transplant center.

2.4.3. Patients Receiving Hydroxyurea Prior to HCT

Hydroxyurea will be discontinued no later than 48 hours prior to administration of alemtuzumab.

2.4.4. Alemtuzumab (Campath-1H)

Patients that receive > 75% of dose (e.g., 36 mg) will be evaluated for outcome. If a patient cannot receive the complete dose, the choice of conditioning regimen will be left to the institution; however, the patient must be followed per protocol.

2.4.4.1. Pre-medication

Pre-medication should be commenced 30 minutes prior to each infusion of alemtuzumab and including the test dose and should be continued for at least 48 hours after the last dose of alemtuzumab.

Recommended pre-medication include the following combination of medications:

Diphenhydramine: 1 mg/kg IV or PO q 8 hours (maximum 50 mg/dose)

Acetaminophen: 10-15mg/kg PO q 6 hours (maximum 4 grams qd)

Hydrocortisone: 1-2 mg/kg IV q 6 hours

Meperidine: 0.5 mg/kg IV q 4-6 hours may be used as needed for rigors

#### 2.4.4.2. Test dose and administration

Alemtuzumab doses may be administered between Days –22 and –18 but are required to be on three consecutive days. Alemtuzumab will be administered in-patient and the patient may be discharged the day after completion of Alemtuzumab infusions. The test dose of alemtuzumab (3 mg IV) must be administered over 2 hours and not less than 24 hours prior to administration of the first dose. It is recommended that the test dose be administered Monday through Thursday, in the morning, to allow for time for notification of the Study Chair in the case of a severe adverse reaction.

**If the test dose is not tolerated:**

If the patient has a severe or life threatening adverse reaction to alemtuzumab (e.g., severe hypotension, severe bronchospasm) the adverse event meets expedited reporting requirements (within 24 hours) through the expedited AE reporting system via AdvantageEDC<sup>SM</sup> (see Section 4.3.2). The study chair(s) may also be consulted regarding further doses at the discretion of treating physician.

**If the test dose is tolerated:**

Alemtuzumab will be diluted in 100cc of 0.9% normal saline (NS) and infused intravenously over a minimum of 6 hours each day for three consecutive days beginning between Days –22 and –18.

Patients  $\geq$  10 kg: 10-15-20 mg over 3 days.

*Since serious infusion reactions are not uncommon during alemtuzumab infusion, it is strongly recommended that vital signs should be recorded q 15-30 minutes during each infusion of alemtuzumab.*

#### 2.4.5. Fludarabine

Fludarabine will be administered IV, on Day –8 to Day –4 (for a total of 5 days) given over a minimum of 30 minutes daily. The infusion can take longer per institutional guidelines.

Patients  $\geq$  10 kg: 30 mg/m<sup>2</sup>/day

Preparation, administration, and monitoring will be according to institutional standard practice.

Fludarabine will be adjusted to the ideal body weight (IBW) in children weighing  $>$  125% IBW.

#### 2.4.6. Melphalan

Melphalan will be given IV on Day –3 given over a minimum of 30 minutes. The infusion can take longer per institutional guidelines.

Patients  $\geq$  10 kg dose: 140 mg/m<sup>2</sup>

Melphalan will be adjusted to the ideal body weight (IBW) in children weighing > 125% IBW.

#### 2.4.7. Infusion of Hematopoietic Stem Cells

Under no circumstances is the stem cell product to be irradiated. Vital signs should be monitored before beginning the infusion and periodically during administration. Pre-medications and hydration prior to stem cell infusion will be administered per institutional procedure. Diphenhydramine, epinephrine, and hydrocortisone should be available at the bedside for emergency use if infusion reactions occur. Oxygen with nasal prongs for standby use should be present in the room.

### 2.5. GVHD Prophylaxis

Patients will receive the regimen as described in Table 2.5.

**TABLE 2.5 GVHD PROPHYLAXIS REGIMENS**

Day	Regimen
-3	Tacrolimus or cyclosporine dosed to maintain appropriate levels. Given though Day 100 then taper to Day 180
0	Stem cell infusion
+1	Methotrexate 7.5 mg/m <sup>2</sup> IV
+3	Methotrexate 7.5 mg/m <sup>2</sup> IV
+6	Methotrexate 7.5 mg/m <sup>2</sup> IV
+7	Methylprednisolone 1.0 mg/kg/day IV or Prednisone 1.2 mg/kg/day PO in divided doses  Continued through Day +28, then taper

#### 2.5.1. GVHD Prophylaxis Regimen

##### 2.5.1.1. Tacrolimus (FK 506) or cyclosporine (CSA)

Tacrolimus administration will commence on Day –3, and doses will be adjusted to maintain a level of 8-12 ng/mL by the IMx immunoassay technique (Abbott Diagnostics) or a level of 5-8 ng/mL if measured by a LC-tandem mass-spectrometric assay. If using another method to measure levels then dose should be adjusted to maintain appropriate levels. Tacrolimus can be administered by intermittent infusion over 6 hours twice daily (BID) or by continuous infusion to maintain therapeutic levels per institutional guidelines.

Dose adjustments will be made on the basis of toxicity and tacrolimus levels. Once the patient can tolerate oral medications and has a normal gastro-intestinal transit time, tacrolimus will be converted to an oral form. Tacrolimus dosing will be monitored and altered as clinically appropriate.

From Day 100 to 180, tacrolimus will be gradually tapered in patients without significant acute or chronic GVHD (taper approximately 5-10% per week).

Alternately, cyclosporine (CSA) may be administered beginning on Day –3 and doses will be adjusted to maintain a level of 250-500 ng/mL by TDX method (or 100-250 ng/mL by Tandem MS or equivalent level for other CSA testing methods). CSA can be administered by continuous or intermittent infusion per institutional guidelines.

Dose adjustments will be made on the basis of toxicity and CSA levels. Once the patient can tolerate oral medications and has a normal gastro-intestinal transit time, CSA will be converted to an oral form, preferably microemulsion, at 2-3x the current IV dose. CSA dosing will be monitored and altered as clinically appropriate.

Patients will receive CSA until Day +100 and tapered between Days 100 to 180. If there is no GVHD, the dose will be tapered 10% per week beginning on Day 100.

Centers will declare their preferences of tacrolimus or cyclosporine. However, in the event of toxicity, centers may change calcineurin inhibitors.

#### 2.5.1.2. Methylprednisolone/prednisone

Starting on Day 7, methylprednisolone 1.0 mg/kg/day intravenous (IV) or prednisone 1.2 mg/kg/day (orally [PO]) will be administered in two divided doses daily and continued until Day 28. In the absence of GVHD, prednisone will be tapered 20% per week.

#### 2.5.1.3. Methotrexate

Methotrexate will be given at a dosage of 7.5 mg/m<sup>2</sup> IV on Days 1, 3, and 6. The Day 1 dose of methotrexate should not be administered until 24 hours following completion of the marrow infusion. Leucovorin rescue may be used per institutional practice guidelines.

Methotrexate dose will be adjusted as follows:

	MTX dose		
	Full	50%	Hold dose
Direct bilirubin	< 2.0 mg/dL	2.0-4.0 mg/dL	> 4.0 mg/dL
AST or ALT	< 399 U/L	400-600 U/L	> 600 U/L
Serum creatinine	-	-	> 2.5 mg/dL

## 2.6. Supportive Care

Institutional standard care practice guidelines will be followed after transplantation for nutritional support, treatment of infections, and blood product support. Supportive care guidelines are detailed below.

### 2.6.1. Engraftment Syndrome

Engraftment syndrome is a clinical diagnosis. The most frequently reported manifestations are transient fever, rash, and respiratory symptoms not attributable to infection or GVHD. The pathophysiology is multifactorial mediated by cellular, complement, and cytokine components. Diagnostic criteria include fever (temperature  $>38.5^{\circ}\text{C}$ ) without an identifiable infectious cause within 4 days of the start of neutrophil recovery + an erythematous rash not attributable to GVHD or medications or capillary leak (weight gain, edema, ascites, effusions) or respiratory symptoms not attributable to IPS. Mild symptoms may not require therapy due to the self-limiting nature of this syndrome. For progressive symptoms, methylprednisolone at 2 mg/kg/day is recommended for 5 days. If recurrent or prolonged, investigation for GVHD is recommended.

### 2.6.2. Venous Access

Recipients will have appropriate long-term central venous access placed, per institutional standard practice, prior to beginning the conditioning regimen. The placement of a double lumen tunneled catheter is recommended.

### 2.6.3. Seizure Prophylaxis

Prophylaxis against seizures is mandatory in all recipients and should be commenced at the start of conditioning with alemtuzumab. Suitable drugs for prophylaxis include gabapentin or levetiracetam, and should be administered according to institution guidelines. Seizure prophylaxis should be continued for 180 days after transplant or until tacrolimus is discontinued, whichever is later.

Serum magnesium level should be maintained  $> 1.5\text{ mg/dL}$  during the period of treatment with calcineurin inhibitors cyclosporine or tacrolimus to reduce the risk of seizures.

### 2.6.4. Blood Pressure Monitoring and Control

Blood pressure should be **strictly** controlled to prevent CNS toxicity. Blood pressure should be monitored closely (every four hours) and both systolic and diastolic hypertension should be treated **promptly** to maintain blood pressure within 10% above the baseline age-related median systolic and diastolic pressure as described in children with SCD in *Pegelow et al Am J Med* 1997; 102: 171-77. Table I provides age-related medians of blood pressure in patients with sickle cell disease as published in the manuscript. In addition, close attention should be paid to

fluid balance since fluid overload (patient weight is > 10% of base weight in euvoletic state at the time of admission to the BMT unit) may contribute to elevations in blood pressures.

**TABLE I**

**Medians and 90th Percentiles of Systolic and Diastolic Blood Pressure for Sickle Cell Anemia Subjects in the Cooperative Study of Sickle Cell Disease (CSSCD)**

Age (Years)		2-3	4-5	6-7	8-9	10-11	12-13	14-15	16-17	18-24	25-34	35-44
Females N		257	97	72	68	57	71	89	81	227	199	66
SYS	Median	90	95	96	96	104	106	110	110	110	110	110
BP	90th	100	110	110	110	110	118	120	122	122	125	130
DIA	Median	52	60	60	60	60	62	70	70	64	68	70
BP	90th	62	70	70	70	74	74	80	78	80	80	84
Males N		276	111	78	66	75	61	75	53	179	166	41
SYS	Median	90	95	100	100	100	110	108	112	112	114	110
BP	90th	104	110	108	116	112	120	120	128	130	130	132
DIA	Median	54	60	60	60	60	64	64	70	68	70	70
BP	90th	66	68	68	70	70	72	78	80	80	80	84

SYS BP = systolic blood pressure; DIA BP = diastolic blood pressure.

#### 2.6.5. Growth Factor

G-CSF (5 µg/kg/day) will be given subcutaneously or IV starting Day 7, after completion of methotrexate doses. G-CSF will be continued until the absolute neutrophil count (ANC) is > 1500/µL for 3 measurements taken on different days after the nadir.

#### 2.6.6. Blood Products

The hemoglobin level must be maintained between 9.0 and 11.0 g/dL for at least 100 days post-transplant and platelet count > 50,000/µL post-transplant and post-alemtuzumab administration until recovered to avoid neurological adverse events, as described previously.<sup>25</sup> Irradiated blood products should be administered universally, and CMV negative blood products are recommended for CMV sero-negative recipients.

#### 2.6.7. Treatment of Fever/Infections

Patients should be monitored closely for clinical manifestations of infection and treated per institution guidelines with broad spectrum antibacterial, antiviral and antifungal agents. Early and severe immunosuppression of the patient necessitates prompt and adequate treatment of infections to prevent systemic spread. Since patients receive alemtuzumab, they are especially susceptible to bacterial and viral infections in the early post-transplant period.

## 2.6.8. Infection Surveillance and Prophylaxis

### 2.6.8.1. HSV prophylaxis

Acyclovir prophylaxis is recommended for 6 months for patients who are sero-positive for HSV or VZV. If unable to tolerate PO medications, IV therapy will be necessary.

### 2.6.8.2. PCP prophylaxis

Trimethoprim-sulfamethoxazole or an equivalent drug should be administered beginning after neutrophil recovery and continued post-transplant for 1 year.

### 2.6.8.3. Fungal prophylaxis

Due to the level of immune suppression, anti-fungal prophylaxis against *Aspergillus sp.* is recommended with agents such as itraconazole, voriconazole, or posaconazole until Day 180. Frequent monitoring of tacrolimus or CSA levels will be necessary during azole therapy to avoid toxic drug levels.

### 2.6.8.4. Bacterial prophylaxis

Prophylaxis against bacterial infections is recommended with a broad spectrum antibiotic such as ciprofloxacin after alemtuzumab administration to Day 180.

### 2.6.8.5. CMV surveillance

All recipients must be tested weekly using the PCR method beginning a week after commencing alemtuzumab until Day 100. From Day 100 to Day 180 all patients should be tested at least twice monthly. Antiviral therapy for CMV reactivation should commence pre-emptively if CMV testing reveals a high or rising viral load. If CMV reactivation occurs at or before engraftment, foscarnet may be considered to prevent marrow suppression.

### 2.6.8.6. Adenovirus and EBV intervention guidelines

#### *Adenovirus:*

Testing for adenovirus infection in the blood by PCR method is recommended in the event of symptoms suspicious for infection such as diarrhea, hepatic dysfunction, or respiratory symptoms. If an active systemic infection is diagnosed, therapy should be instituted with cidofovir or other active agents per institution guidelines.

#### *EBV:*

Patients will have EBV DNA quantitative PCR testing on peripheral blood every two weeks from Day 14 to Day 100. In the event of persistent EBV viremia or signs/symptoms consistent with EBV-related PTLN (adenopathy, fever, etc.) therapy with rituximab is recommended.

### 2.6.9. Intravenous Immune Globulin

Intravenous immune globulin may be administered according to institutional practice guidelines.

### 2.6.10. Guidelines for Infusing a Second Stem Cell Product or Donor Cellular Infusion

A second transplant or donor cellular infusion (DCI) should not be considered unless the patient has < 20% donor chimerism. If donor chimerism declines to less than 20% then the patient may be treated per institutional guidelines.

### 2.6.11. Supportive Care Guidelines for CNS Toxicities

Patients with sickle cell disease and cerebral vasculopathy have a high incidence of new CNS toxicities (seizures, labile hypertension, RPLS, PRES, intracranial hemorrhage, stroke, etc.) during the entire transplant process, beginning with the conditioning regimen and lasting through the time that immunosuppression is eventually discontinued. In order to minimize or avoid these risks, adherence to the following guidelines **is necessary** for all BMT CTN #0601 patients:

1. The baseline blood pressure in patients with sickle cell disease is often less than “normal” for age. Elevations in blood pressure can ensue following fluid infusions, or with the use of medications such as corticosteroids and calcineurin inhibitors, even after short term use. Blood pressure (both systolic and diastolic) **should** be monitored closely (at least every 4 hours) and **strictly** maintained within 10% above the median baseline blood pressure documented for that age in *Pegelow et al Am J Med 1997; 102: 171-77* (see section 2.6.4 for table). Aggressive (and often parenteral) use of anti-hypertensive drugs **will** be required to control elevations in blood pressure. In addition, close attention should be paid to fluid balance since fluid overload (patient weight is > 10% of base weight in euvolemic state at the time of admission to the BMT unit) may contribute to elevations in blood pressure.
2. Seizure prophylaxis should be commenced with conditioning therapy i.e., from the date of the first alemtuzumab infusion (the test dose) and continued at least until immunosuppression is withdrawn. If neurontin is used as the prophylactic antiseizure medication, it is recommended that it be started several days before the test dose of alemtuzumab so that the patient can be on the full dose by the day of administration of the test dose.
3. Platelet counts should be monitored frequently after starting the conditioning therapy (including alemtuzumab) and platelet transfusions should be administered as needed to keep levels > 50,000/ $\mu$ L.

## 2.7. Toxicities

### 2.7.1. Pancytopenia

The administration of fludarabine and melphalan is expected to produce pancytopenia with ANC < 500/ $\mu$ L, hemoglobin < 7-8 gram/dL and platelet < 50,000/ $\mu$ L for at least a week in many patients. Most patients will require transfusions of red blood cells and platelets during this period. In addition, many patients will develop fever and approximately 30% will develop a documented infection during the period of neutropenia. Complications related to pancytopenia may be life-threatening or fatal.

### 2.7.2. Alemtuzumab

Administration can cause fevers, rigors, nausea, vomiting, hypotension, fatigue, rash, urticaria, dyspnea, headache, cough, pruritis, diarrhea, pain, anorexia, increased sweating, sepsis, myalgia, asthenia, hypertension, pharyngitis, abdominal pain, back pain, dizziness, anemia, infections, neutropenia, and thrombocytopenia.

- Fever and chills: These are regularly observed, particularly during initial infusions of alemtuzumab. They probably result from a breakdown of cells binding the antibody.
- Skin rash and itching: A complication that is probably due to minor allergic reactions to the antibody. These symptoms will usually be prevented by or controlled with anti-histamines as well as with concomitant administration of corticosteroids.
- Anaphylaxis: A rare but severe allergic reaction which may cause a life threatening drop in blood pressure, wheezing and difficulty breathing, and severe hives or skin exfoliation. This complication can be treated with anti-histamines and steroids.
- Platelet and white cell count depression: These are frequently observed and are probably caused by the binding of the antibody to human blood elements. Platelet transfusions will be administered to reduce the chance of bleeding or life threatening hemorrhage.

### 2.7.3. Fludarabine

Administration can cause hemolytic anemia, neutropenia or thrombocytopenia, low blood counts secondary to bone marrow suppression, nausea, vomiting, diarrhea, stomatitis, skin rash, pneumonitis, edema, fever, chills, fatigue, blurred vision, peripheral neuropathy, confusion, coma, decreased immunity, and rarely encephalopathy (in very high doses).

### 2.7.4. Melphalan

Administration can cause decreased blood counts, nausea, vomiting, diarrhea, oral ulcers, decreased immunity, sterility, interstitial pneumonitis, lung fibrosis, allergic reactions, and rarely seizures (with very high doses).

#### 2.7.5. Hematopoietic Cell Infusion

Infusion of allogeneic BM cells can result in shortness of breath, fever, hemolysis with renal dysfunction and back pain or anaphylaxis. To reduce the risk of reactions to product infusion, patients will be hydrated before and after administration of allogeneic BM, and will be monitored closely before, during, and after infusion.

#### 2.7.6. Growth Factor or G-CSF (Filgrastim, Neupogen)

G-CSF may cause: bone pain, insomnia, headaches, dyspnea, body aches, rash, fever, splenomegaly allergic reaction, fatigue, edema, and nausea/vomiting.

#### 2.7.7. Tacrolimus

Tacrolimus can cause predisposition to infection, renal insufficiency, hypertension, cholestatic hepatic toxicity, gingival hyperplasia, thrombotic microangiopathy, seizures, tremors, hirsutism, anorexia, nausea, and possibly later B-cell lymphomas. To reduce the risk of toxicity, blood pressure, tacrolimus levels, renal function, and liver enzymes will be monitored closely and vital signs aggressively maintained at baseline.

#### 2.7.8. Cyclosporine

Cyclosporine may cause: nephrotoxicity, seizures, hypertension, hirsutism, thrombotic microangiopathy, electrolyte imbalances, paresthesias/neuropathy, gingival hyperplasia, transient-blindness, and hepatic dysfunction.

#### 2.7.9. Methylprednisolone and Prednisone

Methylprednisolone and prednisone can cause predisposition to infection, electrolyte disturbances, gastritis with GI bleeding, insomnia and mental status changes, fluid retention, edema, fat accumulation causing a change in facial appearance, and aseptic necrosis. To reduce the risk of steroid toxicity, the medication will be tapered early per protocol in the absence of GVHD.

#### 2.7.10. Methotrexate

The most methotrexate sensitive organs are the bone marrow and the gastrointestinal mucosa, so that myelosuppression and stomatitis are the most common dose-limiting side effects. At the dosage used in this study, no other significant side effects are anticipated.

## CHAPTER 3

### 3. STUDY ENDPOINTS

#### 3.1. Primary Endpoint

The primary endpoint will be event-free survival at 1 year post-transplant.

##### 3.1.1. Event-free Survival

Event-free survival is defined as the time interval to an event. Primary or late graft rejection, disease recurrence, or death will count as events for this endpoint.

##### 3.1.2. Graft Rejection

###### *Primary Graft Rejection*

Primary graft rejection is defined as the presence of < 20% donor cells assessed by bone marrow or peripheral blood chimerism assays on Day 42. Infusion of a second stem cell product on or prior to Day 42 will be considered primary graft rejection.

###### *Late Graft Rejection*

The presence of < 20% donor derived hematopoietic cells in peripheral blood or bone marrow after Day 42 in a patient with prior evidence of > 20% donor cells will be considered late graft rejection.

Infusion of a second stem cell product beyond Day 42 will be considered late graft rejection. The Study Chair(s) should be contacted prior to any decisions regarding infusion of additional donor cells/second transplant.

##### 3.1.3. Disease Recurrence

Disease recurrence is defined as the return of sickle erythropoiesis (Hb S level > 70%) with or without recurrence of clinical complications of sickle cell disease such as stroke, acute chest syndrome, and veno-occlusive crisis (VOC).

#### 3.2. Secondary Endpoints

Secondary endpoints are evaluations of the effects of HCT on clinical and laboratory manifestations of SCD at 2 years and evaluation of other transplant-related outcomes. These will include the following.

##### 3.2.1. Overall Survival

Overall survival is defined as time from transplant to death or last follow-up.

### 3.2.2. Cumulative Incidence of Neutrophil and Platelet Engraftment

#### *Neutrophil Engraftment*

Time to ANC engraftment is defined as the first of three measurements on different days that the patient has an absolute neutrophil count of  $\geq 500/\mu\text{L}$  following conditioning regimen induced nadir.

#### *Platelet Engraftment*

Platelet engraftment will be defined as the first day of a minimum of three measurements on different days that the patient has achieved a platelet count  $> 50,000/\mu\text{L}$  AND is platelet transfusion independent for a minimum of seven days following conditioning regimen induced nadir.

Subjects must not have had platelet transfusions during the preceding 7 days after the day of engraftment, unless the platelet transfusion is being given specifically to achieve a platelet threshold to allow an elective invasive procedure, such as a central catheter removal.

### 3.2.3. Grade II-IV and Grade III-IV Acute GVHD

Incidence of grade II-IV and III-IV acute GVHD will be graded according to the BMT CTN Manual of Procedures (MOP).

### 3.2.4. Chronic GVHD

Incidence and severity of chronic GVHD will be scored according to the BMT CTN MOP.

### 3.2.5. Frequency of Transplant-related Complications

#### 3.2.5.1. Idiopathic pneumonia syndrome (IPS)

1. Evidence of widespread alveolar injury
  - a. Radiographic evidence of bilateral, multi-lobar infiltrates (by chest X-ray or CT scan), AND
  - b. Evidence for abnormal respiratory physiology, based upon a room air oxygen saturation ( $\text{SpO}_2$ )  $< 93\%$ , or the need for supplemental oxygen to maintain an oxygen saturation  $\geq 93\%$
2. Absence of active lower respiratory tract infection

#### 3.2.5.2. Veno-occlusive disease

Veno-occlusive disease (VOD) is diagnosed by the presence of two or more of the following with no other identifiable cause for liver disease:<sup>75</sup>

1. Jaundice (direct bilirubin  $> 2 \text{ mg/dL}$  or  $> 34 \mu\text{mol/L}$ )

2. Hepatomegaly with right upper quadrant pain
3. Ascites and/or weight gain (> 5% over baseline)

### 3.2.5.3. CNS toxicity

CNS toxicity will be defined as patient experiencing seizures, CNS hemorrhage, or RPLS.

*Reversible posterior leukoencephalopathy syndrome (RPLS).<sup>76, 77</sup> or Posterior Reversible Encephalopathy Syndrome (PRES)*

An increased diffusion coefficient in areas of T2 hyperintensities on diffusion-weighted imaging in the context of clinical symptoms or physical findings including headache, seizures, visual disturbances, and altered level of consciousness.

### 3.2.5.4. Infection

Infection is defined as CMV reactivation with/without clinical disease, adenovirus infection, EBV, and invasive fungal infections.

## 3.2.6. Frequency of Disease-related Complications

### 3.2.6.1. Stroke

An overt stroke is defined as a focal neurologic event and neurologic deficit lasting more than 24 hours with neuroimaging changes. Children with new MRI lesions and ongoing neurologic injury to the brain that does not result in focal motor impairment are referred to as having silent cerebral infarcts. These lesions are defined as a MRI signal abnormality measuring at least 3 mm visible on two views on T-2 weighted images. Both forms of neurologic injury that develop as a new event post-transplant will be considered a disease related complication.

### 3.2.7. Health-related Quality of Life (QOL)

Health-related QOL will be assessed using the Child Health Questionnaire (CHQ). The CHQ Parent Form 50 (PF50) and the CHQ Child Form 87 (CF87) will be used. It will only be assessed in English speaking patients and patients with English speaking parents. Additional details are described in Appendix F.

### 3.2.8. Immune Reconstitution

Lymphocyte subpopulations (absolute number of CD3, CD4, CD8, CD 16+56, and CD19 cells) will be measure by flow cytometry. Immunoglobulin levels (IgG, A, and M) will also be quantified. Splenic function will be measured by quantifying the pitted red cell count.

### 3.2.9. Neurocognitive Testing

Age-appropriate standard neurocognitive tests for recipients aged between 3.0 years and 19.75 years are administered prior to transplant and at 2 years. These tests are validated for children with sickle cell disease and are in application in clinical trials involving chronic transfusion therapy for sickle cell disease. Additional details are provided in Appendix G.

## CHAPTER 4

### 4. PATIENT REGISTRATION, ENROLLMENT AND EVALUATIONS

#### 4.1. Enrollment Procedures

##### 4.1.1 Screening and Eligibility Procedures

Patients will be identified at the participating institutions. Eligibility will be reviewed and confirmed by a majority of an expert panel prior to enrollment. See Appendix E for details regarding the eligibility review panel.

1. Before a patient can be enrolled in this study, the patient must be evaluated for eligibility by the eligibility review panel, which is an external expert panel familiar with the natural history of sickle cell disease and unconnected with this study. The eligibility screening (Segment 0) includes a question confirming that the patient (or legal guardian) signed the informed consent. Transplant centers will enter screening information using the BMT CTN AdvantageEDC<sup>SM</sup> Electronic Data Capture System. At least two weeks prior to the initiation of the conditioning therapy, an authorized user at the transplant center enters the patient demographics and Segment 0 of the Enrollment Form. In addition, the transplant center will provide the patient's and donor's HLA typing results (HLA-A, -B, -C, and -DRB1 at high resolution using DNA-based typing) to the DCC.
2. The DCC distributes this screening information to the eligibility review panel. The review panel has one week to determine the patient's eligibility. The DCC will distribute the HLA typing results to the Protocol Chairs and Protocol Officer to determine that the patient and donor are appropriately HLA-matched.
3. If the patient is eligible the DCC contacts the transplant center coordinator and requests that they complete the Segment A and Segment B enrollment forms. The Segment B enrollment form must be completed prior to initiation of conditioning regimen.
4. A visit schedule based on transplant date is displayed for printing and is referred to as 'Segment B Follow-up.'

#### 4.2. Patient Assessments

##### 4.2.1. Pre-transplant Evaluations

The following observations are required within the following time frames.

##### 4.2.1.1. Required $\leq$ 90 days prior to initiation of alemtuzumab

A liver biopsy should be done if the patient has been transfused for greater than one year PLUS their serum ferritin is  $> 1000$  ng/mL. If the patient does not meet this criterion, then the biopsy will be optional.

4.2.1.2. Required  $\leq 60$  days prior to initiation of alemtuzumab

1. Neurocognitive testing (see Appendix G).
2. Pitted red cell count (see Appendix C).
3. Baseline quality of life (QOL) assessments (CHQ PF50-parent form and CF87). The CF87 is completed by patients  $> 10$  years of age. If  $\leq 10$  years of age, the PF50 is completed by a parent (for English speaking patients and families only). This must be administered prior to alemtuzumab and may be administered up to 60 days prior to initiation of conditioning therapy.

4.2.1.3. Pre-transplant evaluations required  $\leq 30$  days prior to initiation of alemtuzumab

1. History, physical examination, height, and weight.
2. Lansky/Karnofsky performance status.
3. Hb-electrophoresis: Hb F, Hb S, Hb A2.
4. CBC with differential and platelet count, serum creatinine, bilirubin, alkaline phosphatase, ALT, and AST, serum ferritin, magnesium.
5. Radionuclide GFR is strongly recommended as the test of choice. Measurement of 24-hour urine creatinine clearance is acceptable if the former is not available.
6. CMV antibody test, hepatitis panel (HepA Ab, HepB sAb, HepB sAg, HepB Core Ab, HepC Ab), herpes simplex, syphilis, HIV and HTLV1 I/II antibody, and varicella zoster virus antibody.
7. HLA typing at HLA -A, -B, -C, and -DRB1 at high resolution using DNA-based typing, ABO and Rh typing, if not already performed.
8. Baseline peripheral blood samples for chimerism analysis by molecular methods (STR/VNTR).
9. Baseline EKG.
10. Baseline echocardiography for left ventricular ejection fraction (LVEF), left ventricular shortening fraction and presence or absence of tricuspid regurgitation. If present, measure jet velocity as a measure of pulmonary hypertension. It is strongly recommended that these tests be performed  $\leq 30$  days prior to initiation of the conditioning regimen. However, they can be done Day  $-60$  to Day  $-30$  prior to initiation of the conditioning regimen, provided the patient has been asymptomatic since the time of the tests.
11. Pulmonary function testing: FEV1, FVC, and DLCO. Record oxygen saturation by pulse oxymetry. It is strongly recommended that these tests be performed  $\leq 30$  days prior to the initiation of the conditioning regimen. However they can be done Day  $-60$  to Day  $-30$  prior to the initiation of the conditioning regimen, provided the patient has been asymptomatic since the time of the tests.
12.  $\beta$ -HCG serum pregnancy test for females of childbearing potential.

13. Chest X-ray or CT scan. It is strongly recommended that the X-ray or scan be performed  $\leq 30$  days prior to the initiation of the conditioning regimen. However, it can be done Day -60 to Day -30 prior to the initiation of the conditioning regimen, provided the patient has been asymptomatic since the time of the X-ray or scan.
14. MRA/MRI of the brain to assess for radiologic disease. Images will be sent for central review.
15. Total nucleated cell count and CD34+ count of the infused product on Day 0.

#### 4.2.2. Post-transplant Evaluations

The following evaluations are considered standard evaluations for transplant patients:

1. History and physical exam to assess GVHD and other morbidity weekly until Day 100 post-transplant, then at six months, one year, and then two years post-transplant. GVHD evaluation and grading to be in keeping with BMT CTN MOP.
2. CBC at least three times a week from Day 0 until neutrophil engraftment. Platelet count at least three times a week from Day 0 until platelet engraftment. Thereafter, CBC and platelet count twice weekly until Day 28, then weekly until 12 weeks, then six months, one year, and two years post-transplant.
3. Quantitative hemoglobin electrophoresis (Hb F, Hb A, Hb A2, and Hb S) on Day 100, 6 months, one year, and 2 years post-transplant.
4. Creatinine, bilirubin, alkaline phosphatase, ALT, AST, twice a week until Day 28, then weekly until 12 weeks, and then at six months, one year, and two years post-transplant. Serum ferritin at 1 and 2 years post-transplant.
5. Peripheral blood sample for post-transplant chimerism assay by molecular methods collected between Day 28-42, Day 100, 6 months, 1 and 2 years. Fractionated chimerism examining the myeloid and lymphoid fractions is preferred but total chimerism is acceptable.
6. Oxygen saturation by pulse oxymetry 1 and 2 years post-transplant, pulmonary function testing with FEV1, FVC, DLCO in whom pulmonary function testing can be performed.
7. Echocardiography for left ventricular shortening fraction and presence or absence of tricuspid regurgitation. If present, measure jet velocity as a measure of pulmonary hypertension at 1 and 2 years post-transplant.
8. Immune reconstitution, (absolute lymphocyte numbers) by flow cytometry for lymphocyte subpopulations (CD3, CD4, CD8, CD19, and CD16+56 cell subsets), immunoglobulin levels (G, A, and M) at 6 months, 12 months (and 24 months if 12 month result is abnormal or GVHD developed in the past year). Pitted red cell count – Blood samples will be sent at 2 years post-transplant for analysis of pitted red cell counts as a measure of splenic function (see Appendix C for details of procedure).
9. MRA/MRI of the brain at 2 years post-HCT to assess for radiologic disease or transplant induced CNS toxicity. Images will be sent for central review.

10. Neurocognitive follow-up testing (same battery used pre-transplant) 2 years post-transplant (see Appendix G). Pre- and post-transplant test results will be collated for all patients for central review.
11. Health-related quality of life (QOL) measures (CHQ PF50 and CF87) will be completed by the parent and/or child during scheduled clinic visits at Day 100, 6 months, 1 year, and 2 years post-transplant. Please see Appendix F for additional details.

#### 4.2.3. Summary of Patient Clinical Assessments

Table 4.2.3. Summarizes patient clinical assessments over the course of the study.

**TABLE 4.2.3: SUMMARY OF PATIENT CLINICAL ASSESSMENTS**

	Baseline <sup>1</sup>	Days Post-transplant																
		7	14	21	28	35	42	49	56	63	70	77	84	91	100	6 months	1 year	2 years
History, physical exam, weight, height, and Lansky/Karnofsky performance status	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Health-related Quality of Life Assessment (Child Health Questionnaire Parent Form 50 & Child Form 87)	X <sup>2</sup>														X	X	X	X
Cerebral MRA/MRI	X																	X
Neurocognitive assessment <sup>A</sup>	X <sup>2</sup>																	X
Pitted red cell count	X <sup>2</sup>																	X
Hb-electrophoresis Hb F, Hb S, Hb A2, Hb A	X														X	X	X	X
CBC <sup>3</sup> , differential, platelet count, and blood chemistries <sup>4</sup>	X	X	X	X	X	X	X	X	X	X	X	X	X			X	X	X
Serum ferritin	X																X	X
Radionuclide GFR	X																	
Infectious disease titers <sup>5</sup>	X																	
HLA typing <sup>6</sup>	X																	
Peripheral Blood Sample for Chimerism <sup>7</sup>	X						X								X	X	X	X
EKG	X																	
Cardiac Function by 2D echocardiography LVEF or shortening fraction, tricuspid regurgitation, and jet velocity(if present) <sup>B</sup>	X																X	X
FEV1, FVC, DLCO, O <sub>2</sub> saturation <sup>B</sup>	X																X	X
β-HCG serum pregnancy test	females of childbearing potential only																	
Chest X-ray or CT scan <sup>B</sup>	X																	
Liver biopsy	A liver biopsy is required within 90 days of starting conditioning regimen (alemtuzumab) in patients on chronic transfusion therapy ≥ 1 year + clinical evidence of iron overload (serum ferritin level of > 1000 ng/mL)																	
Nucleated cell CD34+ cell counts	X																	
Immune reconstitution assays <sup>8</sup>																X	X	X
GVHD and other morbidity assessments <sup>9</sup>		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Toxicity assessments	X				X				X						X			
Serious adverse events		From initiation of alemtuzumab test dose until 2 years post-transplant																

Table notes on the next page.

Notes for Table 4.2.3:

- <sup>1</sup> Baseline assessments should be made within a 30 day window prior to initiation of the conditioning regimen, unless otherwise noted.
- <sup>2</sup> These assessments should be made within a 60 day window prior to initiation of the conditioning regimen.
- <sup>3</sup> CBC performed at least three times a week from Day 0 until neutrophil engraftment. Platelet counts performed at least three times a week from Day 0 until platelet engraftment. Thereafter, CBC and platelet count performed twice weekly until Day 28. CBC and platelet count performed weekly after Day 0 and neutrophil engraftment.
- <sup>4</sup> Blood chemistries include: serum creatinine, bilirubin, alkaline phosphatase, AST, and ALT, and magnesium (where standard of care should be according to institutional guidelines). Blood chemistries performed twice weekly until Day 28. Blood chemistries performed weekly after Day 28 until 12 weeks post-transplant, 6 months, 1 and 2 years post-transplant.
- <sup>5</sup> Infectious disease titers include: CMV, Hepatitis panel (HepA, Ab, HepB sAb, HepB sAg, HepB Core Ab, HepC Ab), herpes simplex virus, syphilis, HIV and HTLV I/II antibody, and varicella zoster.
- <sup>6</sup> HLA typing at -A,-B, -C, and -DRB1 at high resolution using DNA-based typing and patient must also have ABO and Rh typing performed, if not already performed.
- <sup>7</sup> Chimerism will be measured by molecular methods. Fractionated chimerism for the lymphoid and myeloid fractions is preferred but total chimerism is acceptable.
- <sup>8</sup> The 24 month tests are only required if 12 month test result is abnormal or if GVHD developed in the past year.
- <sup>9</sup> GVHD and other morbidity assessments performed weekly until Day 100 post-transplant, and then at 6 months, 1 year and 2 years.
- <sup>A</sup> The same battery must be used for pre-transplant and 2-year follow-up testing. If a patient has crossed age groups between two testing periods, the tests will still be administered in the age-appropriate manner.
- <sup>B</sup> It is strongly recommended that these tests be performed  $\leq 30$  days prior to the initiation of conditioning regimen. However, provided the patient has been asymptomatic since the time of the tests, they can be done up to Day -60 prior to the initiation of conditioning regimen.

### 4.3. Study Monitoring

**Criteria for Forms Submission:** Criteria for timeliness of submission for all study forms are detailed in the Data Management Handbook. Forms that are not entered into the web-based data entry system within the specified time will be considered delinquent. A missing form will continue to be requested either until the form is entered into the web based data entry system and integrated into the DCC's master database, or until an exception is granted and entered into the Missing Form Exception File, as detailed in the Data Management Handbook.

**Reporting Patient Deaths:** Recipient Death Information must be entered into the web-based data entry system on both the Death and Unexpected Grade 3-5 Adverse Event (AE) Forms within 24 hours of knowledge of the patient's death. If the cause of death is unknown at that time, it need not be recorded at that time. However, once the cause of death is determined, the form must be updated in the web-based data entry system.

#### 4.3.1. Follow-up Schedule

The follow-up schedule for scheduled study visits is outlined in Table 4.3.1. A detailed description of each of the forms and the procedures required for forms completion and submission can be found in the Data Management Handbook and User's Guide.

**TABLE 4.3.1: FOLLOW-UP SCHEDULE**

Study Visit	Target Day Post-transplant
1 week	7 ± 3 days
2 week	14 ± 3 days
3 week	21 ± 3 days
4 week	28 ± 3 days
5 week	35 ± 3 days
6 week	42 ± 3 days
7 week	49 ± 3 days
8 week	56 ± 3 days
60 days	60 ± 3 days
9 week	63 ± 3 days
10 week	70 ± 3 days
11 week	77 ± 3 days
12 week	84 ± 3 days
13 week	91 ± 3 days
14 week	98 ± 3 days
100 day	100 ± 3 days
6 month	180 ± 28 days
12 month	365 ± 28 days
24 month	730 ± 28 days

Laboratory samples are due  $\pm$  one month from the target date. Samples should still be collected even if outside target window.

**CIBMTR Data Reporting:** Centers participating in BMT CTN trials must register pre- and post-transplant outcomes on all consecutive hematopoietic stem cell transplants done at their institution during their time of participation to the Center for International Blood and Marrow Transplant Research (CIBMTR). Registration is done using procedures and forms of the Stem Cell Transplant Outcomes Database (SCTOD). (Note: Federal legislation requires submission of these forms for all US allotransplant recipients.) Enrollment on BMT CTN #0601 must be indicated on the SCTOD pre-transplant registration form, if applicable. Additionally, CIBMTR pre- and post-transplant Report Forms must also be submitted for all patients enrolled on this trial. CIBMTR forms will be submitted directly to the CIBMTR at the times specified on the Form Submission Schedule.

**Weekly GVHD Monitoring:** GVHD should be monitored in accordance with BMT CTN guidelines as specified in the Manual of Procedures. Patients should be assessed weekly until Day 100 post-transplant for GVHD. After Day 100 patients will be assessed at each follow-up visit (Day 180, 365, and 730) for the presence of GVHD.

#### 4.3.2. Adverse Event Reporting

Unexpected, grade 3-5 adverse events (AE) will be reported through an expedited AE reporting system via AdvantageEDC. Unexpected, grade 4-5 AEs must be reported within 24 hours of knowledge of the event. Unexpected, grade 3 AEs must be reported within three business days of knowledge of the event.

Expected AEs will be reported using NCI's Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0 at regular intervals as defined on the Form Submission Schedule.

Serious Adverse Events will be consistent with BMT CTN procedures (BMT CTN Administrative Manual of Procedures, Chapter 6) and reported through an expedited Adverse Event (AE) reporting system. Serious and unexpected Grades 3-5 adverse events should be reported within three working days. Deaths are to be reported within 24 hours. Other SAEs will be tracked periodically as defined in the Form Submission Schedule, and staged according to CTCAE, Version 4.0.

In addition to the standard BMT CTN Unexpected Grade 3-5 Adverse Event reporting, all central nervous system (CNS) events, or events requiring intensive care (ICU) or advanced care interventions are required to be reported via the Unexpected Grade 3-5 Adverse Event forms in AdvantageEDC for this study. All event reporting is to be expedited. Grade 4-5 events should be reported within 24 hours of knowledge of the event. Grade 3 events should be reported within three business days of the event.

For the purposes of this protocol, examples of "ICU" or "advanced care treatment" are one or more of the following:

- The patient is transferred to an advanced care unit *or* the ICU\* (see footnote below)

- Continuous Renal Replacement Therapy (CRRT) by dialysis (CAVHD) or filtration (CAVH)
- Non-invasive positive pressure ventilation: continuous positive airway pressure (CPAP), bi-level positive airway pressure (BIPAP), intermittent positive pressure ventilation (IPPV)
- Endotracheal intubation with mechanical ventilation
- Invasive blood pressure monitoring
- Pressor support
- Continuous cardiac monitoring
- Organ failure – acute renal failure, acute liver failure, acute respiratory distress syndrome, respiratory failure, or multi-organ failure

**\* Elective transfer to an advanced care/ICU unit for a scheduled intervention does not need to be reported via the Unexpected Grade 3-5 Adverse Event forms in AdvantageEDC.**

## CHAPTER 5

### 5. STATISTICAL CONSIDERATIONS

#### 5.1. Study Design

The study is a Phase II, single arm, multi-center trial. It is designed to estimate the efficacy and toxicity of a HCT procedure for SCD patients using unrelated BM grafts. The study population is children with SCD. The sample size is 30 BM recipients for this trial.

##### 5.1.1. Accrual

It is estimated that four years of accrual will be necessary to enroll the targeted sample size. Accrual will be reported by gender and age.

##### 5.1.2. Study Duration

Patients will be followed for a minimum of two years post-transplant.

##### 5.1.3. Randomization

There is no randomization aspect to this trial.

##### 5.1.4. Primary Endpoint

The primary objective is to assess event-free survival (EFS) probability 1 year post-transplant. Death, primary or late graft rejection, or recurrence of disease will be considered events for this endpoint.

#### 5.2. Sample Size and Power Considerations

The sample size is 30 patients for this trial. Ninety-five percent confidence intervals were calculated for varying probabilities based on the sample size. Table 5.2 provides confidence intervals for a variety of observed proportions. Of particular interest is where the EFS probability is 75%, which is the anticipated 1 year EFS probability based on the available data showing that EFS for HLA-matched sibling donor transplants for SCD is 85%.

For this setting, the confidence interval length is 31%. The percentages above and below 75% are meant to represent other plausible EFS percentages.

**TABLE 5.2: CONFIDENCE INTERVAL LENGTHS AND POSSIBLE CONFIDENCE INTERVALS FOR VARIOUS UNDERLYING EVENT-FREE SURVIVAL PROBABILITIES**

N (BM)	Event-free Survival %	Length of 95% Confidence Interval	Possible Confidence Intervals	
30	90	20.7	79.3	100.0
	85	25.6	72.2	97.8
	80	28.6	65.7	94.3
	75	31.0	59.5	90.5
	70	32.8	53.6	86.4
	60	35.0	42.5	77.5

The EFS probability estimate will be based on the Kaplan-Meier product limit estimator using Greenwood's formula as the variance estimate. In the absence of censoring, the Kaplan-Meier estimate reduces to the simple binomial proportion.

### 5.3. Interim Analysis and Stopping Guidelines

Monitoring of key safety endpoints (overall mortality, graft rejection, and GVHD) will be conducted. The stopping guidelines serve as a trigger for consultation with the DSMB for additional review.

A truncated Sequential Probability Ratio Test (SPRT) for a binomial outcome will be used to monitor each type of toxicity as described below. This sequential testing procedure conserves type I error across all of the monitoring looks for each toxicity, but not across the three toxicity outcomes. Thus for a single toxicity the type I error is approximately 10%, and across all the toxicities, the study-wide type I error is < 30%. A type I error rate of 10% per toxicity was selected to be more conservative with regard to the safety of a new treatment in a less well studied disease indication.

The rationale for not conserving type I error across multiple toxicities is twofold. First, adjusting the size of the test for multiple comparisons would reduce statistical power to detect adverse outcomes, which seems imprudent. Secondly, the procedure is a guideline for requiring additional review by the Data and Safety Monitoring Board, and is not a formal "stopping rule" that would mandate automatic closure of study enrollment.

The SPRT can be represented graphically. At each interim analysis, the total number of patients enrolled is plotted against the total number of patients who have experienced toxicity. The continuation region of the SPRT is defined by two parallel lines. Only the upper boundary will be used for monitoring the study to protect against high incidences of toxicity. If the graph falls above the upper boundary, the SPRT rejects the null hypothesis and concludes that the toxicity rate is higher than predicted by the observed number of patients enrolled on study. Otherwise, the SPRT continues until enrollment reaches the target goal.

The usual measures of performance of an SPRT are the error probabilities  $\alpha$  and  $\beta$  of rejecting  $H_0$  when  $\theta = \theta_0$  and of accepting  $H_1$  when  $\theta = \theta_1$ , respectively, and the expected sample size  $E(N|\theta_i)$ . Note that since the test uses only the upper boundary, and is truncated by a finite sample size, the size of the test will be slightly lower than the nominal level given %. The tests to be used in this protocol were developed from SPRT's described in more detail in the following subsections.

### 5.3.1. Overall Mortality

Overall mortality in this trial is projected to be  $\leq 15\%$  at 100 days. The stopping rule for overall mortality will be triggered if there is significant evidence that the 100 day overall mortality rate exceeds 15% based on the truncated SPRT. This truncated SPRT is based on contrasting 15% versus 30% 100 day mortality, with nominal type I and II errors of 14% and 10%, respectively. The common slope of the parallel lines is 0.219 and the intercept for the upper boundary is 2.097. The stopping rule is summarized in the following table.

**TABLE 5.3.1a: STOPPING RULE FOR OVERALL MORTALITY BY DAY 100  
(ALSO APPLIES TO GRADE III-IV aGVHD BY DAY 100)**

<b>Number of <u>Evaluable</u> Patients Enrolled<sup>1</sup></b>	<b>Number of Deaths</b>
3-4	3
5-8	4
9-13	5
14-17	6
18-22	7
23-26	8
27-30	9

<sup>1</sup> Evaluable patients are defined as those who proceed to transplant.

The actual operating characteristics of this stopping rule, shown in the table below, were determined in a simulation study that assumed uniform accrual of 30 individuals over a four-year time period.

**TABLE 5.3.1b: OPERATING CHARACTERISTICS OF STOPPING RULE FOR OVERALL MORTALITY FROM A SIMULATION STUDY WITH 10,000 REPLICATIONS**

<b>True 100-Day Rate</b>	<b>15%</b>	<b>25%</b>	<b>30%</b>	<b>35%</b>
Probability Reject Null	0.08	0.48	0.70	0.85
Mean Month Stopped	46.0	37.2	30.7	25.2
Mean # Endpoints in 100 Days	4.5	5.9	5.9	5.6
Mean # Patients Enrolled	29	23	19	16

The testing procedure for overall mortality rejects the null hypothesis in favor of the alternative 8% of the time when the true 100 day overall mortality rate is 15%, and 85% of the time when the rate is 35%. This corresponds to a type I error rate of  $\alpha = 0.08$  and a type II error rate of  $\beta = 0.15$ . When the true 100 day mortality rate is 35%, on average, the stopping rule will be triggered 25 months after opening, when 6 events have been observed in 16 patients.

### 5.3.2. Graft Rejection

Failure to engraft donor cells (defined as < 20% donor chimerism) by Day 100 should occur in < 20% of patients with this approach. The stopping rule will be triggered if there is significant evidence that the Day 100 graft rejection rate exceeds 20% based on the truncated SPRT. This truncated SPRT is based on contrasting 20% versus 35% Day 100 graft rejection, with nominal type I and II errors of 12% and 20%, respectively. The common slope of the parallel lines is 0.271 and the intercept for the upper boundary is 2.473. The stopping rule is summarized in the following table.

**TABLE 5.3.2a: STOPPING RULE FOR GRAFT REJECTION BY DAY 100**

<b>Number of <u>Evaluable</u> Patients Enrolled<sup>1</sup></b>	<b>Stop if Graft Rejection Occurs in</b>
3-5	4
6-9	5
10-13	6
14-16	7
17-20	8
21-24	9
25-27	10
28-30	11

<sup>1</sup> Evaluable Patients are defined as those who proceed to transplant.

Operating characteristics of the stopping rule for graft failure were determined in a simulation study that assumed uniform accrual of 30 individuals receiving unrelated donor BM over a four-year time period.

**TABLE 5.3.2b: OPERATING CHARACTERISTICS OF STOPPING RULE FOR GRAFT FAILURE (GF) FROM A SIMULATION STUDY WITH 10,000 REPLICATIONS**

True 100-Day Rate	20%	30%	35%	40%
Probability Reject Null	0.08	0.41	0.64	0.82
Mean Month Stopped	46.3	39.2	33.6	28.0
Mean # Endpoints in 100 Days	5.9	7.5	7.5	7.1
Mean # Patients Enrolled	29	25	21	17

The testing procedure for graft failure rejects the null hypothesis in favor of the alternative 8% of the time when the true Day 100 graft failure rate is 20%, and 82% of the time when the rate is 40%. This corresponds to a type I error rate of  $\alpha = 0.08$  and a type II error rate of  $\beta = 0.18$ . When the true Day 100 graft failure rate is 40%, on average, the stopping rule will be triggered 28 months after opening, when 7 non-engraftment events have been observed in 17 patients.

Secondary graft rejection will be monitored and reported to the DSMB.

### 5.3.3. Grade III-IV Acute GVHD

Grade III-IV acute GVHD should occur in  $\leq 15\%$  of patients by Day 100 with this approach. The grade III-IV acute GVHD rates are not expected to differ substantially by stem cell source, so that the monitoring rule will include both stem cell sources together. The stopping rule will be triggered if there is significant evidence that the rate of acute GVHD on Day 100 is more than 15% based on the truncated SPRT. The stopping rule and operating characteristics are identical to that for overall mortality and are not reproduced here.

## 5.4. Demographic and Baseline Characteristics

Demographics and baseline characteristics will be summarized for all patients. Characteristics to be examined are: age, gender, stem cell source, performance status, HLA match, disease symptoms, serum bilirubin level, serum creatinine level, pulmonary function tests, cardiac function, donor age, donor gender, donor ethnicity, splenic function, hemoglobin electrophoresis, cerebral MRI, serum ferritin, QOL, neurocognitive function.

## 5.5. Analysis of Primary Endpoint

The primary analysis will consist of estimating 1 year EFS probability based on the Kaplan-Meier product limit estimator. The 1 year EFS probability and confidence interval will be

calculated. All transplanted patients will be considered for this analysis. In addition, the frequencies of each component of the composite endpoint (primary graft failure, secondary graft failure, and disease recurrence) will be described.

## 5.6. Analysis of Secondary Endpoints

**Overall survival:** The survival distribution will be estimated by the Kaplan-Meier curve. All patients will be followed for a minimum of two years post-transplant for mortality.

**Incidence of neutrophil and platelet engraftment:** To assess the incidence of each type of engraftment from day of transplant, a cumulative incidence curve will be computed along with a 95% confidence interval at 100 days post-transplant. Death prior to each type of engraftment will be considered as a competing risk.

**Time to acute GVHD:** To assess the incidence of grades II-IV or grades III-IV acute GVHD from day of transplant, the first day of acute GVHD onset at either grades II-IV or grades III-IV will be used to calculate a cumulative incidence curve for that acute GVHD grade. A 95% confidence interval at 100 days post-transplant will be computed. Death prior to development of acute GVHD will be considered as a competing risk.

**Time to first clinical onset of chronic GVHD:** To assess the incidence and severity of chronic GVHD from day of transplant, a cumulative incidence curve will be computed along with a 95% confidence interval at two years post-transplant. Death prior to occurrence of chronic GVHD will be considered as a competing risk.

**Transplant-related complications:** The frequency of transplant-related complications, both overall and by type of complication, will be described using proportions (along with 95% confidence intervals).

**Disease-related complications:** The frequency of disease-related complications (stroke) will be described using proportions (along with 95% confidence intervals).

**Immune reconstitution:** Descriptive statistics will be computed for all the immune reconstitution assays.

**Health-related QOL:** Mean QOL scores and confidence intervals will be computed at each time point. For both parent and child reported health-related QOL mean scores, a paired student T test will be used to look for differences in mean scores between baseline and 2 years after the HCT. To determine the magnitude of differences, standardized effect sizes (or z-scores) will be calculated. This will be done by taking the difference between the mean domain scores of the baseline score and the follow-up scores and dividing by the standard deviation of the baseline. Domain scores will also be compared between baseline and 2 years post-transplant with a Bonferroni correction for multiple testing. In addition, mixed models for repeated measures data will be used to assess whether QOL is changing significantly over each time point among survivors.

Pearson correlation coefficients will be used to determine the association between child and parent health-related QOL scores. Fisher's z-transformation will be used to test whether this correlation is significantly different from 0 and to construct confidence intervals for the correlation coefficient. A paired t-test will be used to test the difference between the mean CHQ scores of the child and parent at each time point.

**Neurocognitive evaluation:** All patients will be assessed with a cognitive battery at study entry and 2-year follow-up. The major indicator of cognitive loss will be decline in general intellectual abilities (IQ) as assessed by the Wechsler Abbreviated Scale of Intelligence (WASI) or Wechsler Preschool and Primary Scale of Intelligence (WIPPSI-III).

Other secondary outcomes, including the Behavior Rating Inventory of Executive Function (BRIEF) Continuous Performance Test Second Edition (CPT-II), VMI, Children's Memory Scale (CMS), and California Verbal Learning Test (CVLTC) will be compared in the same manner by comparing the composite t-score for each age-appropriate cognitive domain test over time.

A paired t-test will be used to assess the change from baseline in WASI IQ and change from baseline in BRIEF and other age-appropriate tests T-Scores over time. In addition to using statistical techniques to evaluate changes in IQ, clinical significance will also be evaluated.

Functional scores and confidence intervals will be computed at each time point in age-appropriate fashion. Mixed models for repeated measures data will be used to assess whether functional scores change significantly after transplant over time.

Secondary analysis of the secondary outcome will be performed. This secondary analysis will be an adjustment of the secondary study outcome for potential confounding factors (e.g., adjust the secondary study outcome for confounding factors such as gender, age, medical history, co-morbidities, etc.). Since the secondary study outcome is a continuous measure, we will be able to make these adjustments with the use of generalized linear models (e.g., analysis of covariance). Generalized linear models can help to determine whether the apparent effect of one variable (e.g., treatment) on the outcome of interest (e.g., the mean change from baseline of IQ after 2-years of follow-up) is accounted for by differences in other patient characteristics (e.g., treatment compliance, gender, age, medical history, co-morbidities, etc.).

## **5.7. Safety Analysis**

The DSMB will review the study for safety endpoints at least twice per year or more often if stopping guidelines are reached. Accrual will not stop during this evaluation. The reporting of serious adverse events will be consistent with standard BMT CTN procedures. The type and severity of adverse events will be analyzed.

## **APPENDIX A**

### **HUMAN SUBJECTS**

#### **1. Subject Consent**

A conference will be held with the patient and family to discuss this study and alternative treatments available for the treatment of the underlying disease. The Principal Investigator or another designated physician will conduct the conference. All potential risks associated with the use of conditioning regimen and GVHD prophylaxis medications should be discussed as objectively as possible.

The consent document should be reviewed with the patient and family prior to proceeding to transplantation. Informed consent from the patient and the patient's parent/guardian will be obtained using a form approved by the Institutional Review Board of the institution enrolling the patient.

#### **2. Confidentiality**

Confidentiality will be maintained by individual names being masked and assigned a patient identifier code. The code linking the patient's identity with the ID code will be kept separately at the center. The ID code will be transmitted to the BMT CTN Data Coordinating Center upon enrollment.

#### **3. Participation of Women and Minorities and Other Populations**

Women and ethnic minorities and other populations will be included in this study. Accrual of women and minorities at each center will be monitored to determine whether their rates of enrollment are reflective of the distribution of potentially eligible women and minorities expected from data reported to the CIBMTR. Centers will be notified if their rates differ significantly from those expected and asked to develop appropriate recruitment strategies.

It is expected that the number of minority patients on this study will be large given the disease being studied.

#### **4. Ethical Considerations**

Patients are referred to the Transplant Center for consideration of hematopoietic cell transplantation. While there will be every effort to seek out and include females and minority patients, the patient population is dependent upon the referral pattern and the ability to identify suitable donors. Female and minority patients are eligible for all aspects of the study and their participation will be actively encouraged.

## **APPENDIX B**

### **CONSENT AND ASSENT FORMS**

## ***Informed Consent to Participate in Research***

### **Unrelated Donor Reduced Intensity Bone Marrow Transplant for Children with Severe Sickle Cell Disease**

This is a clinical trial, which is a research study to answer specific medical questions. The information from this study may also help future patients. The Study doctor (the person in charge of the research) will explain the study to you and your child. This research study will include only people who choose to take part. Please take your time to make your decision about allowing your child to take part. You may discuss your decision with family and friends. You and your child should also discuss this with your child's health care team. If you, or your child, have any questions, you and your child can ask the Study doctor for more explanation.

This is a consent for a research study. Your child is being invited to participate in this study because your child has severe sickle cell disease that may be treated with a bone marrow transplant. This form is intended to give you and your child information to help you decide if you and your child want to participate in this study. You should read this form and ask any questions you or your child may have before agreeing to be in the study.

Children with sickle cell disease are born with a defect in hemoglobin. Hemoglobin is a protein in red blood cells that carries oxygen to vital organs such as the brain, heart, lungs and kidneys. Defective hemoglobin damages red blood cells and makes them sticky. This causes them to look like a sickle. The damaged or sickled red blood cells can block blood flow in blood vessels and block oxygen and nutrients from reaching organs. As a result almost all the organs in the body can be damaged but it is especially bad when vital organs are involved. When the flow of blood is blocked in a blood vessel in the brain, a stroke occurs. When blood flow is blocked in a bone or muscle, this causes severe pain. If it occurs in the lung, this complication is called acute chest syndrome and causes chest pain and a low oxygen level. Sickle cell disease can also cause anemia.

It is possible to replace the defective red blood cells with normal blood cells in children with severe sickle cell disease by performing a bone marrow transplant. We know this from recent studies where bone marrow from a brother or sister has replaced the defective sickled red cells after transplantation. Blood vessels are no longer blocked, and the anemia goes away.

However, a bone marrow transplant is an intensive medical procedure and there are serious risks involved. Therefore, it is usually offered only to children who have had severe complications of sickle cell disease such as stroke, frequent painful crises, or repeated episodes of acute chest syndrome. In most cases, it is offered only to children who have a healthy brother or sister who

is HLA-identical, which means that the donor has the same ‘tissue’ type as the person receiving the transplant.

Most children with severe sickle cell disease do not receive a transplant because they do not have the same tissue type as their healthy brothers or sisters. These children are often treated with medicines and regular red blood cell transfusions for many years to try to control symptoms and organ damage. However, in children with sickle cell disease who have had a stroke, 20% (1 in 5) will develop a second stroke and, of that group, 30% (1 in 3) children will develop a third stroke even if they are receiving regular blood transfusions.

Cells in the bone marrow that make healthy red cells are called blood stem cells.

This study is a clinical trial for children with severe sickle cell disease that do not have a brother or sister with the same tissue type who can serve as their donor. Although previous studies have shown that a bone marrow transplant is possible for patients with a healthy related bone marrow donor, this study will determine if this is a good treatment option for patients transplanted with cells from an unrelated donor. Patients who participate in this study will also receive lower intensity treatment (conditioning) before transplant with bone marrow donated by a healthy adult donor.

This lowered intensity conditioning treatment (reduced intensity conditioning regimen – RIC) is being used to decrease the side effects or toxicities of the conditioning treatment. It is not known whether this RIC will be successful in allowing donor cells to settle in the patient and grow successfully. This is the research question that is being asked in this study.

Before you decide whether or not to have your child join the study, please read all the information in this consent form. Feel free to ask questions to understand your child’s rights and protections. The choice to take part in this study is completely voluntary.

**Sponsor and source of funding:**

This study is sponsored by the National Institutes of Health (NIH) and the National Marrow Donor Program® who is the primary source of funding. Additional NIH sponsors include the Sickle Cell Disease Clinical Research Network (SCD-CRN) and the National Center on Minority Health and Health Disparities. The NIH is a government program that funds the Blood and Marrow Transplant Clinical Trials Network (BMT CTN), a group of transplant programs conducting research in transplantation for several diseases including severe sickle cell disease.

**If you decide to allow your child to take part in this research study:**

- You or your child's insurance company will be responsible for all medical bills from your child's transplant.
- You or your child will not pay for any extra tests that are being done for the study (these are lab tests, scans and other special tests that are not part of regular, routine care). The extra research tests that are being done for this study are a blood test to measure function of the spleen, a special scan of the brain called an MRA/MRI (magnetic resonance angiography and imaging after injection of contrast), measurement of brain function using performance tests that are age-dependent, and question sheets that measure quality of life. The tests that measure learning and brain function will be done before transplant and again 2 years after transplant. Quality of Life question/answer sheets will be completed before transplant and at 3, 6, 12 and 24 months after transplant.
- You or your child will not be paid to take part in this study.

**If you decide not to allow your child to participate in this study,** your child's study doctor will discuss other treatment options with you.

**What other choices does your child have if your child does not take part in the study?**

Your child's other choices may include:

- Treatment with drugs such as hydroxyurea that may lessen complications of severe sickle cell disease.
- Experimental treatments with new drugs for severe sickle cell disease.
- A transplant using bone marrow without being part of this study where the choice of medicines before and after transplant, and other guidelines may be different.
- Other treatments such as regular RBC transfusions to try to control symptoms related to your child's severe sickle cell disease. However, RBC transfusion therapy may not prevent further strokes in all children who have had an initial stroke.

It is important that you talk to your child's study doctor about your child's treatment choices before you decide to have your child participate in this study.

**Why is this study being done?**

This study is being done to determine if blood cell transplants, with bone marrow from unrelated donors, are effective in children with severe sickle cell disease and if this treatment approach has acceptable risks and side effects. The study will also look into the risk of death associated with this treatment approach and if it is acceptable considering the potential benefit of avoiding the long-term consequences of severe sickle cell disease. Transplant studies for sickle cell disease have previously used high dose chemotherapy to prepare patients for transplant (myeloblastic conditioning). This has been successful mostly in patients who had tissue-matched family members such as brothers or sisters, as donors. In this study, the success of transplant for sickle cell disease is being measured after using reduced intensity conditioning treatment to reduce toxicities of treatment using tissue-matched unrelated donor cells. This is being done to try to avoid the toxicities of more intense conditioning treatment.

This research study is being done to answer the following questions:

- Is it safe to transplant children who have severe sickle cell disease with bone marrow from a healthy, unrelated donor?
- Are the drugs given to patients in this study (reduced intensity conditioning) effective in destroying sickle cells and allowing blood cells from a healthy donor to grow in the recipient?
- After transplant, will children with sickle cell disease make healthy red blood cells and avoid the health problems caused by sickle cell disease?
- After transplant, will children with sickle cell disease experience any side effects that shorten their life or worsen their quality of life?

### **How many children will take part in the study?**

Thirty (30) children in the U.S. will take part in this study. To be part of the study your child must:

- Be between the ages of 3 and 19 years
- Have severe sickle cell disease with one or more of the following sickle cell complications:
  - Stroke or other severe sickle cell disease complication affecting the brain
  - Repeated acute chest syndrome episodes, despite treatment
  - Repeated severe pain episodes despite treatment
- Not have a healthy brother or sister who has the same tissue type who is able and willing to donate
- Have an acceptable unrelated marrow donor available
- Provide a signed consent for participation in the study (your child signs an assent form if they are old enough to understand the risks and benefits)

### **What will happen if your child takes part in this research study?**

#### **Before the transplant:**

The first step in considering your child for an unrelated blood or marrow transplant is to have an eligibility review panel (a group of 5 sickle cell disease experts and blood and marrow transplant physicians unconnected with this trial) review your child's medical history. Once they have confirmed that your child is eligible for this trial we will proceed with the pre-transplant work-up and research tests listed below. In the unlikely event that they determine that your child is not eligible for this trial, your doctor will discuss other transplant and treatment options with you.

The following research tests will be performed:

- Question/answer sheets to measure quality of life before transplant.
- Pitted red cell count before transplant to measure spleen function which is decreased by sickle cell disease.
- Special tests of learning and brain function called 'Neurocognitive testing'. These tests are done to learn about any effects of sickle cell disease on brain function before the transplant, and compare 2 years after transplant to see if there is a change.
- MRA and MRI tests before transplant will be performed as part of the research for this study.

These tests will be done as an outpatient before the transplant over the course of several days.

To help with the administration of medicines, blood transfusions and obtaining blood for lab tests, a central venous catheter (also known as a ‘Hickman’ or ‘Broviac’ catheter) will be placed before the transplant. This is a hollow tube that is inserted by a surgeon or radiologist usually in the operating room. The doctor performing the procedure will explain it in more detail before the procedure. Your child’s anesthesiologist will describe the risks of a general anesthetic. The tube is placed in the chest and allows medicines, transfusions, etc. to be given painlessly into the vein without the need for repeated sticking of needles in your child’s arms. Once the central venous catheter is placed, it will need daily care at home with cleaning and injection of medications to prevent catheter-related blood clots.

**Transplant/conditioning:**

Conditioning is the chemotherapy and other medicines given to prepare your child to receive donor cells. It prevents your child’s immune system from rejecting donor cells. Conditioning will start 22 days before transplant.

The medicines used are alemtuzumab, fludarabine, and melphalan. Alemtuzumab will be given intravenously (through your child’s central venous catheter) once a day for 3 days (after a small test dose) beginning 3 weeks before the transplant. The test dose is given to make sure that your child will not have a bad reaction to the full dose. If your child has a bad reaction, your doctor will discuss the best way to proceed. Your child will be admitted during alemtuzumab infusions and may be discharged if well the day after the infusions are completed.

Next, your child will return to the hospital for admission to the bone marrow transplant unit to receive fludarabine and melphalan starting 8 days before the transplant. Fludarabine will be given intravenously once a day for 5 days. Finally, melphalan will be given intravenously 3 days before the transplant. This will be followed by 2 “rest days” when your child will not receive any chemotherapy.

Day	Treatment
24 hours before 1 <sup>st</sup> dose of Alemtuzumab	Alemtuzumab test dose
21 days before transplant	Alemtuzumab
20 days before transplant	Alemtuzumab
19 days before transplant	Alemtuzumab
8 days before transplant	Fludarabine
7 days before transplant	Fludarabine
6 days before transplant	Fludarabine
5 days before transplant	Fludarabine
4 days before transplant	Fludarabine
3 days before transplant	Melphalan
2 days before transplant	Rest
1 day before transplant	Rest
Transplant day	Bone marrow infusion

**Bone marrow transplant:**

Your child will be given the marrow transplant on Day 0. The donated marrow will come from a suitably tissue-matched unrelated person. The donor cells will be given through the central venous catheter, in a manner similar to a blood transfusion.

**Immunosuppression to prevent graft-versus-host disease (GVHD):**

Three days before the transplant, your child will begin to receive medicines to hold back the immune system and these medicines will continue after the transplant. This may make your child develop more infections than usual. However, these drugs are important to allow donor cells to accept their new role and to lower the chance of their injuring your child's organs by causing graft-versus-host disease (GVHD). GVHD is an attack by the donor cells against your child's body. GVHD is discussed in greater detail below. Your child will receive one or more standard drugs to prevent GVHD and these will be given for at least 6 months after the transplant. These drugs include tacrolimus (also called FK 506 or Prograf®), methylprednisolone or prednisone, methotrexate, and cyclosporine (also called Gengraf® or Neoral®) and may be used in different combinations. Choice of drugs may depend on the preference of the transplant team at your hospital.

**Post-transplant follow-up and care:**

The conditioning regimen will destroy your child's blood and marrow cells. This will cause low counts of white blood cells, red blood cells, and platelets. Blood stem cells from the donor will produce new blood cells to replace the destroyed recipient cells. To speed this process along after the transplant, your child will receive granulocyte-colony-stimulating factor (also called G-CSF or Neupogen). G-CSF is a natural protein made in the body that increases the white blood cell count and that is used to help protect against infections. Your child will start receiving G-CSF one week after the transplant. It is given either by injection under the skin or intravenously. Your child will receive it daily until the white blood cell count has recovered.

Your child will stay in the hospital after the transplant until the doctor feels it is safe for your child to go home. During that time, your child will be carefully watched for signs of infection and other problems. A physical exam and blood tests will be done daily. Additional blood tests, medications, and procedures may be required if problems arise.

After leaving the hospital, your child will need to visit the transplant clinic at least once a week for check-ups, blood counts, and chemistries to make sure that he/she is doing well medically. Eventually the visits will be less frequent. Your child will be examined at 100 days, 6 months, 1 year and 2 years after transplant to check your child's progress and treat any problems. The following tests will be performed during these visits:

### CLINICAL TESTS

Test	Purpose	When Performed
Blood Tests (1-3 tablespoons or 10-30 ml)	Check number of donor cells in your child, hemoglobin level, and recovery of immunity	28 days, 42 days, 100 days, 180 days, one year, and two years after transplant
Lung and Heart Function	Check the health of heart and lungs	One year and two years after transplant

### TESTS FOR RESEARCH

Test	Purpose	When Performed
MRA/MRI Scan	Check structure and flow of blood vessels in the brain	2 years after transplant
Neurocognitive Testing	Detect any changes in memory and learning abilities	2 years after transplant
Quality of Life Questionnaire	Check child's quality of life compared to before transplant	Before transplant, and 100 days, six months, one year and two years after transplant

You may decide to take your child out of any research test at any time.

In addition to these, your doctor will decide if other tests and treatments that are not part of this research study are necessary for good medical care.

### How long will your child be in the study?

Your child will be in the study for 2 years. Please notify your child's transplant doctor if you move or change your child's primary care doctor so that we will be able to obtain all the necessary information about your child's health.

**Can your child stop being in the study?**

You can decide to stop your child's participation at any time. Tell your child's doctor if you or your child are thinking about stopping or decide to stop. The doctor will tell you and your child how to stop safely. It is important to note that once your child receives the medicines for the conditioning regimen, he/she must receive the unrelated donor marrow in order for the blood counts to recover in a timely fashion.

If you decide to withdraw your child, or your child's doctor withdraws your child from the study, we will ask your permission to use all the information about your child that has already been collected as part of the study. We will also ask your permission to continue to allow your child's doctor to tell us about his/her progress until at least two years post-transplant. You can choose to give or not to give this permission.

**Can the doctor who is the Principal Investigator withdraw your child from this study?**

Your child can be taken off the study (with or without your consent) for any of the following reasons:

- Your child needs a medical treatment not allowed in this study
- The investigator decides that continuing in the study would be harmful to your child
- Your child becomes pregnant and the study treatment could be harmful to the fetus
- The study is cancelled by the Food and Drug Administration (FDA) or the National Institutes of Health (NIH)

**What are the risks of being in this study?**

**Catheter placement:** Pain at insertion site, minor bleeding and infection may happen. Rare side effects associated with this procedure include lung puncture and severe bleeding. A chest X-ray or CT scan will be obtained to confirm the catheter location before it is used for the first time.

***POTENTIAL SIDE EFFECTS OF STUDY DRUGS***

The most common side effects of the treatments to be used in this study are listed below. There is also the risk of very uncommon or previously unknown side effects.

**Alemtuzumab**

<b>Likely</b> <i>("Likely" refers to a side effect that is expected to occur in more than 20% of patients.)</i>	<b>Less Likely</b> <i>("Less likely" refers to a side effect that is expected to occur in 20% or fewer patients.)</i>	<b>Rare, but Serious</b> <i>(These possible risks have been reported in rare occurrences, typically less than 2% of patients. They may be serious if they occur.)</i>
<ul style="list-style-type: none"> <li>• Fever</li> <li>• Chills</li> <li>• Anemia due to decreased number of red cells</li> <li>• Infection due to decreased number of white blood cells</li> <li>• Bleeding due to decreased numbers of platelets</li> <li>• Weakened immune system</li> </ul>	<ul style="list-style-type: none"> <li>• Nausea</li> <li>• Vomiting</li> <li>• Diarrhea</li> <li>• Rash</li> <li>• Headache</li> <li>• Sweating</li> <li>• Back pain</li> <li>• Severe itching</li> <li>• Allergic reaction of skin and blood vessels</li> <li>• Tiredness</li> <li>• Loss of appetite</li> </ul>	<ul style="list-style-type: none"> <li>• Abdominal pain</li> <li>• Dizziness</li> <li>• High blood pressure</li> <li>• Blisters</li> <li>• Pain in the muscles</li> <li>• Herpes simplex infection</li> <li>• Inflammation of the throat</li> </ul>

**Fludarabine**

<b>Likely</b> <i>("Likely" refers to a side effect that is expected to occur in more than 20% of patients.)</i>	<b>Less Likely</b> <i>("Less likely" refers to a side effect that is expected to occur in 20% or fewer patients.)</i>	<b>Rare, but Serious</b> <i>(These possible risks have been reported in rare occurrences, typically less than 2% of patients. They may be serious if they occur.)</i>
<ul style="list-style-type: none"> <li>• Anemia due to decreased number of red cells</li> <li>• Infection due to decreased number of white blood cells</li> <li>• Bleeding due to decreased numbers of platelets</li> <li>• Tiredness</li> <li>• Nausea</li> <li>• Vomiting</li> <li>• Weakened immune system</li> </ul>	<ul style="list-style-type: none"> <li>• Pneumonia</li> <li>• Diarrhea</li> <li>• Mouth sores</li> <li>• Skin rash</li> <li>• Fever</li> <li>• Swelling of hands and feet</li> </ul>	<ul style="list-style-type: none"> <li>• Numbness and tingling in hands and/or feet related to irritation of nerves of the hand and/or feet</li> <li>• Changes in vision</li> <li>• Agitation/nervousness</li> <li>• Confusion</li> <li>• Cough</li> <li>• Difficulty breathing</li> <li>• Weakness</li> <li>• Severe brain injury and death</li> </ul>

**Melphalan**

<b>Likely</b> <i>("Likely" refers to a side effect that is expected to occur in more than 20% of patients.)</i>	<b>Less Likely</b> <i>("Less likely" refers to a side effect that is expected to occur in 20% or fewer patients.)</i>	<b>Rare, but Serious</b> <i>(These possible risks have been reported in rare occurrences, typically less than 2% of patients. They may be serious if they occur.)</i>
<ul style="list-style-type: none"> <li>• Loss of appetite</li> <li>• Nausea</li> <li>• Vomiting</li> <li>• Skin breakdown if drug leaks from vein</li> <li>• Anemia due to decreased number of red cells</li> <li>• Infection due to decreased number of white blood cells</li> <li>• Bleeding due to decreased numbers of platelets</li> <li>• Mouth sores</li> <li>• Temporary hair loss</li> </ul>	<ul style="list-style-type: none"> <li>• Diarrhea</li> <li>• Inflammation of the lung</li> <li>• Weakness</li> <li>• Weight loss</li> </ul>	<ul style="list-style-type: none"> <li>• Low blood pressure</li> <li>• Excessive perspiration</li> <li>• Allergic reaction</li> <li>• Damage/ scarring of lung tissue</li> <li>• Sterility</li> <li>• Seizure</li> </ul>

**G-CSF (Filgrastim)**

<b>Likely</b> <i>("Likely" refers to a side effect that is expected to occur in more than 20% of patients.)</i>	<b>Less Likely</b> <i>("Less likely" refers to a side effect that is expected to occur in 20% or fewer patients.)</i>	<b>Rare, but Serious</b> <i>(These possible risks have been reported in rare occurrences, typically less than 2% of patients. They may be serious if they occur.)</i>
	<ul style="list-style-type: none"> <li>• Local irritation (skin) at injection site</li> <li>• Ache or pain inside the bones. Increased levels of liver enzymes and uric acid in the blood</li> <li>• Bleeding due to decreased numbers of platelets</li> </ul>	<ul style="list-style-type: none"> <li>• Allergic reaction, low fever</li> <li>• Enlargement or rupture of the spleen</li> <li>• Worsening of pre-existing skin rashes</li> <li>• Temporary hair loss</li> <li>• Inflammation of a blood vessel in the skin</li> </ul>

***Potential Risk of RPLS/PRES***

The Data Safety and Monitoring Board (DSMB) of the Blood and Marrow Transplant Clinical Trials Network is a group of transplant, sickle cell disease and other experts that ensure the safety of patients treated on this and other trials. This group carefully monitors the experience of patients to make sure that the side effects that they experience are not unusual or more frequent or more severe than would be expected.

The DSMB has noted that children transplanted on the clinical trial BMT CTN 0601 have a higher than expected occurrence of a usually uncommon (< 5%) complication called reversible posterior leukoencephalopathy syndrome (RPLS) also known as posterior reversible encephalopathy syndrome (PRES). Patients with RPLS/PRES have confusion and other changes in their ability to think. Sometimes, they experience seizures, sleepiness or, rarely, loss of consciousness. RPLS is diagnosed with an MRI of the brain. It is a disorder that is sometimes seen in patients with sickle cell disease even if they do not have a transplant. In transplant patients, it is usually caused by some of the drugs used to prevent or treat graft versus host disease. It can often, but not always, be prevented by very careful control of blood pressure. It is treated by changing graft versus host disease drugs, controlling blood pressure and/or giving anti-seizure medicines. About a quarter of the patients on BMT CTN 0601 have developed RPLS/PRES; all were successfully treated for this complication. Thus far, no RPLS/PRES has been observed in any patient more than 6 months from their date of transplant. We believe that children who are on prednisone or other corticosteroids, or immunosuppressive drugs such as cyclosporine or tacrolimus or have high blood pressure are more likely to develop RPLS/PRES.

**If your child experiences any of these side effects or changes in mental status, you should contact your child's transplant physician right away, since early treatment is important. It is also important that any blood pressure medication be taken as prescribed to decrease the risk of RPLS/PRES.**

**RISKS AND TOXICITIES RELATED TO STANDARD TRANSPLANT PROCEDURES**

**Graft-versus-Host Disease (GVHD):** This condition results from white cells called T cells in the donor's bone marrow cells recognizing your child's body as foreign and attacking it. Your child is more likely to get GVHD if the donor's tissue type does not match your child's tissue type well.

There are two forms of GVHD: acute GVHD (usually occurs in the first 3 months after transplant) and chronic GVHD (usually occurs later and lasts longer). Acute GVHD may produce skin rash, nausea, vomiting, diarrhea, abdominal pain, abnormalities of liver function and an increased risk of infection. Chronic GVHD may produce skin rashes, hair loss, thickened skin, joint stiffness, dry eyes, dry mouth, liver disease, weight loss, diarrhea and an increased risk of infection. To confirm the diagnosis of acute or chronic GVHD, your child may be asked to have a skin biopsy (i.e., taking a small sample of skin tissue to look at under the microscope) and possibly an intestinal biopsy and rarely a liver biopsy.

There is a 10-20% chance that your child will develop GVHD after the transplant. Your child will be watched closely for this complication and given treatment to treat it further if it occurs despite the medicines given to prevent it. In most cases, GVHD can be successfully treated. If GVHD does not respond to the medicines listed above, treatment can involve combinations of many other medicines with different side effects. Treatment may be necessary for many years as GVHD symptoms can last for many months or years. Prolonged treatment for chronic GVHD can result in a weak immune system and infections and may need frequent medical care and hospitalization. Sometimes GVHD is severe or difficult to treat and may lead to death.

### ***SIDE EFFECTS OF MEDICINES USED TO PREVENT GVHD***

The side effects listed below are usually reversible once the medicines are discontinued.

**Cyclosporine:** This drug may be used for all patients.

<b>Likely</b> <i>("Likely" refers to a side effect that is expected to occur in more than 20% of patients.)</i>	<b>Less Likely</b> <i>("Less likely" refers to a side effect that is expected to occur in 20% or fewer patients.)</i>	<b>Rare, but Serious</b> <i>(These possible risks have been reported in rare occurrences, typically less than 2% of patients. They may be serious if they occur.)</i>
<ul style="list-style-type: none"> <li>• High blood pressure</li> <li>• Kidney problems</li> <li>• Headaches</li> <li>• Nausea</li> <li>• Vomiting</li> <li>• Stomach pain or indigestion</li> <li>• Swelling of the hands or feet</li> </ul>	<ul style="list-style-type: none"> <li>• Tremors</li> <li>• Increased hair growth</li> </ul>	<ul style="list-style-type: none"> <li>• Muscle cramps</li> <li>• Numbness and tingling of the hands or feet</li> <li>• Seizure</li> </ul>

**Tacrolimus:** This drug may be used for all patients.

<b>Likely</b> <i>("Likely" refers to a side effect that is expected to occur in more than 20% of patients.)</i>	<b>Less Likely</b> <i>("Less likely" refers to a side effect that is expected to occur in 20% or fewer patients.)</i>	<b>Rare, but Serious</b> <i>(These possible risks have been reported in rare occurrences, typically less than 2% of patients. They may be serious if they occur.)</i>
<ul style="list-style-type: none"> <li>• Anemia</li> <li>• Loss of appetite</li> <li>• Diarrhea</li> <li>• High potassium levels</li> <li>• High blood pressure</li> <li>• Nausea</li> <li>• Fever</li> <li>• Headache</li> <li>• High blood sugar</li> </ul>	<ul style="list-style-type: none"> <li>• Hair loss</li> <li>• Vomiting</li> <li>• Tingling sensation in the extremities</li> <li>• Itching</li> <li>• Rash</li> <li>• Abdominal pain</li> </ul>	<ul style="list-style-type: none"> <li>• Confusion</li> <li>• Painful joints</li> <li>• Increased sensitivity to light</li> <li>• Blurred vision</li> <li>• Insomnia</li> <li>• Infection</li> <li>• Jaundice</li> <li>• Kidney injury</li> <li>• Seizures</li> </ul>

**Methotrexate:** This drug will be used as part of GVHD prophylaxis Regimen 1 for bone marrow recipients.

<b>Likely</b> <i>("Likely" refers to a side effect that is expected to occur in more than 20% of patients.)</i>	<b>Less Likely</b> <i>("Less likely" refers to a side effect that is expected to occur in 20% or fewer patients.)</i>	<b>Rare, but Serious</b> <i>(These possible risks have been reported in rare occurrences, typically less than 2% of patients. They may be serious if they occur.)</i>
<ul style="list-style-type: none"> <li>• High levels of liver enzymes</li> </ul>	<ul style="list-style-type: none"> <li>• Nausea</li> <li>• Vomiting</li> <li>• Loss of appetite</li> <li>• Diarrhea</li> <li>• Mouth sores</li> <li>• Sensitivity to sunlight</li> <li>• Increased risk of sunburn</li> <li>• Decreased number of red and white blood cells and platelets</li> </ul>	<ul style="list-style-type: none"> <li>• Hair loss</li> <li>• Dizziness</li> <li>• Redness, tenderness, darkening, and peeling of skin</li> <li>• Blurred vision</li> <li>• Allergic reaction</li> <li>• Damage to nerve tissue</li> <li>• Kidney damage</li> <li>• Seizures</li> <li>• Decreased lung function</li> <li>• Decreased liver function - temporary</li> <li>• Bone and tissue damage</li> <li>• Loss of memory, concentration, balance, and walking</li> <li>• Poor nervous system function</li> </ul>

**Methylprednisolone:** This drug will be used as part of GVHD prophylaxis Regimen 1 for bone marrow recipients.

<b>Likely</b> <i>("Likely" refers to a side effect that is expected to occur in more than 20% of patients.)</i>	<b>Less Likely</b> <i>("Less likely" refers to a side effect that is expected to occur in 20% or fewer patients.)</i>	<b>Rare, but Serious</b> <i>(These possible risks have been reported in rare occurrences, typically less than 2% of patients. They may be serious if they occur.)</i>
<ul style="list-style-type: none"> <li>• Water retention</li> <li>• Overeating</li> <li>• Weakened immune system</li> <li>• Temporary personality changes</li> <li>• Abnormal hormone production</li> <li>• High blood sugar</li> <li>• Slowed growth</li> <li>• Decreased bone density</li> </ul>	<ul style="list-style-type: none"> <li>• Headaches</li> <li>• Poor wound healing</li> <li>• Stomach swelling or pain</li> <li>• Tissue swelling</li> <li>• High blood pressure</li> <li>• Stomach ulcer</li> <li>• Muscle weakness</li> <li>• Cataracts</li> </ul>	<ul style="list-style-type: none"> <li>• Difficulty in falling asleep</li> <li>• Worsening of diabetes</li> <li>• Inflammation of pancreas</li> <li>• Personality disturbances</li> <li>• Bleeding in the stomach and intestines</li> <li>• Increased pressure within the eye</li> <li>• Disturbance of bone calcium which can lead to possible fractures or permanent bone damage</li> </ul>

**Damage to the vital organs in your body.** The conditioning or GVHD treatment could result in problems in the heart, lungs, liver, intestine, kidneys and bladder, brain etc. Lung problems can be the result of infections or chemotherapy. Some patients can have veno-occlusive disease of the liver (VOD). This complication usually results from high doses of chemotherapy. Patients with VOD become jaundiced (yellowish skin), have liver function abnormalities, fluid retention, abdominal swelling, and abdominal pain. If organ damage symptoms are severe, your child may have to stay in the hospital longer or be re-hospitalized after transplant. Although many patients recover completely, these complications may cause permanent damage or even death.

**Serious infections.** Full and complete recovery of your child's immune system may take many months following the initial recovery of your child's cell counts. During this time, there is an increased risk of infections. Your child will be prescribed certain medications to reduce the chance of those infections. However, preventive treatments are not always effective. If your child has an infection, he/she may have to stay in the hospital longer or be re-hospitalized after transplant. Although most infections can be successfully treated, some infections are fatal.

**Recurrence of disease and graft rejection.** Since the study uses a conditioning treatment regimen of reduced intensity, it may not allow donor cells to grow and your child may partially or fully reject the donor's bone marrow. If this happens, your child's blood cells will grow back again and the severe sickle cell disease may persist or come back even if the transplant is initially successful.

**Central venous catheter complications.** The most common complications associated with central venous catheters are blood clots in the catheter and infection. If clots form, a medicine will be injected to dissolve the clot. If it cannot dissolve, the catheter may need to be replaced. Infections will be treated with medicines; sometimes, removal of the infected catheter is required and a new catheter will need to be placed.

**Impact on reproductive hormone function and sexuality.** High doses of chemotherapy can cause sterility (inability to have children) and decreased hormone levels. Some patients with chronic GVHD have reported impaired sexual function due to decreased sexual desire and vaginal dryness. Since the chemotherapy doses used in the preparative regimen for this study are lower, the risk of sterility may be lower. Some patients treated with this preparative regimen have had children after their transplant. However, it is difficult to know the exact risk of sterility after transplant with the use of this conditioning regimen.

**Risk of death.** Some of the side effects of an unrelated donor transplant may be very severe and may cause death of the recipient despite using all supportive care. Though all precautions will be taken to make the transplant as safe as possible for your child, there is still a 10% chance of the patient's death following unrelated donor transplantation.

**Quality of life surveys.** Completion of the quality of life surveys will not cause you or your child any physical discomfort, although it is possible that you or your child will find some of the questions or topics upsetting. You or your child may experience emotional distress or feel a loss of privacy. If you do, there will be someone available to speak with you and your child. They will be able to refer you to appropriate counselors or other support people.

**Are there benefits to taking part in the study?**

Your child may or may not benefit from taking part in this study. If the transplant is successful, your child may benefit by not having further symptoms and complications of severe sickle cell disease. The information obtained from your child's participation in this study will help doctors treat future patients with severe sickle cell disease who require a transplant using unrelated donor bone marrow.

**What are the costs of taking part in this study?**

Most of the care given in this study is standard care; it will be billed to you or your child's insurer in the usual way. Standard costs include those of your child's hospitalization, doctor's visits, standard laboratory tests, medications, and the cost of the donor's bone marrow. There will be no charge for research tests.

**What happens if your child is injured because of participation in this study?**

In the event that this research activity results in an injury, treatment will be available, including first-aid, emergency treatment and follow-up care as needed. Care for such injuries will be billed in the ordinary manner, to your child's insurance company. If you or your child thinks that your child has suffered a research-related injury, let the study doctors know right away. It is important that you tell your child's doctor, \_\_\_\_\_ [investigator's name], if you or your child feel that your child has been injured because of taking part in this study. You can

tell the doctor in person or call him/her at \_\_\_\_\_ [telephone number]. Your child will receive medical treatment if injured as a result of taking part in this study. You or your child's insurance will be charged for this treatment.

**What are your child's rights if your child takes part in this study?**

You may choose to allow your child to either take part or to not take part in the study. If you decide to allow your child to take part in this study, your child may leave the study at any time. No matter what decision is made, there will be no penalty and your child will not lose any of his or her regular benefits. If your child leaves the study, he/she can still get medical care from your child's doctor and transplant center. We will tell you and your child about new information or changes in the study that may affect your child's health or your willingness to continue in the study. In the case of injury resulting from this study, your child does not lose any legal rights to seek payment by signing this form.

**Who can answer your and your child's questions about the study?**

You and your child can talk to your child's doctor about any questions or concerns about this study. Contact your child's doctor \_\_\_\_\_ [name(s)] at \_\_\_\_\_ [telephone number].

For questions about your child's rights while taking part in this study, call the \_\_\_\_\_ [name of center] Institutional Review Board (a group of people who review the research to protect your child's rights) at \_\_\_\_\_ (telephone number).

**Will your child's medical information be kept private?**

We will do our best to make sure that the personal information in your child's medical record be kept private. However, we cannot guarantee total privacy. Your child's personal information may be given out if required by law. If information from this study is published or presented at scientific meetings, your child's name and other personal information will not be used.

A description of this clinical trial will be available on <http://www.ClinicalTrials.gov>, as required by U.S. Law. This Web site will not include information that can identify your child. At most, the Web site will include a summary of the results. You can search this Web site at any time.

Organizations that may look at and/or copy your child's medical records and protected health information for research, quality assurance, and data analysis include:

- Members of the Blood and Marrow Transplant Clinical Trials Network (BMT CTN), which is conducting this study
- The EMMES Corporation, a research organization that is helping to coordinate this study
- The National Marrow Donor Program (NMDP) and the Center for International Blood and Marrow Transplant Research (CIBMTR), organizations involved in research on blood and marrow transplantation and in the coordination of this study
- The National Heart, Lung, and Blood Institute (NHLBI), the National Cancer Institute (NCI) and other government agencies, like the Food and Drug Administration (FDA), involved in keeping research safe for people

- The Sickle Cell Disease Clinical Research Network (SCD CRN)
- Researchers and staff members at Washington University for central review of MRI images

**Expiration date for retention of records:**

The study results will stay in your child's research record at (*insert Institution*) for at least six years or until after the study is completed, whichever is longer. At that time either the research information not already in your child's medical record will be destroyed or your child's name and other identifying information will be removed from such study results. Research information in your child's medical record will be kept indefinitely.

**How will the researcher(s) benefit from your child being in this study?**

In general, presenting research results helps the career of a scientist. Therefore, the Principal Investigator may benefit if the results of this study are presented at scientific meetings or in the scientific press. In addition, the sponsor (the NIH) is paying the Principal Investigator to conduct this study. The investigators have no financial interest in the drugs used in the study.

**HIPAA<sup>1</sup> authorization to use and disclose individual health information for research purposes:**

- Purpose:** As a research participant, I authorize the Principal Investigator and the researcher's staff to use and disclose my child's individual health information for the purpose of conducting the research study entitled *Unrelated Donor Hematopoietic Cell Transplantation for Children with Severe Sickle Cell Disease Using a Reduced Intensity Conditioning Regimen*.
- Individual Health Information to be Used or Disclosed:** My child's individual health information that may be used or disclosed to conduct this research includes: demographic information (e.g., age, date of birth, sex, weight), medical history (e.g., diagnosis, complications with prior treatment), physical examination findings, and laboratory test results obtained at the time of work up and after transplantation (e.g., blood tests, biopsy results). The identities of individuals such as names and addresses will not be shared or de-identified to make sure information cannot be linked to you.
- Parties Who May Disclose My Child's Individual Health Information:** The researcher and the researcher's staff may obtain my child's (my) individual health information from:  
(*list: hospitals, clinics or providers from which health care information can be requested*)

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<sup>1</sup> HIPAA is the Health Insurance Portability and Accountability Act of 1996, a federal law related to privacy of health information

d. Parties Who May Receive or Use My Child's Individual Health Information: The individual health information disclosed by parties listed in item c and information disclosed by my child during the course of the research may be received and used by the following parties:

- Members of the BMT CTN Data and Coordinating Center and BMT CTN #0601 Protocol Team
  - National Heart, Lung, and Blood Institute (NHLBI) and the National Cancer Institute (NCI), both of the National Institutes of Health (NIH), study sponsors
  - The National Marrow Donor Program and the Center for International Blood and Marrow Transplant Research
  - U.S. government agencies that are responsible for overseeing research such as the Food and Drug Administration (FDA) and the Office of Human Research Protections (OHRP)
  - U.S. government agencies that are responsible for overseeing public health concerns such as the Centers for Disease Control (CDC) and federal, state and local health departments
  - The Sickle Cell Disease Clinical Research Network (SCD CRN)
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e. Right to Refuse to Sign this Authorization: I do not have to sign this Authorization. If I decide not to sign the Authorization, my child will not be allowed to participate in this study or receive any research-related treatment that is provided through the study. However, my decision not to sign this authorization will not affect any other treatment, payment, or enrollment in health plans or eligibility for benefits.

f. Right to Revoke: I can change my mind and withdraw this authorization at any time by sending a written notice to the Principal Investigator to inform the researcher of the decision. If I withdraw this authorization, the researcher may only use and disclose the protected health information already collected for this research study. No further health information about my child (me) will be collected by or disclosed to the researcher for this study.

g. Potential for Re-disclosure: My child's individual health information disclosed under this authorization may be subject to re-disclosure outside the research study and no longer protected. Examples include potential disclosures for law enforcement purposes, mandated reporting or abuse or neglect, judicial proceedings, health oversight activities and public health measures.

h. This authorization does not have an expiration date. However, you can elect at any time to withdraw your authorization to participate in the study.

You will receive a copy of this form. If you (or your child) need more information about this study, ask the study doctor.

\*\*\*\*\*

**SIGNATURE**

**I have read the information in this consent form and have had the study explained to me. My questions have been answered to my satisfaction. I agree to allow my child to participate in the study.**

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**Signature of Subject's Mother/Guardian**

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**Date**

---

***Printed Name of Subject's Mother/Guardian***

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**Signature of Subject's Father/Guardian**

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**Date**

---

***Printed Name of Subject's Father/Guardian***

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**Signature of Patient/Study Subject (if greater than or equal to 18 years of age)**

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***Printed Name of Patient/Study Subject (if greater than or equal to 18 years of age)***

**I certify that the nature and purpose, the potential benefits, and possible risks associated with participation in this research study have been explained to the above individual(s) and that any questions about this information have been answered.**

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**Signature of Physician Obtaining Consent**

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**Date**

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***Printed Name of Physician Obtaining Consent***

## Assent to Participate in Research (Ages 7 to 11 years old)

### Unrelated Donor Reduced Intensity Bone Marrow Transplant for Children with Severe Sickle Cell Disease

You are being invited to be in a research project. This research project is about seeing if transplants can help children who have severe sickle cell disease. People with sickle cell disease do not make healthy red blood cells. In this research project, doctors will see if a bone marrow transplant can help children with sickle cell disease make healthy red blood cells. You should talk to your parents about this research project. If you have questions, ask your parents or your doctor.

Before the transplant, your doctors will give you medicines so that your body will let the new cells grow. These medicines are not as strong as the ones that have been used before and are called reduced intensity medicines. The medicines may make you throw up, lose your hair or have mouth sores.

After the medicines, you will get a transplant of new cells from an unrelated donor. An unrelated donor is a person you do not know. The cells will come from the donor's bone marrow. The cells should make new and healthy red blood cells in your body. Sometimes the donor's cells can cause a problem called GVHD. GVHD can give you diarrhea, a skin rash, or make you not feel hungry. Your doctors will give you medicines to try to make sure that you don't get GVHD. Sometimes the donor cells may not establish themselves and grow in your body. If donor cells are rejected from your body, your own blood cells will grow again and your sickle cell disease problems will come back.

You will stay in the hospital for several days before your transplant and for about four weeks after your transplant. After you go home, you will need to go back to see your doctors often.

You don't have to be in this research project. Your doctors and nurses will not be mad at you if you don't want to be in the research project. If you decide you don't want to be in this research project, you should talk to your doctor and parents about other things to do for your disease.

Sign your name on the line below if you want to be in this research project. You can keep a copy of this form at home.

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Minor's Signature

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Date

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*Print Name of Minor*

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*Age of Minor*

Certification of Counseling Healthcare Professional: I certify that the nature and purpose, the potential benefits, and possible risks associated with participation in this study have been

explained to the above individual and that any questions about this information have been answered.

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Counseling Healthcare Professional

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Date

## **Assent to Participate in Research (Ages 12 to 17 years old)**

### **Unrelated Donor Reduced Intensity Bone Marrow Transplant for Children with Severe Sickle Cell Disease**

Patients with sickle cell disease have a defect in hemoglobin. Hemoglobin is a protein in red blood cells (RBCs) that carries oxygen to vital organs such as the brain, heart, lungs and kidneys. When hemoglobin is damaged the RBCs are sticky and look like a sickle. The damaged RBCs cannot flow well through blood vessels, and block oxygen and nutrients from reaching organs. This can damage almost all the organs in the body. It is especially bad when vital organs are involved.

Recent studies have shown that a bone marrow transplant from a brother or sister can replace the damaged RBCs with healthy red blood cells. Because of the risks of a bone marrow transplant, transplants are usually only given to patients with severe complications. Usually a bone marrow transplant is only done if the patient has a brother or sister as a donor who has the same ‘tissue’ type. Before a transplant, patients receive high doses of chemotherapy called conditioning to allow donor cells to grow in the patient. Since most patients with severe sickle cell disease do not have a brother or sister with the same tissue type, they do not receive a transplant. These patients often receive medicines and red cell transfusions to try to control symptoms and organ damage. These treatments may be given for many years.

This is a research study of unrelated donor transplantation in patients with severe sickle cell disease. There will be as many as 30 patients with sickle cell disease participating in this research study. It is being done to learn if the intensity of the conditioning can be reduced to reduce the side effects or toxicities and if bone marrow transplants from unrelated donors can help patients with sickle cell disease make healthy red blood cells. In this study, patients who do not have a brother or sister with the same tissue type can receive a transplant from an unrelated donor. The cells for the transplant can come from bone marrow. Bone marrow cells are donated by volunteers who agree to donate some of the cells made in their bone marrow.

You are being invited to join this research study because you have severe sickle cell disease. Because your red blood cells cannot flow well through your blood vessels, this has caused problems with your brain, lungs, or other parts of your body. That is the reason that you have pain, breathing difficulties or weakness. Your doctors think that a transplant may be an option for you.

This form gives you information to help you decide if you want to be in this study. You should read this form and ask any questions you have before agreeing to be in the study. It is up to you to decide if you want to be in the study.

**What other choices do I have if I do not take part in the study?**

If you decide not to participate in this study, your doctor will discuss other treatment options with you and your parents. Other choices may include:

- Treatment with drugs such as hydroxyurea that can lessen complications of severe sickle cell disease
- Experimental treatments with new drugs for severe sickle cell disease
- A transplant using bone marrow without being part of this study
- Other treatments such as regular RBC transfusions to try to control symptoms related to your severe sickle cell disease

**Why is this study being done?**

This research study is being done to answer the following questions:

1. Is it safe to do a transplant in patients who have severe sickle cell disease using bone marrow from healthy unrelated donors?
2. Is a “reduced intensity” transplant effective and safe in performing unrelated donor transplants? This “reduced intensity” approach will use a new combination of drugs at reduced doses compared to those previously used for traditional transplants.
3. After the transplant, will the patients with severe sickle cell disease make healthy red blood cells and be protected from health problems of severe sickle cell disease?

**What will happen if I take part in this research study?****Before enrolling on study:**

Your doctor will check to see if you have a type matched bone marrow donor available for your transplant.

**Before the transplant:**

You will have several tests done to check your organ function. These tests will check your heart, lungs, and brain. Most of these tests are X-rays or scans, questions, or blood tests. The doctors will look at the results of all these tests to make sure that it is okay for you to have a transplant.

A central line will be placed in your chest in the operating room (you will be asleep for this). A central line makes it easier for you to receive drugs and for drawing blood for tests (you will not be poked for blood tests or receive shots).

**Preparation for the transplant:**

Before the transplant, you will need to receive medicines so that your body can accept the new bone marrow cells. This is called a ‘preparative regimen.’ Before the transplant, you will be given 3 drugs. You will get the first drug called alemtuzumab for 4 days about 3 weeks before your transplant. You will go home and return to the hospital to stay 8 days before your transplant. At that time you will get two more drugs called fludarabine and melphalan. All these drugs are given through your central line.

Three days before the transplant, you will also get medicines to suppress your immune system.

These medicines are given to allow the donor cells to grow. There are many drugs that can be used to suppress your immune system. The names of these medicines are tacrolimus, methylprednisolone/prednisone, methotrexate, and cyclosporine.

**Bone marrow - infusion of cells:**

Bone marrow from an unrelated donor will be used for the transplant. These cells will be given through the central venous catheter, just like a blood transfusion. On the day of your transplant, the new bone marrow cells will be given through your central line.

**Post-transplant follow-up and care:**

After the transplant you will continue to get medicines to help the donor cells grow. These drugs will also help lower the chance of getting graft versus host disease (GVHD). GVHD is a complication that happens when the donor's cells attack your body. You will receive one or more medicines to prevent GVHD. You will continue to receive these drugs for at least 6 months after the transplant.

You will be in the hospital for about four weeks after your transplant. You will be allowed to go home from the hospital when your doctor feels it is safe. After you go home you will need to return to visit your doctors so they can check your recovery. Your doctors will need to check your blood and bone marrow after the transplant to make sure the new blood cells are growing in your body. Your doctors will also do blood tests and other tests to make sure your organs are working well. When blood is needed for these tests it will be drawn through the central line.

**Can the doctor who is the Principal Investigator withdraw me from this study?**

You can be taken off the study (with or without your consent) for any of the following reasons:

- You need a medical treatment not allowed in this study
- The investigator decides that continuing in the study would be harmful to you
- You become pregnant and the study participation could be harmful to the fetus
- The study is cancelled by the Food and Drug Administration (FDA) or the National Institutes of Health (NIH)

**What are the risks of being in this study?**

The drugs may cause a skin rash, hair loss, nausea and vomiting, diarrhea and infections. Your blood counts will fall and you may get fevers, infections or start bleeding. You may also get mouth sores. These are temporary and you will feel better as your new bone marrow grows.

Since you will not be able to fight infections while your new bone marrow is growing back, you may need to get antibiotics. You may also need to get blood transfusions since your new bone marrow will not be making new blood cells right away.

Even with medicines to prevent it, you may get GVHD. This can cause skin rash, vomiting, diarrhea, stomach pain, lung and liver problems, swelling of the hands and feet, dry eyes, stiff joints, and tiredness. These problems are usually mild but can become very serious and prolonged. Medicines are given to prevent GVHD during and after transplant. If GVHD occurs even after taking these medicines, other medicines will need to be started and hospital stays may

be necessary. The medicines used to treat GVHD also have side effects. They can cause tiredness, depression, sleep problems and mood swings. They can also make you get severe infections very easily. Your doctors will do their best to make you feel better and keep you safe. Often this may require many hospital stays. However, it is important to understand that there is a small risk (about a 1 in 10 chance) that you may die as a result of one or more of the complications of unrelated donor transplantation.

It is possible that instead of new bone marrow, your old red blood cells will grow back. If it does, you will continue to have severe sickle cell disease and its problems.

**Are there benefits to taking part in the study?**

You may or may not benefit from taking part in this study. If the transplant is successful, you may benefit by not having further symptoms and complications of severe sickle cell disease. The knowledge gained from this study may help other patients with severe sickle cell disease.

**What are your rights if you decide to take part in this study?**

It is up to you if you want to participate in this research study. If you leave the study you can still get medical care from your doctor and transplant center. You will be told about new information or changes in the study that may affect your health or your willingness to continue in the study.

**Will your medical information be kept private?**

We will do our best to make sure that the personal information in your medical record be kept private. However, we cannot guarantee total privacy. Your personal information may be given out if required by law. If information from this study is published or presented at scientific meetings, your name and other personal information will not be used.

You will receive a copy of this form. If you need more information about this study, ask the study doctor.

\*\*\*\*\*

**Minor's Assent**

I have read the information in this consent form and have had the study explained to me. My questions have been answered to my satisfaction. I agree to participate in the study.

\_\_\_\_\_  
Signature of Minor\_\_\_\_\_  
Date\_\_\_\_\_  
*Print Name of Minor*\_\_\_\_\_  
*Age of Minor***Certification of Counseling Healthcare Professional**

I certify that the nature and purpose, the potential benefits, and possible risks associated with participation in this study have been explained to the above individual and that any questions about this information have been answered.

\_\_\_\_\_  
Counseling Healthcare Professional\_\_\_\_\_  
Date

## APPENDIX C

### LABORATORY PROCEDURES

#### Red Blood Cell Pit Score Studies

**Pitted red cell count:** Blood samples will be drawn from recipients within 60 days of conditioning, fixed in formalin, and sent to Children's Hospital of Oakland for central analysis. Testing will be repeated 2 years post-transplant in similar fashion to assess splenic function, and samples will be processed as described below:

#### SAMPLE COLLECTION AND SHIPPING INSTRUCTIONS

Children's Hospital Oakland will provide the collection tubes.

*Please keep tubes refrigerated until use.*

#### To collect sample:

- Immediately after blood draw, add one small drop (about 30 µl) of whole EDTA blood to collection tube. Please **DO NOT** add more than one (1) small drop. Make sure blue cap is on securely.
- **MIX GENTLY BUT THOROUGHLY**. If this is not done, cells will clump together and sample will be unreadable.
- Store at 4°C.

#### To ship sample:

Ship overnight Monday through Thursday (for receipt Tuesday through Friday) in Styrofoam box with **WET** ice to:

**Attn: Mahin Azimi, MT**  
**Hemoglobinopathy Lab – Rm 202**  
**Children's Hospital Oakland**  
**747 52<sup>nd</sup> Street**  
**Oakland, CA 94609**  
**(510) 450-7688**

- Be sure and mark outside of box: **DO NOT FREEZE**.
- Please notify Mahin Azimi, MT prior to sending samples.

**APPENDIX D****LANSKY/KARNOFSKY PERFORMANCE STATUS SCALES****LANSKY SCALE < 16 YEARS**

Percentage	
100	Fully Active
90	Minor restriction in physically strenuous play
80	Restricted in strenuous play, tires more easily, otherwise active
70	Both greater restrictions of, and less time spent in, active play
60	Ambulatory up to 50% of time, limited active play with assistance/supervision
50	Considerable assistance required for any active play; fully able to engage in quiet play
40	Able to initiate quiet activities
30	Needs considerable assistance for quiet activity
20	Limited to very passive activity initiated by others (e.g., TV)
10	Completely disabled, not even passive play
0	Dead

**KARNOFSKY SCALE  $\geq$  16 YEARS**

Percentage	
100	Normal, no complaints, no evidence of disease
90	Able to carry on normal activity; minor signs or symptoms of disease
80	Normal activity with effort; some signs or symptoms of disease
70	Cares for self; unable to carry on normal activity or do active work
60	Requires occasional assistance, but is able to care for most of his/her needs
50	Requires considerable assistance and frequent medical care
40	Disabled; requires special care and assistance
30	Severely disabled, hospitalization indicated. Death not imminent
20	Very sick, hospitalization necessary, active supportive treatment necessary
10	Moribund, fatal processes, progressing rapidly
0	Dead

## **APPENDIX E**

### **ELIGIBILITY REVIEW PANEL**

Patients will be screened for protocol eligibility by a five-member review panel composed of experts in hemoglobinopathies and transplantation. Approval for transplant is based on data contained in the patient registration and pre-transplant forms, as well as in supporting documentation. If the majority of panel review members and the Principal Investigators agree that a patient meets eligibility criteria, the Protocol Coordinator will contact the Transplant Center Coordinator and confirm that the patient can be enrolled.

The Review Panel will include: hemoglobinopathy experts, Dr. George Buchanan (Dallas), Dr. Jim Eckman (Emory, Atlanta), Dr. Alexis Thompson (Northwestern Chicago); and, transplant physicians Dr. John DiPersio (St. Louis) and Dr. Catherine Wu (Boston). Review materials will be sent to the panel at least two weeks prior to enrollment to confirm eligibility. A panel member will recuse him/herself if a patient from his/her center is being discussed.

HLA typing will be reviewed by the Protocol Chairs and Protocol Officer to determine that the patient and donor are appropriately HLA-matched.

## APPENDIX F

### HEALTH-RELATED QUALITY OF LIFE ASSESSMENT

The CHQ Parent Form 50 and Child Form 87 health-related QOL measures will be administered *pre-transplant, at Day 100, 6 months, 1 year, and 2 years post-transplant* to all parents of children 5 years of age or greater and to children greater than 10 years of age<sup>78, 79</sup>. The forms will be self completed by the parent and/or child during the routine clinic visit. It is expected that it will take approximately 20 minutes for the parents to complete the form and 30 minutes for the child.

Child Health Questionnaire Parent Form 50: To be administered to all parents with children who are 5 to 18 years of age at the time of the QOL assessment.

Child Health Questionnaire Child Form 87: To be administered to all children who are 10 to 18 years of age at the time of the QOL assessment. The form is intended to be self completed by the child without help from the parent. If the child needs help completing the survey, this should be done with the aid of a research assistant who can read the questions and responses to the child.

The information below from the User's Manual of the CHQ should aid in administering the questionnaires.

#### ADMINISTERING THE CHILD HEALTH QUESTIONNAIRE

(From Landgraf, Abetz, and Ware, Child Health Questionnaire (CHQ): A User's Manual, 1999)

The Child Health Questionnaire (CHQ) is intended to be completed by the individual without the help of an administrator. The administrator should present the importance of the questionnaire and why their responses to the questions will be helpful in understanding the quality of life of children with silent stroke. The administrator should also ensure the questionnaires are completed and done correctly.

The CHQ is designed to be able to be read and understood by all those with a 3<sup>rd</sup> grade reading level. If the person completing the questionnaire is not able to read at this level, the form should be read to them using the interview script designed for the questionnaire (please see Interview Script section).

Each questionnaire should contain a unique identifier (this should be the subject's study ID number) that will be used to keep the information confidential yet linked to each study subject.

## INTRODUCING THE CHQ

Spouses, or other family members, or visitors, **should not** assist parents in completing the CHQ.

*The CHQ was designed to provide reliable information about the everyday functioning and well-being of children in ways that matter most to them and their families. The CHQ asks questions about your child's physical wellness, his/her feelings, behavior, and activities at school and with family and friends. The parent-completed CHQ also asks a few questions about you.*

*The CHQ is simple to complete. Be sure to read the instructions [point to them]. The CHQ contains questions that ask how you feel. Remember, there are no right or wrong answers. This is not a test. Choose the response that best represents the way you feel. Please do not share or compare responses with your child or other family members.*

*Please fill out the questionnaire now. I will be nearby in case you want to ask me any questions. Return your completed questionnaire to me.*

## ADMINISTERING AND COMPLETING THE HEALTH QUESTIONNAIRE

Provide a firm writing surface such as a clipboard or tabletop. Provide a number 2 pencil.

When the parent returns the CHQ, check the questionnaire for completeness. Note whether the questionnaire is complete by simply scanning the pages of the questionnaire. If it is not complete, bring the missing section or questions to the respondent's attention. If they chose not to answer a series of questions, gently encourage them to do so. If there are more than half of the items missing for a scale the data will be discarded and it will not be possible to calculate their responses. If the parent/child has an objection or difficulty completing any items or sections, simply record their reason(s) for non-completion. Never force someone to answer if they do not feel comfortable doing so on their own

### Closing

Be sure to put the completed questionnaires in a safe and secure place to ensure confidentiality.

Finally, thank the parent using the following exit script (or a variation appropriately reworded to sound more like your style of speech):

*Thank you for taking the time to complete this questionnaire. You will complete this questionnaire again at the end of the study.*

## ADDRESSING PROBLEMS AND QUESTIONS

### ***What to do if the CHQ parent doesn't want to complete the CHQ?***

If the parent is able to self-administer the CHQ but declines to participate, tell the parent that its completion is voluntary. They are being asked to complete the CHQ because it will provide helpful information for clinicians, school nurses, teachers, and others. The goal is to better understand the physical, mental, and social health problems of children

Emphasize that this information is as important as any of the other medical information. Responses are essential so that a complete picture of the child's health and its effect on the parent and family may be obtained. Emphasize that the CHQ is simple to complete. Suggest that it is possible that this questionnaire is different from others they have filled out in the past, and that they may even enjoy completing it. If parent still declines, retrieve the CHQ, record the reason for the decline, and thank the parent

***What to do if the CHQ parent starts to complete the CHQ, but doesn't want to finish?***

If non-completion is a result of the parent having trouble understanding particular items, ask the parent/child to explain the difficulty. Reread the question for them verbatim, but do not rephrase the question. If the parent is still unable to complete the CHQ, accept it as incomplete, and indicate on the CHQ itself that the parent was unable to complete the questionnaire due to difficulty understanding questions.

If the parent is unable to self-administer the questionnaire, document the reason. If the reason is health related, indicate the specific physical problem/condition.

***What to do if the CHQ parent is concerned that someone will look at their answers?***

Emphasize that all parents' responses to the CHQ are to be kept confidential. You are not allowed to read the responses other than to check that all responses are answered. Point out that their names do not appear anywhere on the questionnaire, so that their results will be linked with an ID number and not their name. If this is for a clinical study, tell parents that their answers to the questionnaire will be pooled with other parents' answers and that they will be analyzed as a group rather than as individuals

***What to do if the CHQ parent asks the meaning of an item?***

While completing the questionnaire, some parents might ask the meaning of specific items so that they can better understand and respond, if this happens, you can assist the parent/child by rereading the question for them *verbatim*. If the parent/child asks you to interpret the meaning of an item, do not try to explain it, but suggest that he/she use his/her own interpretation of the question. All parents should answer the questions based on what *they* think the questions mean.

Sometimes parents may have trouble with the *response choices*. They may say "I don't know" or something different than what is stated on the questionnaire. In these circumstances, it is important to gently guide the parent to respond in one of the pre-set categories by saying something like:

*"I know that it may be hard for you to think this way, but which of these categories most closely expresses what you are thinking or feeling?"*

If the parent does not like a question, or thinks it is unnecessary or inappropriate, emphasize that all questions in the CHQ are very important and included for different reasons. They should try to answer all of the questions.

Rewording items, paraphrasing or interpreting items for the parent can bias results. Thus, it is important that differences in answers due to rewording of items be minimized. If the parent has difficulty completing the CHQ and you feel you cannot address their concerns adequately with the instructions and guidance provided, thank them, retrieve the CHQ, and record the difficulty

***What to do if the CHQ parent wants to know the meaning of their answers?***

If a parent asks you to interpret responses or asks for a "score" on the CHQ, tell him/her that you are not trained in interpreting or scoring the information. Emphasize that their responses are to be kept confidential. You are not allowed to read the responses other than to check that all responses are answered. If this is for a clinical study, tell parents that their answers to the questionnaire will be pooled with other parents' answers and that they will be analyzed as a group rather than as individuals.

***What to do if the CHQ parent asks why they must fill out the CHQ additional times?***

Optional - only appropriate in situations or studies with multiple administration of the CHQ.

Explain to the parent that the reason they are being asked to complete the CHQ more than once is to determine if the child's health or well-being has changed over time. Monitoring changes provides a more complete and appropriate representation of the child's health and its impact on their everyday functioning and well-being and that of their family

## CHQ ADMINISTRATION DO'S AND DO NOT'S

DO's	DO NOT's
<b>Do</b> check to make sure that all items, response choices, and scales on your questionnaire are the verbatim replicas of the CHQ <b>before</b> fielding your questionnaire	Do <b>not</b> reorder items, response choices, or scales in the CHQ
<b>Do</b> have the parent answer, the CHQ <b>before</b> they fill out any health data forms and they see their physicians (if appropriate)	Do <b>not</b> discuss the child's health, or emotions with the child or parent <b>before</b> they complete the CHQ
<b>Do</b> be warm, friendly, and helpful	Do <b>not</b> minimize the importance of the CHQ
<b>Do</b> request and encourage the parent to complete the CHQ	Do <b>not</b> force or command the parent to complete the CHQ
<b>Do</b> read and repeat a question <b>verbatim</b> for the parent/child	Do <b>not</b> interpret or explain a question
<b>Do</b> tell the parent to answer a question based on what <b>they think</b> the question means	Do <b>not</b> accept an incomplete questionnaire without first encouraging the parent to fill out unanswered questions
<b>Do</b> have the parent fill out the CHQ <b>by themselves</b>	Do <b>not</b> allow spouses or family members to help the parent fill out the CHQ
<b>Do</b> encourage the parent to complete all questions	Do <b>not</b> force or command the parent to complete a particular question
<b>Do thank</b> the parent for completing the CHQ	
Optional: <b>Do</b> inform parent that they will be asked to complete the CHQ more than once-at the beginning and end of the study	

## APPENDIX G

### NEUROCOGNITIVE TESTING

**Neurocognitive assessment:** Age-appropriate neurocognitive evaluation will be performed in all patients prior to, and 2 years after transplant to document effects of transplant on cognitive function. If a patient has crossed age groups between two testing periods, the child will be administered the same battery of tests completed at pre-screening but at the age-appropriate level. Please see the CRFs for the sub tests of each tool.

Time scheduled for each assessment: 2 hours

**For children  $\geq 6$  years:** Wechsler Abbreviated Scale of Intelligence (WASI) (Block Design, Similarities, Matrix Reasoning, Vocabulary), Continuous Performance Test-Second Edition (CPT-II), Berry-Buktenica Developmental Test of Visual-Motor Integration (VMI), Children's Memory Scale (CMS), California Verbal Learning Test (CVLTC), Adaptive Behavior system-Second Edition (ABAS-II), and BRIEF.

**Age 4 and 5 years:** Wechsler Preschool and Primary Scale of Intelligence (WIPPSI-III) (Block Design, Information, Matrix Reasoning, Vocabulary, Picture Concepts, Word Reasoning), ABAS-II, VMI, and BRIEF-P (parent to complete).

**Age 3 years:** Wechsler Preschool and Primary Scale of Intelligence (WIPPSI-III) (Receptive Vocabulary, Block Design, Information, Object Assembly, Picture Naming, ABAS-II, VMI, and BRIEF-P (parent to complete).

## APPENDIX H

### CRANIAL MAGNETIC RESONANCE IMAGING (MRI) PROTOCOL

Cranial Magnetic Resonance Imaging (MRI) and Magnetic Resonance Angiography (MRA) will be performed within 30 days of commencing conditioning for transplant according to this protocol. A similar scan will be repeated at 2 years post-transplant to evaluate change in previously existing lesions or identify and define the presence of any new onset lesions.

#### Preparing MRI Imaging CD

Images will be saved to CDs in Digital Imaging and Communications in Medicine (DICOM) format. DICOM is a standard format for handling, storing, printing, and transmitting information in medical imaging. The imaging CD will be shipped with a copy of the patient's signed informed consent form to Washington University for central review at the following address:

Washington University Laboratory: MIR  
Attn: Robin Haverman  
510 South Kingshighway, Box 8131  
St. Louis, Missouri 63110  
Phone: 314-747-1624

#### Study Description: MRA/MRI

Protocol Scan Time = 15 to 20 minutes.

Standard Head Coil (circularly polarized or quadrature)

**1. Scout Localizer (3 planes) Time: 9 sec**

**2. Fast/Turbo FLAIR T2-weighted Axial and Coronal Acquisition** (Must cover the whole brain)

Time: 5:42 min approx (for TR 9000ms)

<p>Alignment AC-PC line (undersurface of the genu &amp; splenium of the corpus callosum)</p> <p>TR = 10,000ms (acceptable value 9000- 10000 ms)</p> <p>TE = 125ms(acceptable range: 90ms- 130ms)</p> <p>TI = Dependent on TR. Set to null CSF signal (acceptable range 2000-3000)</p> <p>[for TR of 9 seconds, TI = 2500ms; for TR of 10 seconds, TI = 2308ms)</p> <p>Echo Train Length = 7 (acceptable range is 5 - 11)</p>	<p>Echo Train Flip Angle: 180 degrees</p> <p>1 acquisition</p> <p>Matrix 256 (AP) x 192 (L-R)</p> <p>FOV 210mm (acceptable range is 210 –230 mm)</p> <p>Slice Thickness = 5mm</p> <p>No intersection gap is desirable (acceptable intersection gap 0 - 1mm)</p>
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**3. T1-weighted Acquisition (for segmentation and volumetrics)**

<b>Option 1:</b> 3-D T1-weighted fast gradient echo: (MPRAGE or IRprep-3DSPGR) Time: 7:07 min approx (for TR 1900)	<b>Option 2:</b> T1-weighted Sagittal and Axial Acquisitions Time: 1:38 min approx (for TR 500ms) for Sagittal Time: 1:38 min approx (for TR 500ms) for Axial	
Orientation = Sagittal TR= 1900 ms TE= ~4ms	<b>Orientation 1</b> = Sagittal (Must cover the whole brain)	<b>Orientation 2</b> = Axial (Must cover the whole brain)
TI= 1100 ms Slab Thickness=160mm (acceptable range = 160-180mm)	TR 500ms (acceptable range is 400-800ms)	Aligned to match the orientation of the FLAIR acquisition
Slab Partitions = 128 (acceptable range = 120-144) Slab Partition Thickness= 1.25 mm (acceptable range 1.25 – 1.5mm)	TE 12ms (use minimum value, acceptable range 10-30ms)	TR 500ms (acceptable range is 500-800ms)
FOV= 256 mm Matrix= 222 x 256	1 acquisition Matrix 256 (AP) x 192 (L-R)	TE 12ms (use minimum value, acceptable range 10-30ms)
Resolution = 1.0 x 1.0 x 1.25mm (acceptable limit = 1.0 x 1.0 x 1.5)	FOV 210mm (acceptable range is 210 –230 mm)	1 acquisition
	Slice Thickness = 5mm No intersection gap is desirable (acceptable intersection gap 0 - 1mm)	Matrix 256 (AP) x 192 (L-R) FOV 210mm (acceptable range is 210 –230 mm)
		Slice Thickness = 5mm No intersection gap is desirable (acceptable intersection gap 0 - 1mm)

**4. Fast/Turbo Spin Echo T2-weighted Axial Acquisition (Must cover the whole brain)**

Time: 1:16 min approx (for TR 4000ms)

Aligned to match the orientation of the FLAIR acquisition TR = 5000ms (acceptable range: 3000-6000ms) TE = 100ms (acceptable range: 90ms - 110ms) Flip Angle = 180 degrees 1 acquisition	Echo Train Length = 7 (acceptable range is 5 - 11) Matrix 256 (AP) x 192 (L-R) FOV 210mm (acceptable range is 210 –230 mm) Slice Thickness = 5mm No intersection gap is desirable (acceptable intersection gap 0 - 1mm)
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**Extended MRI Protocol**

Extended Protocol Scan Time = 15 to 20 minutes (As time allows for appropriate subjects)

**5. Echo-Planar Axial Diffusion Acquisition** (Repeated 2 times. Do not use signal averaging.)

Time: 45 sec (for TR 6000ms) x 2 = 1:30 min approx.

Aligned to match the orientation of the FLAIR acquisition (if possible) TR = 6000ms (allowable range: 3000ms – 6000ms) TE = 90ms (minimum value desirable, allowable range: 75 – 140ms) 1 acquisition Matrix: 128x128	FOV: 230 mm Slice Thickness = 5mm No gap between slices: (must not allow an intersection gap) b values: 0, 1000 s/mm <sup>2</sup> Gradient Orientation: Minimum X, Y, Z for computation of the trace of the tensor Desirable: 6 or more directions to compute the tensor
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**6. 3D Time-of-Flight Magnetic Resonance Angiography**

Time: 9:12 min approx (for 192x512 matrix)

Axial slab centered on the supraclinoid internal carotid artery. (MOTSA = multiple thin slab acquisition strategy is desirable) Slab partitions > 60, with resolution < 1mm. Number of Slabs= 3 TE minimum (< 5ms)	Flip Angle = 25 degrees, optimized for TR (TONE or variable RF pulse flip angle desirable). Matrix: 192 x 512 (acceptable range up to 512 x 512) FOV: minimum allowable for TE < 5ms, and for head size. MT pulse is acceptable to improve background suppression. Venous (Cephalad) presaturation pulse
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Protocol Scan Time = 30-40 minutes

## APPENDIX I

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