Transplantation Using Reduced Intensity Approach for Patients With Sickle Cell Disease From Mismatched Family Donors of Bone Marrow (TRANSFORM)

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SYNOPSIS

Transplantation using **R**educed Intensity **A**pproach for Patie**n**ts with **S**ickle Cell Disease from Mismatched **F**amily Don**or**s of Bone **M**arrow (TRANSFORM Study)

Study Chairpersons:

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Study Sites:

Children's Healthcare of Atlanta; Winship Cancer Institute, Emory University

Primary Objective: The primary objective is to estimate the event-free survival (EFS) at 1 year after allogeneic in children and young adults with severe sickle cell disease (SCD) following hematopoietic cell transplantation (HCT) from a haploidentical family donor.

Secondary Objectives: Secondary objectives focus on the incidence of transplant-related toxicities. The latter include: time to neutrophil and platelet recovery; grades II-IV and III-IV acute graft-versus-host disease (GVHD); chronic GVHD; hepatic veno-occlusive disease (VOD); idiopathic pneumonia syndrome (IPS); central nervous system (CNS) toxicity (posterior reversible encephalopathy syndrome [PRES], hemorrhage, and seizures); the incidence of significant bacterial, fungal and viral infection (e.g., cytomegalovirus [CMV] reactivation and infection; adenovirus infection; Epstein Barr virus [EBV] post-transplant lymphoproliferative disease[PTLD]); and the incidence of stable mixed chimerism.

Study Design: We will enroll and take to transplant 15 subjects with severe SCD who have a haploidentical donor.

Accrual Objective: The sample size includes 15 transplant recipients.

Accrual Period: The estimated accrual period is 3 years.

Eligibility Criteria:

a) Sickle cell disease, Transfusion dependent Thalassemia, sideroblastic anemia or diamond blackfan anemia

- Patients with severe SCD with any clinically significant sickle genotype, for example, Hemoglobin SS (Hb SS), Hemoglobin SC (Hb SC), Hemoglobin S Beta thalassemia (Hb Sβ), Hemoglobin S-OArab genotype, or Hemoglobin SD] who have 1 or more of the following (i-v):
  - **i.** Clinically significant neurologic event (stroke) or any neurological deficit lasting > 24 hours;
  - **ii.** History of two or more episodes of acute chest syndrome (ACS) in the 2-year period preceding enrollment despite the institution of supportive care measures (i.e. asthma therapy and/or hydroxyurea);
  - **iii.** History of an average of three or more severe pain crises per year in the 2-year period preceding enrollment despite the institution of supportive care measures (i.e. a pain management plan and/or treatment with hydroxyurea);
  - **iv.** Administration of regular RBC transfusion therapy, defined as receiving 8 or more transfusions per year for ≥ 1 year to prevent vaso-occlusive clinical complications (i.e. pain, stroke, and ACS). Patients on chronic transfusion who have to discontinue transfusion because of allo-sensitization will be eligible.
  - **v.** An echocardiographic finding of tricuspid valve regurgitant jet (TRJ) velocity ≥ 2.7 m/sec. Patients under the age of 18 years must have cardiac catheterization proven pulmonary arterial hypertension to qualify on this eligibility criterion.

vi. Ongoing high impact chronic pain on a majority of days per month for ≥ 6 months as defined as ONE or more of the following: Chronic pain without contributory SCD complications[2], OR Mixed pain type in which chronic pain is occurring at site(s) (arms, back, chest, or abdominal pain) unrelated to any sites associated with Contributory SCD complications[2] (e.g. leg ulcers and/or avascular necrosis).

b) Age: Patients must be 3 - 40 years of age inclusive.

c) Adequate physical function as measured by:

i. Karnofsky/Lansky performance score ≥ 60

ii. Cardiac function: Left ventricular ejection fraction (LVEF) > 40% or LV shortening fraction > 26% by cardiac echocardiogram or by MUGA scan.

iii. Pulmonary function: Pulse oximetry with a baseline O_2 saturation of ≥ 85% and DLCO > 40% (corrected for hemoglobin).

iv. Renal function: Serum creatinine ≤ 1.5 x the upper limit of normal for age as per local laboratory and 24-hour urine creatinine clearance > 70 mL/min/1.73 m^2 or GFR > 70 mL/min/1.73 m^2 by radionuclide GFR.

v. Hepatic function: Serum conjugated (direct) bilirubin < 2 x upper limit of normal for age as per local laboratory and ALT and AST < 5 x upper limit of normal as per local laboratory. Patients with hyperbilirubinemia as a consequence of hyperhemolysis, or who experience a sudden, profound change in the serum hemoglobin after an RBC transfusion, are not excluded.

vi. For patients with a suitable donor who meet eligibility criteria and are willing to proceed to HCT, if they have received chronic transfusion therapy for ≥ 1 year and have clinical evidence of iron overload by serum ferritin or MRI, an evaluation by liver biopsy is required. Histological examination of the liver must document the absence of cirrhosis, bridging fibrosis and active hepatitis. The absence of bridging fibrosis will be determined using the histological grading and staging scale as described by Ishak and colleagues (1995).

d) Suitable Donor: To undergo transplantation on this study, patients must have a first degree relative who shares at least 1 HLA haplotype with the patient, does not have SCD or other hemoglobinopathy, and is in good health; if these criteria are met, they will be allowed to serve as donors. Relatives with sickle cell trait are not excluded as donors. ABO mismatch, CMV status, donor age, parity, gender and the presence of donor specific antibodies will be considerations in selecting a donor. When more than 1 donor is available, the donor with the fewest HLA allele mismatches will be chosen, unless the patient had donor anti-HLA antibodies, a major ABO mismatch, or there was a medical reason to exclude the donor. If donor anti-HLA antibodies are detected, the next best related match will be chosen. When a pediatric and adult donor are available and if all other criteria are equal, an adult donor will be preferred over a pediatric donor. Umbilical cord blood or peripheral blood stem cell donors will not be accepted.

Exclusion criteria for proceeding to HCT on this study:

a) Availability of HLA matched sibling

b) Presence of donor specific antibodies in the patient

c) Histological examination of the liver must document the absence of cirrhosis, bridging fibrosis and active hepatitis. The absence of bridging fibrosis will be determined using the histological grading and staging scale as described by Ishak and colleagues (1995). The presence of bridging fibrosis will be an exclusion criterion.

d) Uncontrolled bacterial, viral or fungal infection in the 6 weeks before enrollment

e) Seropositivity for HIV

f) Previous HCT less than one year prior to enrollment.

g) Participation in a clinical trial in which the patient received an investigational drug or device or off-label use of a drug or device within 3 months of enrollment
h) Demonstrated lack of compliance with prior medical care
i) Unwilling to use approved contraception for at least 6 months after transplant
j) A history of substance abuse in the last 5 years that interferes with care
k) Pregnant or breast-feeding females at the time of consideration for HCT

Treatment Description: The HCT preparative regimen will consist of Hydroxyurea 30 mg/kg administered as a single daily dose PO on Days -100 through -10 (in patients not already receiving hydroxyurea as part of routine clinical care); Fludarabine 30 mg/m^2 administered IV on Days -6 through -2 (total dose 150 mg/m^2); antithymocyte globulin (ATG; rabbit) 0.5 mg/kg on day -9 and 2 mg/kg on days -8 and -7 (total ATG dose 4.5 mg/kg); Thiotepa 10 mg/kg IV on day -7; Cyclophosphamide 14.5 mg/kg IV on days -6 and -5 (total dose 29 mg/kg); and total body irradiation 2 Gy on day -1. Bone marrow will be collected and infused on day 0. GVHD prophylaxis will consist of Cyclophosphamide 50 mg/kg on days +3 and +4, Sirolimus starting on day +5 and continued for 1 year, and mycophenolate mofetil (MMF) 15 mg/kg/dose TID starting on day +5 and continued until day +35. Patients unable to tolerate Rabbit ATG will receive an equivalent dose of horse ATG.

Stem cell source: Bone marrow grafts will be collected from haploidentical donors. The nucleated cell target range is between 8 to 16 × 10^8/kg recipient ideal body weight, with the volume not to exceed 20 mL/kg donor's weight once the minimum target of 8 × 10^8/kg is reached.

Study Duration: Patients will be followed for 1-year post-transplant for study primary endpoints, functional measures and toxicity. Patients who meet a primary endpoint such as graft failure will be followed only for overall survival at one-year time point.

Data collection: Clinical events that occur during the one-year period of evaluation will be monitored, collected and analyzed. In addition, routine laboratory testing results that are performed in the course of standard medical monitoring will be collected.

Sample size calculations for primary endpoint of 1-year EFS: For this primary endpoint, we are focusing on the lower 95% exact binomial confidence bound to support our sample size of 15 subjects with severe SCD undergoing haploidentical HCT. We believe that if we observe 70% of the patients alive without events (defined as primary or late graft rejection, disease recurrence, or death) at one-year post-transplant, the outcomes are acceptable.

2. INTRODUCTION

Sickle cell disease (SCD) is a hereditary anemia that affects tens of thousands of Americans and millions of individuals worldwide. It is associated with early mortality and a diminished quality of life, with intermittent episodes of pain that are accompanied by progressive damage to vital organs, such as the lung, brain, spleen and kidney. Supportive health care measures instituted during childhood, which include newborn screening and pneumococcal prophylaxis, the administration of hydroxyurea and regular red blood cell (RBC) transfusions, have decreased the risk of serious infections and other life-threatening complications, resulting in improved survival to adulthood. This has, in part, shifted the demographics of SCD to include a growing proportion of young adults with chronic health impairments. As an alternative to chronic supportive care, hematopoietic cell transplantation (HCT) from a human leukocyte antigen (HLA)-identical sibling donor has been used sparingly in children but is curative in the majority of children treated[2]. As the outcomes from HCT have improved and the debilitating nature of SCD has been defined more completely, there is greater interest in expanding the applicability of HCT as a curative option for patients with SCD. A BMT Clinical Trials Network (BMTCTN) sponsored multicenter trial of unrelated donor HCT for children with severe SCD has recently completed enrollment. However, matched sibling or unrelated donors are available only to a minority of patients with SCD, the overwhelming majority of who belong to minority ethnic groups that are

underrepresented in bone marrow donor registries. It is estimated that approximately 14% of eligible patients will have a matched sibling donor[3] and 19% will find an 8/8 HLA antigen matched unrelated donor[4]. Thus, over two thirds of patients who may be considered for HCT are unlikely to be able to access this curative treatment because of the lack of a suitable donor. Recent advances in the conditioning regimen and strategies for the prevention of graft versus host disease (GVHD) have made the use of haploidentical related donors feasible. The group from Hopkins has demonstrated that high dose cyclophosphamide administered early after bone marrow transplant effectively modulates alloreactivity associated with haploidentical donors[5,6]. Since hematopoietic stem cells have high levels of aldehyde dehydrogenase, which is the enzyme responsible for metabolizing cyclophosphamide, they are relatively protected from high dose cyclophosphamide, which simultaneously is highly toxic to lymphocytes. This permits the administration of the drug on days 3 and 4 following the infusion of bone marrow to target the proliferating alloreactive T cells while sparing the donor hematopoietic stem cells. Using a strategy of post HCT high dose cyclophosphamide, Bolanos-Meade et al have demonstrated the safety of haploidentical donor transplantation in a case series of adult patients with SCD[10]. While the treatment appeared to be well tolerated with minimal GVHD and treatment related complications, the failure of engraftment with autologous recovery occurring in 43% of patients remains a concern. The Hopkins group of investigators carried out a series of modifications of the protocol in response to the problems seen; these include the substitution of stimulated bone marrow as the stem cell source, the replacement of calcineurin inhibitors by sirolimus, and the addition of rabbit anti thymocyte globulin (ATG) as GVHD prophylaxis. Subsequent experience from Hopkins suggests that GCSF priming does not increase engraftment and may be associated with more GVHD (R Brodsky, personal communication). While most clinical trials of HCT for SCD have reported HCT specific outcomes such as overall survival (OS), event free survival (EFS) and transplant related complications, there has been relatively limited study of sickle cell specific endpoints after HCT. This, on the one hand limits our ability to determine the impact of HCT on the biological course of SCD, and on the other hand, represent a lost opportunity to study the mechanisms of the development or regression of sickle cell related complications such as chronic pain syndromes or chronic organ dysfunction. We hypothesize that HCT from 3-5 HLA antigen matched family donors for patients with severe SCD is feasible, has an acceptable safety profile, will generate a two-year disease-free survival of at least 80%, and result in the stabilization or improvement of functional status related to SCD.

3. BACKGROUND

3.1. Rapid disease progression in adults with SCD

Natural history studies show that in contrast to the improvements in outcomes in childhood, there is a rapid progression in organ damage, morbidity and premature mortality in adulthood. Progression of organ damage in adulthood is marked by pulmonary hypertension, which occurs in 20-40% of adult patients with a 10 fold increase in risk of premature mortality[7], renal insufficiency with proteinuria in 70% and progressing to renal failure in 11%, abnormal pulmonary function in 90% and progression to irreversible organ damage in 50% of patients by 50 years of age[8]. One third of adults with SCD develop chronic pain syndrome and only 20% are employed[9,10]. Death in adulthood is frequently related to organ damage which is not preventable or easily managed with current medical measures[8]. SCD related complications such as leg ulcers, stroke, priapism, vascular necrosis, anxiety, and depression further worsen the health-related quality of life. The mortality rate of patients with SCD is 5.8-20% in the first 10 years after transition to adult care[9,10]. Premature death occurs at a median age of 38 years, a statistic that has not changed in 20 years [8,11,12] (**Figure 1**). In a long-term follow-up study of patients with symptomatic SCD who were eligible to participate in the multicenter study of hydroxyurea (MSH), the annual mortality rate was 4.4 per 100 person-years among adults with SCD who satisfied eligibility criteria (4.4%). Thus, the inexorable progression of disease and premature mortality in adulthood provides the rationale for expanding the applicability of HCT as a curative option for patients with SCD.

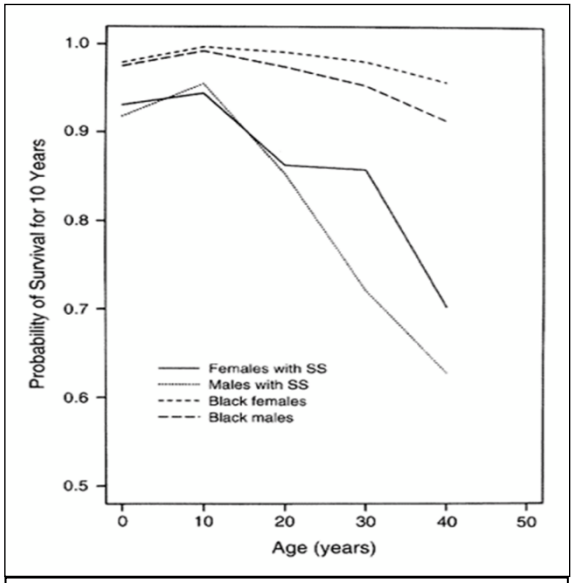

Figure 1: Steep decline in probability of survival in adulthood in patients with SCD[1].

3.2. Lack of curative treatments for adults with SCD provides the rationale for expanding the applicability of HCT

HCT is the only curative treatment for this genetic blood disorder with excellent outcomes in children 16 years and younger. Kaplan-Meier probabilities of survival and EFS are 93% and 85%, respectively, with a median follow-up of 6.3 years (range 3-12.4 years)[2]. Following successful HCT, there is a resolution of complications related to SCD with protection from episodes of pain, stroke, or ACS, and in most patients, a stable appearance of cerebral MRI and pulmonary function tests and regeneration of the spleen[13]. The prevailing framework of clinical research in SCD shifts in the transition from childhood to adulthood. Among pediatric hematologists, the dominant view is that survival to adulthood is excellent, and that children, on average, have a very good quality of life as a result of supportive care measures such as antibiotics, family education, and the judicious use of transfusions. In addition, a great deal of effort has focused on identifying children who have high-risk features, so that the risk of any specific intervention might be balanced by the severity of disease in that individual. Thus, clinical research studies in children with SCD have focused primarily on safety and efficacy, often in the setting of a high-risk population, such as children at risk for a stroke. While HCT has curative potential, its routine application remains quite limited to children with SCD with matched sibling donors, which is due in part to its toxicities which include acute toxicity, a risk of secondary leukemia and infertility due to chemotherapy and a risk of dying from the procedure itself. As a result, clinical studies of transplantation in children with SCD have suffered from poor accrual, and despite excellent EFS, HCT is not routinely considered in children with SCD.

In contrast, the prevailing view among clinicians who care for adults is that SCD is, on average, a severe disease with a significant risk of sudden death and the development of chronic medical problems, and that supportive care options for young adult patients are not adequate to address the overwhelming nature of this disease. Thus, clinical studies in adults with SCD have focused on interventions that prolong survival

and improve the quality of life. Unlike children, adults with SCD are much more likely to have a debilitating complication. As a result, the risk/benefit ratio of HCT is more favorable in adults, particularly if an approach to HCT that defines an acceptable level of toxicity can be established. Across the age span, the lack of access to a suitable HLA matched donor remains the single most critical barrier to the application of HCT to this condition. If successful, an approach to HCT using haploidentical donors could significantly improve the outlook of many patients with SCD and broaden the therapeutic choices.

3.3. Critical barriers to the application of allogeneic HCT to patients with SCD

The excellent outcomes in children with SCD treated by conventional myeloablative HCT stimulated the initiation of a trial of myeloablative HCT for adults in the early 1990s. Unacceptably high transplantation related morbidity and mortality was observed, which precipitated the early closure of HCT studies in young adults with SCD and set back the field by more than a decade. In addition to the usual transplant-related toxicities, sickle cell recipients are susceptible to unique toxicities that include posterior reversible encephalopathy syndrome (PRES)[14] and intracranial hemorrhage. Unlike in malignant diseases, GVHD does not provide any survival advantage in patients with SCD and is the major cause of mortality after HCT in these patients. Thus, the possibility that a patient may exchange the chronic morbidity of SCD with that of chronic GVHD is a barrier to acceptability of HCT by intended recipients and their providers. In addition to concerns about transplant-associated toxicities, there are several other important barriers to broadly applying a HCT-based approach to treatment of adults with SCD; these include: (1) Increased risk of non-engraftment after HCT likely due to the fact that patients with SCD have not been previously exposed to multi-agent chemotherapy, are not immunosuppressed and have a hyperplastic bone marrow. The risk of graft rejection after HCT is approximately 10% when myeloablative chemotherapy is employed and higher when non-myeloablative (NMA) regimens are utilized. (2) High risk of infertility as evidenced by the observation post-HCT of primary ovarian failure in most post-pubertal females and oligo/azoospermia with FSH elevation despite relatively preserved testosterone production in pubertal males.[2] This could also be an important obstacle to the acceptability of HCT by intended recipients. (3) Limited donor availability because of the underrepresentation of minorities in the bone marrow donor registries. In 2003, approximately 59.7% of SCD patients and 80.2% of thalassemia patients were predicted to identify at least one potential marrow or umbilical cord blood (UCB) donor matched at 6/6 HLA loci at low resolution[15]. All patients are likely to find at least one marrow donor or an UCB unit that is potentially matched at 5/6 HLA loci[16]. However, for African-Americans, the likelihood of finding an 8/8 HLA matched donor using the current standard for selection of donors especially is only 19%.[4] (4) Slow accrual is a concern in clinical trials in SCD. Dr. Kathryn Hassell analyzed 14 SCD studies that were successfully conducted outside an established clinical research network. These studies involved a median of 23 centers and 235 study participants, with a median time of 44 months to enroll (personal communication). This requirement was fairly consistent across all types of studies, except for pediatric studies, which required fewer sites. Even sites that enrolled as few as 5 - 6 subjects were needed to complete enrollment. There was no relationship between the number of study sites and the time to enrollment, however, there was a trend to more rapid enrollment with more available sites. The multicenter cooperative study of HCT in children with SCD spanned 9 years to recruit 59 subjects[17] and the recent BMTCTN trial of URD HCT for children with SCD enrolled 30 subjects over 7 years. (5) Lack of empirically derived eligibility criteria for HCT for SCD. In SCD there is considerable variability in clinical phenotype, with few validated and specific predictors of disease progression or premature mortality. Ideally, in crafting HCT trials, a study design would rely upon a reliable predictor of early mortality as a method to ensure that the toxicity of transplantation was balanced by the risks of having the underlying disease morbidity. In the pediatric HCT trial, consensus opinion was reached in developing acceptable eligibility criteria. Further, severity criteria for enrollment in a clinical trial represent an attempt to balance the risks of transplant related morbidity and mortality with that of the risks associated with the disease process. As such, eligibility criteria must also be informed by the anticipated outcomes with the particular stem cell source and GVHD prevention strategies. As there are no accepted criteria for haploidentical donor HCT for SCD, a similar process would be required to develop and initiate a multi-center HCT trial in haploidentical donor HCT in patients with SCD with severe disease.

3.4. Rationale for proposed study

3.4.1. Improved survival of children with SCD has uncovered the natural history of the disease in adults

In 1960, SCD was a disease of childhood and few survived to adulthood. Health care initiatives such as newborn screening, penicillin prophylaxis against pneumococcal sepsis and comprehensive programs with a focus on family education together dramatically improved outcomes in children with SCD. Currently, 90% of US newborns with SCD survive to 20 years of age. However, with improved survival to adulthood, chronic health impairments caused by progressive organ damage from SCD and the associated risk of premature death have become the dominant targets of clinical intervention for this disorder[8,18-23].

3.4.2. Adults with SCD have rapid disease progression that dramatically increases the risk of early mortality

There is a steady decline in survival in patients with SCD that is observed by early adulthood[24]. In patients with symptomatic SCD who met disease severity based eligibility criteria and participated in the multicenter study of hydroxyurea (MSH), the annual mortality rate was approximately 4% per year among adults with SCD[25]. In addition to the risk of early mortality, individuals with SCD who survive beyond the second decade of life are at risk of developing chronic health conditions that negatively impact the quality of life. These include progressive vasculopathy, characterized by systemic and pulmonary hypertension, endothelial dysfunction, and intimal and smooth muscle proliferative changes in arterial conduits and major end-organ damage, such as chronic renal failure, hemorrhagic and non-hemorrhagic stroke, avascular necrosis of the bones, and pulmonary hypertension[26,27]. Patients who experience a hemorrhagic stroke have a 26% risk of mortality within the first two-week period following the stroke[28]. Thus, the lack of supportive measures to halt disease progression in adulthood and eliminate premature mortality represents a clear and urgent rationale for developing effective and curative therapies.

3.4.3. Novel approaches to HCT for hemoglobinopathies based on a combination of myeloablation and immunosuppression

NMA and reduced intensity conditioning regimens are now routinely used to establish engraftment of donor hematopoietic cells in adults with hematologic malignancies. The success of immunoablative but reduced-intensity regimens suggests that an immunologic barrier rather than a requirement of “hematopoietic space” is a primary factor to ensuring engraftment of donor hematopoietic stem cells. While NMA conditioning regimens have been successfully applied in adults with hematological malignancies, these have been far less successful in hemoglobin disorders. Over a decade ago, Lucarelli et al. described the effects of conditioning regimen dose intensity on overall transplant outcome in HCT for thalassemia. Conditioning regimens containing less than 200 mg/kg of cyclophosphamide (CY) resulted in decreased transplantation-related mortality with a concomitant increase in graft rejection.[29] For example, in pediatric patients with class III disease, the incidence of graft rejection increased from 10% to 30% after reducing the CY dose to 120-160 mg/kg. Sodani et al. successfully modified this regimen, resulting in a reduced rate of graft rejection from 30% to 8% by introducing a combination of intensive hypertransfusion, hydroxyurea and chelation to reduce erythropoiesis and expansion of thalassemic clones well before transplantation, and by the addition of fludarabine (Flu) and azathioprine to increase the level of immunosuppression.[30] In another series focused on SCD, Krishnamurti et al. reduced the dose of busulfan (Bu) by 50%, substituted Fludarabine (Flu) for CY and included 500 cGy total lymphoid irradiation and ATG[31] in the conditioning regimen. In this series, 6 of 7 patients had stable engraftment after HLA-identical sibling HCT and discontinued immunosuppression with no SCD-related symptoms at 2 to 9 years after HCT. Investigators from Duke University have also demonstrated encouraging results after HCT for SCD using the Bu-Flu backbone in 2 adults, one of whom had end stage renal disease[32]. Shenoy et al. substituted the alkylator Melphalan for BU in combination with Flu and Alemtuzumab observed long term stable engraftment in patients with non-malignant disorders

(including SCD) treated by HCT from related or unrelated donors [33]. Hsieh et al. demonstrated stable donor engraftment in adult SCD patients following NMA HCT from HLA-matched sibling donors. Low-dose total-body irradiation (300 cGy) and alemtuzumab were administered before transplantation, and sirolimus was used for GVHD prophylaxis[34]. However, long-term administration of sirolimus at a median of 30 months post HCT was necessary because patients developed mixed lymphohematopoietic chimerism after transplantation with a perceived risk of late graft rejection. Finally, the application of haploidentical donor transplantation is in the early stages of development, led by the investigative team at Johns Hopkins. Seventeen children received an NMA combination of ATG, Flu, CY and 200 cGy TBI before transplantation and received high-dose CY after transplant to accomplish in vivo T-cell depletion of alloreactive donor T-cells. While this was safe and effectively prevented severe GVHD, graft rejection occurred in 6 of 14 haploidentical donor transplants. This approach is being modified to reduce the rejection incidence. Thus, while encouraging results have been observed after these NMA regimens tested in patients with SCD, the problems of graft rejection and a need for long-term immunosuppressive therapy after transplantation have not been overcome.

Another approach to improve the safety profile of HCT has been to modify the myeloablative Bu/CY backbone of a conventional preparative regimen by substituting Flu for CY. This approach has successfully reduced the toxicity of the conditioning regimen but retains very high rates of stable donor engraftment[35-37]. Taken together, these studies suggest that modified preparative regimens containing a combination of myeloablative and immunosuppressive drugs can be safely and effectively administered in adults with non-malignant disorders.

3.4.4. Safety and efficacy of the post-transplant cyclophosphamide based GVHD prevention strategy for haploidentical HCT

Occurrence of GVHD previously was a major obstacle to the use of haploidentical donors for HCT. The safety and efficacy of haploidentical donor transplants have been greatly enhanced by the development of novel strategies for the prevention of GVHD. Strategies for prevention of GVHD focused on in vitro or in vivo T cell depletion. In vitro T-cell depletion of the graft, primarily through positive selection of $CD34^+$ cells, has been shown to lead to high rates of engraftment and prevented both acute and chronic GVHD in haploidentical HCT[38,39]. However, the application of this approach has been limited by the high rate of transplant related mortality due to delayed recovery of adaptive immunity and risk of infections[39]. Recently a novel method of ex vivo T- and B-cell depletion based on the selective elimination of $\alpha\beta^+$ T cells has been demonstrated to result in high rated of engraftment with limited GVHD and treatment related mortality in children with non-malignant disorders[40]. The general applicability of this approach is however limited by the need for institutional technical expertise in graft manipulation techniques. Haploidentical donor bone marrow transplantation with in vivo T-cell depletion with high-dose Cy has proven effective with very low rates of both acute and chronic GVHD by the Hopkins group in malignant and non-malignant conditions in single and multicenter studies[41]. The use of GCSF mobilized bone marrow and the use of rabbit ATG may have contributed to low rate of GVHD in patients with SCD undergoing haploidentical HCT using the post-transplant CY approach[5]. However, subsequent experience from Johns Hopkins suggests that GCSF priming does not increase engraftment and may be associated with more GVHD (R Brodsky, personal communication). Substitution of calcineurin inhibitors with Sirolimus may have also contributed to decreasing the incidence of PRES. The reported rate of failure of engraftment at 43% remains a significant barrier to the general applicability of this approach, though it is possible that the protocol changes made during the study may mitigate this rate of non-engraftment. Several factors may contribute to the rate of 10% or higher rate of non-engraftment in patients with SCD undergoing HCT, including the lack of prior myelosuppression or immunosuppression due to chemotherapy and the exposure to blood products. In an attempt to provide additional myelosuppression, we propose to add Hydroxyurea (HU) 30mg/kg/day for 30 days prior to starting the remainder of the conditioning regimen. Such an approach has been used successfully to increase engraftment in Pesaro class III or Adult Thalassemia patients[30,42,43]. Since most of the patients may have already previously received HU, we do not anticipate that this will significantly change the profile of tolerability of this regimen. To provide additional myelosuppression, we will add a single dose of Thiotepa 10mg/kg. These drugs have been well tolerated and have been shown to contribute to improved engraftment in Pesaro class III Thalassemia patients. The

regimen proposed is similar to the one that has been piloted by Dr. Josu De La Fuentes in Imperial College, London, U.K. for haploidentical HCT in patients with SCD which has been demonstrated to improve donor engraftment without significantly increasing morbidity or mortality and could dramatically expand curative options for individuals with SCD.[44] In their experience 15 patients (including 2 with previous graft rejection) underwent haplo-BMT with this thiotepa-augmented conditioning regimen. At a median follow-up of 13.3 months (interquartile range [IQR], 3.8 to 23.1 months), 93% (14 of 15) had >95% stable donor engraftment at 6 months, with 100% overall survival.

3.4.5. Immune reconstitution following haploidentical HCT using a reduced intensity conditioning regimen and post-transplant cyclophosphamide

In the experience of the Hopkins group, post-transplantation recovery of lymphocyte subsets was notable for the following[45]: 1) The median lymphocyte count at day 30 after transplantation is 250/ml and recovers to over 1000/ml by day 60; 2) $CD4^+$ T cell counts recover to a median > 150/ml by day 60 after transplantation; and 3) recovery of $CD31^+$ recent thymic emigrants and $CD45RA^+$ naïve T cells is delayed compared to recovery of memory T cells. Patients with a day 30 absolute lymphocyte count (ALC) of > 200 cells/ul had a markedly improved OS ($p = 0.008$) and EFS ($p < 0.0001$) as compared with those patients with an ALC of < 200. In conclusion, immune reconstitution after NMA haploidentical T cell replete HCT with post-transplantation CY compares favorably with other reduced intensity conditioning regimens and T cell depleted haploidentical HCT, especially with regard to $CD3^+$ and B cell numbers and may explain the low infectious complication rate. Analysis on 60 additional patient/donor pairs is currently being performed.

3.4.6. Impaired endocrine function and fertility related to SCD may be exacerbated by HCT

Many sickle cell patients demonstrate a pattern of growth and development consistent with constitutional delay of growth and puberty[46]. This pattern of growth is characterized by short stature during childhood and early adolescence, late onset of puberty with eventual attainment of final height consistent with mid parental height and full progression through puberty. Myeloablative HCT for SCD is associated with infertility in over 80% of patients.[2] For male SCD patients who have undergone HCT, hormone production appears to be fairly well preserved. After Bu and CY, males will typically begin and progress through puberty with normal virilization. Testosterone levels have been variably reported to be low but often this is at the lower limit of normal range and not necessarily low enough to require intervention. Likewise, gonadotropins have been reported to be high but often these are marginally high. When evaluating hormonal profiles in males it is important to standardize the timing of samples and the definition of what values can be uniformly agreed upon to be abnormal. It has become standard practice to base clinical decisions on morning testosterone levels as opposed to random testosterone levels. Only a few studies have evaluated spermatogenesis after HCT. Testicular volume has been reported to be significantly decreased and azoospermia and oligospermia common[47,48]. Long term follow up studies on semen parameters years after transplant have not been reported. In females it is more difficult to separate hormone production and oocyte production. It is unusual to have one process preserved and not the other. Studies of ovarian function in SCD patients following HCT demonstrate rates of 57-87% of patients with amenorrhea or ovarian failure[2,48-50]. There are occasional reports of late (> 10 years) resolution of hypergonadotropism and even reports of pregnancy after HCT for SCD[2,48]. Typical hormonal profiles after HCT for SCD reveal elevated LH and FSH and low estradiol. Anti-mullerian hormone (AMH), considered a marker of ovarian reserve, has been reported to be undetectable after HCT with Bu based preparative regimens. These very low levels of AMH are compatible with severe diminished ovarian reserve. Many patients SCD patients after HCT had menopausal FSH levels and therefore were deemed to have premature ovarian insufficiency (Elchuri, Meacham et al., 2014 abstract SCD BMT).

4. STUDY OVERVIEW

The primary goal of this study is to determine the safety and tolerability **of HCT using a haploidentical donor in SCD patients who lack an HLA identical related donor.** We will determine the 1-year EFS among patients treated by HCT, with events defined as graft failure/recurrent SCD or death. We will determine the

impact of donor engraftment on pulmonary, cardiac, renal and gonadal function and on central nervous system imaging after HCT.

5. STUDY DESIGN

5.1. Study Objectives

5.1.1. Primary Objective

The primary objective of this clinical trial is to estimate the EFS at 1 year after allogeneic Hematopoietic cell transplantation (HCT) in children and young adults with severe SCD following HCT from a haploidentical family donor using a pre-transplant conditioning regimen of HU, Flu, ATG, Thiotepa, CY, and low dose TBI and GVHD prophylaxis with post transplantation CY, Sirolimus and MMF. Events for this endpoint will include primary and late graft failure, recurrence of SCD, and death.

5.1.2. Secondary Objective

The secondary objectives of the clinical trial include determining the toxicity of HCT, for which we will measure the following: time to neutrophil and platelet recovery; grades II-IV and III-IV acute GVHD; chronic GVHD; VOD; IPS; CNS toxicity (PRES, hemorrhage, and seizures); the incidence of significant bacterial, fungal and viral infection (e.g., CMV reactivation and infection; adenovirus infection; EBV PTLD); and the incidence of stable mixed chimerism.

5.2. Patient Eligibility

The PI or co-PI will confirm patient eligibility prior to enrollment.

5.2.1. Eligibility Criteria:

a) Patients with Sickle Cell Disease, Transfusion dependent Thalassemia, sideroblastic anemia or diamond blackfan anemia

I. SCD who have 1 or more of the following disease severity criteria:

- Clinically significant neurologic event (stroke) or any neurological deficit lasting > 24 hours;
- History of ≥ 2 episodes of ACS in the 2-year period preceding enrollment despite the institution of supportive care measures (i.e. asthma therapy and/or HU);
- History of ≥ 3 severe pain crises per year in the 2-year period preceding enrollment despite the institution of supportive care measures (i.e. a pain management plan and/or treatment with HU);
- Administration of regular RBC transfusion therapy, defined as receiving ≥ 8 transfusions per year for ≥ 1 year to prevent vaso-occlusive clinical complications (i.e. pain, stroke, and ACS);
- An echocardiographic finding of TRJ velocity ≥ 2.7 m/sec in adult patients. Pediatric patients with symptomatic right heart catheterization proven PAH may be considered eligible for the study.
- Ongoing high impact chronic pain on a majority of days per month for ≥ 6 months as defined as ONE or more of the following: Chronic pain without contributory SCD complications[2], OR Mixed pain type in which chronic pain is occurring at site(s) (arms, back, chest, or abdominal pain) unrelated to any sites associated with Contributory SCD complications[2] (e.g. leg ulcers and/or avascular necrosis).

b) Age: Patients must be 3- 40 years of age inclusive.

c) Adequate physical function as measured by:

- Lansky or Karnofsky performance score ≥ 60

- Cardiac function: LVEF > 40% or LV shortening fraction > 26% by cardiac echocardiogram or by MUGA scan
- Pulmonary function: Pulse oximetry with a baseline O_2 saturation of ≥ 85% and DLCO > 40% (corrected for hemoglobin)
- Renal function: Serum creatinine ≤ 1.5 x the upper limit of normal for age as per local laboratory **and** one of the following: 24-hour urine creatinine clearance > 70 mL/min/1.73 m^2 or GFR > 70 mL/min/1.73 m^2 by radionuclide GFR
- Hepatic function: Serum conjugated (direct) bilirubin < 2 x upper limit of normal for age as per local laboratory and ALT and AST < 5 x upper limit of normal as per local laboratory. Patients with hyperbilirubinemia as a consequence of hyperhemolysis or who experience a sudden, profound change in the serum hemoglobin after an RBC transfusion **OR** there is evidence of moderate direct hyperbilirubinemia defined as direct serum bilirubin < 5 times ULN and not caused by underlying hepatic disease are not excluded.
- In patients who have received chronic transfusion therapy for ≥ 1 year and who have clinical evidence of iron overload by serum ferritin or MRI, evaluation by liver biopsy is required. Histological examination of the liver must document the absence of cirrhosis, bridging fibrosis and active hepatitis. The absence of bridging fibrosis will be determined using the histological grading and staging scale as described by Ishak and colleagues (1995).

d) Suitable Donor: To undergo transplantation on this study, patients must have a first degree relative who shares at least 1 HLA haplotype with the patient, does not have SCD or other hemoglobinopathy, and is in good health; if these criteria are met, they will be allowed to serve as donors. Relatives with sickle cell trait are not excluded as donors. ABO mismatch, CMV status, donor age, parity, gender and the presence of donor specific antibodies will be considerations in selecting a donor. When more than 1 donor is available, the donor with the fewest HLA allele mismatches will be chosen, unless the patient had donor anti-HLA antibodies, a major ABO mismatch, or there was a medical reason to exclude the donor. If donor anti-HLA antibodies are detected, the next best related match will be chosen. When a pediatric and adult donor are available and if all other criteria are equal, an adult donor will be preferred over a pediatric donor. Umbilical cord blood or peripheral blood stem cell donors will not be accepted

5.2.2. Exclusion criteria:

a) Availability of HLA matched sibling.
b) Presence of donor specific antibodies directed at available family donors
c) Histological examination of the liver must document the absence of cirrhosis, bridging fibrosis and active hepatitis. The absence of bridging fibrosis will be determined using the histological grading and staging scale as described by Ishak and colleagues (1995).
d) Uncontrolled bacterial, viral or fungal infection in the 6 weeks before enrollment
e) Seropositivity for HIV
f) Previous HCT less than one year prior to enrollment
g) Participation in a clinical trial in which the patient received an investigational drug or device or off-label use of a drug or device within 3 months of enrollment
h) Demonstrated lack of compliance with prior medical care
i) Unwilling to use approved contraception for at least 6 months after transplant
j) A history of substance abuse in the last 5 years that interferes with care
k) Pregnant or breast-feeding females

5.3 Donor Selection Criteria

Preference will be given to related marrow donors who are 2-4 (out of 8) HLA antigen mismatched and towards whom the recipient does not have donor specific antibodies. Donors will sign an informed consent

disclosing that the marrow donation will be used by a patient participating in this study. The donor must be matched with the recipient for at least 4 of 8 HLA alleles (HLA -A, -B, -C and -DRB1 by allele-level DNA methodology). The target total nucleated cell count (TNC) is 3.5-8.0 x 10^8/kg of recipient weight. Marrow will be collected without mobilization. Mobilized peripheral blood stem cell (HPC-A) collections will not be permitted. Donors must undergo hemoglobinopathy screening by electrophoresis; donors who have a hemoglobinopathy will be excluded but trait condition is acceptable.

5.4. Treatment Plan

All HCT recipients will receive the preparative regimen as shown in **Table 1**. Hydroxyurea, Thiotepa, Fludarabine, and Cyclophosphamide (pre- and post-transplant) dosing will be based on adjusted ideal body weight (AjBW) in patients weighing >125% IBW. The following are dose adjustment formulas with IBW in kg:
Males: IBW = 50 kg + 2.3 kg for each inch over 5 feet.

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

AjBW = IBW + 0.4(ABW - IBW)

5.4.1. Pre-transplant procedures

5.4.1.1. HbS Level Prior to Start of Conditioning

Prior to the start of conditioning, patients who are not on a chronic transfusion protocol should receive EITHER a partial volume exchange transfusion (no more than 72 hours before the start of conditioning) OR simple transfusion(s) (starting one to two weeks before the start of conditioning). The transfusion should be tailored to achieve a HbS level < 30% and a Hgb to 9-11 by day-9, the day of the first ATG dose.

5.4.1.2. Patients Receiving Iron Chelation Therapy Prior to HCT

Iron chelation therapy will be discontinued no later than 48 hours prior to commencement of the conditioning therapy (ATG). Iron chelation therapy or a program of phlebotomy may be resumed after neutrophil and red cell engraftment at the discretion of the transplanting center based on assessments of iron overload and GVHD (repeating liver iron quantification/biopsy at day 100 or later is recommended).

5.4.1.3. Back-up Bone Marrow

Because of the risk for graft failure, it is recommended that patients have an autologous bone marrow harvest prior to start of the preparative regimen. It is recommended that at least 2 x 10^8 nucleated cells/kg are collected.

Table 1: Schema of Conditioning Regimen for HCT Recipients

Day	Treatment
-100 to -10	Hydroxyurea 30 mg/kg PO Qday
-9	Rabbit ATG 0.5 mg/kg IV
-8	Rabbit ATG 2 mg/kg IV
-7	Rabbit ATG 2 mg/kg IV; Thiotepa 10 mg/kg IV
-6	Fludarabine 30 mg/m^2 IV; Cyclophosphamide 14.5 mg/kg IV
-5	Fludarabine 30 mg/m^2 IV; Cyclophosphamide 14.5 mg/kg IV
-4	Fludarabine 30 mg/m^2 IV
-3	Fludarabine 30 mg/m^2 IV
-2	Fludarabine 30 mg/m^2 IV
-1	TBI 200 cGy
0	Stem cell infusion
+3	Cyclophosphamide 50 mg/kg IV
+4	Cyclophosphamide 50 mg/kg IV
+5	Sirolimus (through day +365); MMF 15 mg/kg/dose TID (through day +35)

5.4.2. Preparative regimen

5.4.2.1 Hydroxyurea

For patients not already receiving hydroxyurea as treatment for SCD, hydroxurea will be administered at a dose of 30 mg/kg PO as a single daily dose for 90 days (from day -100 to day-10). Hydroxyurea dosing will be based on AjBW in patients weighing >125% IBW (see above). Patients will be seen in clinic for a CBC every 2 weeks during this period of time, and the dose will be reduced if ANC <1500 or platelet count <100,000.

For patients who are already receiving hydroxyurea for ≥ 90 days at a maximally tolerated dose (approximately 30 mg/kg/day or >20mg/kg/d in setting of prior hydroxyurea associated toxicity) as treatment for SCD and who are being monitored by a physician for such, their dosing does not require adjustment. These patients will continue routine monitoring (e.g. CBC) with the physician who has prescribed hydroxyurea.

5.4.2.2 Thiotepa
Thiotepa will be administered at a dose of 10mg/kg IV over 2 hours or per institutional guidelines on day -7. Thiotepa dosing will be based on AjBW in patients weighing >125% IBW (see above).

5.4.2.3 Fludarabine
Fludarabine 30 mg/m^2/day will be administered from day –6 to day –2 (for a total of 150 mg/m^2 over 5 consecutive days) and administered IV over a minimum of 30 minutes. The IV infusion can take longer per institutional guidelines. On days -6 and -5, Fludarabine must be administered before cyclophosphamide. Preparation, administration, and monitoring will be according to institutional standard practice. In patients weighing > 125% IBW, Flu will be dosed based upon AjBW (see above).

5.4.2.4. Cyclophosphamide
Cyclophosphamide will be administered on days -6 and -5 prior to bone marrow infusion at a dose of 14.5 mg/kg IV infused over 1-2 hours. The administration of cyclophosphamide should begin just after the fludarabine infusion is complete.

Post bone marrow infusion cyclophosphamide will be infused on days +3 (between 60- and 72-hours post marrow infusion) and +4 (approximately 24 hours after day +3 dose) at a dose of 50 mg/kg IV infused over 1-2 hours. No immunosuppressive drugs, including steroids being used as an anti-emetic, should be given during the period between marrow infusion and 24 hours post day +4 cyclophosphamide.

For patients weighing more than 125% of their ideal body weight, dosing will be based on adjusted ideal body weight (see above). Supportive care for cyclophosphamide (hydration and the use of MESNA) should follow institutional practices.

5.4.2.5. Rabbit ATG
Rabbit ATG will be administered on day -9 at 0.5 mg/kg and on days -8 and -7 at 2 mg/kg (for a total dose 4.5 mg/kg). ATG will be dosed on actual weight. Each vial of rabbit ATG lyophilized powder is reconstituted with 5 mL of soluble water for injection and transferred into the bag of infusion solution (saline or dextrose). It is recommended to use 50 mL of infusion solution for each vial of thymoglobulin. Total volume is usually between 50 to 500 mL. It is infused through a 0.22 micrometer filter into a high-flow vein over a minimum of 6 hours for the first dose and over at least 4 hours for subsequent doses. Premedication with corticosteroids, acetaminophen and diphenhydramine 1 hour prior to the infusion is recommended to reduce the incidence and intensity of side effects during and after the infusion.

5.4.2.6 Total body irradiation
Patients will receive 200 cGy of TBI in a single fraction on day -1. Patients will receive lead shielding to minimize the toxicity of radiation to the gonads.

5.4.3. Infusion of Hematopoietic Stem Cells
Institutional procedures should be followed for requesting and receiving marrow units for infusion. Under no circumstances are the marrow cells to be irradiated. No in-line leukocyte filter should be used and no medications or fluids should be given piggyback through the catheter lumen used for infusion of stem cells. Vital signs should be monitored before beginning the infusion and periodically during administration and in accordance with institutional guidelines. Pre-medications (if any) prior to BM infusion will be at the discretion

of the transplant center. It is recommended that diphenhydramine, epinephrine, hydrocortisone, and oxygen be available at the bedside for emergency use.

5.5. GVHD Prophylaxis

5.5.1. Sirolimus

Sirolimus will be administered beginning on day +5 at least 24 hours following completion of cyclophosphamide. Sirolimus will be continued through 1 year (day 365), at which point it can be discontinued without planned taper. In the case of unstable mixed donor chimerism or GVHD, the duration of Sirolimus administration alone or in combination with additional immunosuppressive agents may be modified at investigator's discretion as dictated by patient's condition. Please see Table 2 for age-based dosing and trough levels. Doses will be adjusted to maintain appropriate levels (HPLC or immunoassay) which should be measured weekly at a minimum.

Table 2. Sirolimus dosing and levels

Age	Loading dose (day +5)	Maintenance dose (day +6)	Trough level
≥18 years	6 mg PO	2 mg PO Qday	5-15 ng/mL
<18 years	3 mg/m^2 PO (max 6 mg)	1 mg/m^2 PO Qday (max 2 mg)	5-12 ng/mL

5.5.2 Mycophenolate Mofetil

MMF will be given at a dose of 15 mg/kg/dose TID beginning on day +5 and continued until day +35. The preferred route of administration is PO but it can be given IV if not tolerated PO.

5.5.3. Biomarkers

A biomarker panel consisting of ST2 and REG3α in a blood sample obtained seven days after HCT can identify patients at high risk for lethal GVHD (Hartwell eat al JCI Insight. 2017 Feb 9; 2(3): e89798.). We will obtain a blood sample for GVHD biomarkers at day seven post HCT. The results will be reviewed by the GVHD group within the Aflac BMT team and consideration will be given to modifying GVHD prophylaxis and treatment. The panel will also be repeated at onset of GVHD and will guide further management.

5.6. Supportive Care

Institutional practice guidelines should be followed after transplantation for nutritional support, treatment of infections, and blood product support. Additional supportive care guidelines are recommended below. Enrollment onto another clinical trial for treatment of transplant related complications (i.e. VOD, GVHD) does not require study pre-approval, but the PI should be notified. Enrollment on a clinical trial for non-treatment related purposes (i.e. addition of an immunosuppressive agent for GVHD prophylaxis or engraftment) does require study pre-approval. These trials are generally not allowed, but will be reviewed by the PI on an individual basis.

5.6.1. 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.

5.6.2. Seizure Prophylaxis and Management of PRES

Prophylaxis against seizures is mandatory in all recipients and should be commenced at the start of inpatient conditioning (e.g. ATG). Suitable drugs for prophylaxis include lorazepam or levetiracetam and should be administered according to institutional guidelines. Serum magnesium level should be maintained >1.7 mg/dL to reduce the risk of seizures. There will be careful monitoring of blood pressure and prompt treatment of hypertension by anti-hypertensive agent(s) will be instituted. Suitable agents include amlodipine and ACE inhibitors; often multi-modal therapy is required to bring the BP in the normotensive range.

5.6.3. Hypertension

Hypertension should be strictly controlled to prevent CNS toxicity. Blood pressure should be monitored closely and both systolic and diastolic hypertension should be treated promptly to maintain blood pressure at the patient's pre-transplant baseline ± 20%. Explicit orders must be written to intervene if systolic or diastolic blood pressure exceeds 20% over baseline.

5.6.4. Blood Products

The hemoglobin level must be maintained between 9.0 and 11.0 g/dL and platelet count > 50,000/mL after transplantation to minimize the risk of neurological adverse events. Irradiated blood products should be administered universally, and CMV negative or leuko-filtered blood products are recommended for CMV sero-negative recipients. In those patients who do not receive chronic RBC transfusions or who have a HbS fraction > 30%, a partial exchange transfusion will be performed to reduce the HbS to ≤ 30% before commencing the conditioning regimen. The Hgb and % HbS should be re-checked 4 hours after the exchange transfusion.

5.6.5. 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. Patients are especially susceptible to bacterial and viral infections in the early post-transplant period.

5.6.6. Infection Surveillance and Prophylaxis

Please use institutional guidelines for infection surveillance and prophylaxis. Where institutional guidelines do not exist, the following guidelines are recommended.

5.6.6.1. HSV prophylaxis

Acyclovir prophylaxis is recommended after transplantation until immune reconstitution in patients who are sero-positive for HSV or VZV. If unable to tolerate PO medications, IV therapy will be necessary.

5.6.6.2. PCP prophylaxis

Trimethoprim-sulfamethoxazole or an equivalent drug should be administered beginning after neutrophil recovery and continued until there is full immune reconstitution.

5.6.6.3. Fungal prophylaxis

Due to the level of immune suppression, anti-fungal prophylaxis for candida and invasive mold infection is recommended with agents such as itraconazole, voriconazole, or posaconazole until day 180. Frequent monitoring of sirolimus levels will be necessary during azole therapy to avoid toxic drug levels. Voriconazole will be started on Day+5 along with Sirolimus. This is to prevent drug interaction between Voriconazole and Cyclophosphamide. From Day -1 to Day -4 antifungal prophylaxis will consist of Casponfungin or Micafungin

5.6.6.4 Bacterial prophylaxis

Prophylaxis against bacterial infections is generally not recommended. However, antibiotic prophylaxis according to institutional guidelines is acceptable.

5.6.6.5. CMV surveillance

All recipients must be tested weekly using the polymerase chain reaction (PCR) method beginning a week after commencing the conditioning regimen and 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 preemptively if CMV testing reveals a high or rising viral load. Treatment of CMV should be undertaken per institutional

guidelines. If CMV reactivation occurs at or before engraftment, foscarnet may be considered as an alternative to mitigate marrow suppression.

5.6.6.6. Adenovirus guidelines

Testing for adenovirus infection in the blood by a 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 institutional guidelines.

5.6.6.7. EBV surveillance

All recipients must 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 PTLD (adenopathy, fever, etc.), therapy with rituximab is recommended.

5.6.7. Intravenous Immune Globulin (IVIG)

IVIG may be administered according to institutional practice guidelines.

5.6.8. Fertility Preservation

Consideration should be made for fertility preservation prior to beginning these treatments. Cryopreservation of semen should be offered to all males Tanner III or more prior to gonadotoxic therapy. Cryopreservation of oocytes is not typically utilized in adolescent oncology patients because the time required to harvest oocytes would cause an unacceptable delay in cancer treatment; for pubertal SCD patients, however, this time delay is not typically prohibitive. Pubertal females, therefore, have the option of cryopreservation of oocytes. For prepubertal boys and girls, cryopreservation of gonadal tissue, which is experimental, is the only option to preserve fertility. Fertility consultation should be arranged for each patient prior to scheduling HCT with either a member of the Aflac Fertility Preservation team (for pediatric patients) led by Dr. [redacted] or by referral to Emory Reproductive Medicine (for adult patients).

5.7. Graft Failure

Graft failure following HCT in patients with SCD is usually associated with autologous reconstitution of the bone marrow with host hematopoiesis. It is associated with a steady decline in donor chimerism, increasing representation of HbS (in the absence of ongoing RBC transfusion therapy) and clinical manifestations of SCD. A second transplant or donor cell infusion should not be considered unless the patient has < 20% donor chimerism.

5.8. Toxicities of HCT

5.8.1. Toxicities related to the preparative regimen

5.8.1.1. Pancytopenia

The administration of Flu and CY is expected to produce pancytopenia with ANC < 500/μL, hemoglobin < 7-8 gm/dL and platelet < 50,000/μL for as long as several weeks in most patients. Thus, these patients will require RBC and platelet transfusions until hematological recovery after HCT. 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.

5.8.1.2. Chemotherapy-specific toxicities

1. 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. Rabbit ATG
Thymoglobulin (rabbit ATG) is a purified, pasteurized, gamma immune globulin obtained by immunization of rabbits with human thymocytes and thus contains cytotoxic antibodies directed against antigens expressed on human T-lymphocytes. Infusional toxicities include leukopenia, malaise, and PTLD. Chills and fever commonly occur in patients receiving ATG. Symptomatic treatment and temporary slowing of the infusion can usually manage minor toxicities. Pruritus and erythema occasionally develop. These symptoms are generally controlled with diphenhydramine. Respiratory distress and hypotension may be signs of anaphylaxis. In such cases, the infusion should be discontinued. If reaction persists, diphenhydramine, epinephrine or hydrocortisone or a combination should be administered. Serum sickness syndrome may present with fever, arthralgias, and rash. Symptoms usually occur with some delay after initial ATG administration and treatment is with steroids. Other toxicities include pain in chest, flank or back which may be a sign of anaphylaxis or hemolysis. Infusion should be discontinued if anaphylaxis is suspected. Patients unable to tolerate rabbit ATG will receive an equivalent dose of horse ATG.

3. Cyclophosphamide
Common side effects include nausea, headache, diarrhea, vomiting, stomach upset, and loss of appetite. Occasional side effects include hemorrhagic cystitis and rare side effects include cardiac toxicity. Late sequelae include risk of infertility and risk of secondary malignancy.

4. Thiotepa
Side effects of thiotepa include fatigue, loss of appetite, nausea, vomiting, myelosuppression, rash, alopecia, pain at injection site, asthenia, dizziness, blurred vision, headache, and seizures (at high doses).

5.8.2. Toxicities related to hematopoietic stem 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.

5.8.3. Toxicities related to GVHD prophylaxis

5.8.3.1. Sirolimus
Increased susceptibility to infection including opportunistic infections such as tuberculosis, fatal infections, and sepsis and the possible development of lymphoma and other malignancies, particularly of the skin, may result from immunosuppression. The rate of lymphoma/lymphoproliferative disease ranges from 0.7-3.2%. Thrombotic microangiopathy may occur particularly when sirolimus is combined with calcineurin inhibitors.

5.8.3.2. Mycophenolate Mofetil
Common side effects include constipation, nausea, headache, diarrhea, vomiting, stomach upset, loss of appetite, gas, tremor, or trouble sleeping.

6. STUDY ENDPOINTS

6.1. Primary Endpoint
The primary endpoint is EFS at 1-year post-transplant.

6.1.1. Event-free Survival

EFS is defined as the survival with stable donor erythropoiesis with no new clinical evidence of SCD. Primary or late graft rejection with disease recurrence or death will count as events for this endpoint.

6.1.2. Graft Rejection

Primary Graft Rejection: Primary graft rejection is defined as the absence of donor cells assessed by peripheral blood chimerism assays on day 42. Primary graft rejection can be accompanied by pancytopenia and marrow aplasia or by autologous hematopoietic reconstitution without aplasia.

Late Graft Rejection: The absence of donor hematopoietic cells in peripheral blood beyond day 42 in a patient who had initial evidence of hematopoietic recovery with > 20% donor cells will be considered a late graft rejection.

6.1.3. Disease Recurrence

In the majority of cases in patients with SCD, failure of the allograft is followed by reconstitution of the bone marrow with autologous hematopoiesis and recurrence of SCD, generally as a late graft rejection as defined above. Marrow aplasia with primary graft failure is uncommon. Disease recurrence is defined as the return of sickle erythropoiesis (HbS level > 70%) and the absence of donor cell representation. This may be accompanied by recurrence of clinical complications of SCD such as stroke, ACS, and VOC.

6.2. Secondary Endpoints

Secondary endpoints are evaluations of the effects of HCT on clinical and laboratory manifestations of SCD at 1 year and evaluation of transplant-related outcomes. Secondary endpoints include the following:

6.2.1. Overall Survival

OS is defined as survival with or without SCD after HCT.

6.2.2. Cumulative Incidence of Neutrophil and Platelet Engraftment

Neutrophil Engraftment: Time to neutrophil engraftment is defined as the first of 3 measurements on different days when the patient has an absolute neutrophil count of $\geq$ 500/μL after conditioning.

Platelet Engraftment: Time to platelet engraftment will be defined as the first day of a minimum of 3 measurements on different days that the patient has achieved a platelet count > 50,000/μL AND did not receive a platelet transfusion in the previous 7 days. The exception is the case in which a subject is given a platelet transfusion specifically to achieve a platelet threshold to allow an elective invasive procedure, such as a central catheter removal.

6.2.3 Chimerism following HCT for SCD

Genomic DNA extracted from peripheral blood will be analyzed for variable number of tandem repeats (VNTR) to detect donor engraftment in myeloid and lymphoid fractions.

6.2.4. 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 CIBMTR consensus criteria (please see Appendix A).

6.2.5. Chronic GVHD

Incidence and severity of chronic GVHD will be scored according to the NIH consensus criteria.

6.2.6. Frequency of Transplant-related Complications

1. IPS
IPS is diagnosed by evidence of widespread alveolar injury:

a. Radiographic evidence of bilateral, multi-lobar infiltrates (by chest x-ray or CT scan); AND
b. Evidence of abnormal respiratory physiology based upon oxygen saturation (SpO2) < 93% on room air or the need for supplemental oxygen to maintain oxygen saturation ≥ 93%; AND
c. Absence of active lower respiratory tract infection

2. VOD
VOD is diagnosed by the presence of ≥ 2 of the following with no other identifiable cause for liver disease:

a. Jaundice (direct bilirubin ≥ 2 mg/dL or > 34 µmol/L)
b. Hepatomegaly with right upper quadrant pain
c. Ascites and/or weight gain (> 5% over baseline)

3. CNS toxicity
CNS toxicity will be defined as seizures, CNS hemorrhage, or PRES. PRES is defined as an increased diffusion coefficient in areas of T2 hyperintensity on diffusion-weighted imaging in the context of clinical symptoms or physical findings including headache, seizures, visual disturbances, and altered level of consciousness.

4. Infection
Significant infections will be recorded including but not limited to bacterial or fungal sepsis, CMV reactivation with/without clinical disease, adenovirus infection, EBV PTLD, other significant viral reactivations or community-acquired viral infections and invasive mold infections.

6.2.7. Frequency of Stroke
An overt stroke is defined as a focal neurologic event and neurologic deficit lasting > 24 hours with neuroimaging changes. Patients 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 an MRI signal abnormality measuring at least 3 mm visible on two views on T2 weighted images. Silent and overt strokes that occur post-transplant will be judged a disease related complication unless there is a transplant-related event related to these findings.

7. HUMAN SUBJECT PROTECTION AND PATIENT ASSESSMENTS

7.1 Human Subject Protection

7.1.1. Risks to Subjects
Subjects Involvement and Characteristics: 15 patients with severe SCD who are 5 - 40 years of age inclusive or younger than 15 years but pubertal who undergo HCT will be recruited.

Recruitment of Women and Minorities as Research Subjects: Equal number of women and men are likely to be recruited. The majority of patients will be of minority ethnic origin because of the demographics of distribution of SCD. The racial, gender and ethnic characteristics of the proposed subject population reflects the demographics of the study sites. We shall attempt to recruit subjects in respective proportion to these demographics. No exclusion shall be based on race, ethnicity, or gender.

Inclusion of Children as research subjects: Previous studies have demonstrated the benefit of HCT for children from matched sibling donor in children up to age 16 years. There is currently an ongoing study of HCT for children up to age 16 years from unrelated donors. The risk involved in the participation of the study is greater than minimal but has the potential for direct benefit to the individual. The risk is justified by the

extent of potential benefit to the involved children and the relation of the risk to the potential benefit is at least as favorable to the subject as that presented by alternative approaches.

Expertise of the investigative team in dealing with patients of the specific age ranges: Investigators are Board Certified in Pediatric or Adult Hematology/Oncology/HCT with experience in taking care of hemoglobinopathy patients and HCT patients. The PI of the study has previously conducted IRB approved multicenter trials of HCT for SCD.

Potential Risks: HCT is the only modality that can cure SCD. If HCT is successful, it is anticipated that patients will not have symptoms related to the disease, have no requirement of transfusion and have stabilization of organ damage. The risk of mortality is low in patients < 16 years of age receiving HCT from matched siblings (4-5%)[51]. Risks are higher among older patients and those receiving HCT from an unrelated donor. Published data from Hopkins suggest that this protocol is well tolerated in recipients of HCT from a mismatched family donor. However, serious viral or bacterial infections and acute and chronic GVHD pose significant risks of morbidity and mortality even in patients receiving HCT following an NMA conditioning regimen. While there is no information available on the effect of this regimen on fertility, the use of high doses of alkylator (CY alone) in HCT conditioning regimens was associated with recovery of ovarian function in all patients under the age of 26 years and in 61% of patients over the age of 26 years[52,53]. At least half of the patients with aplastic anemia transplanted and survived ≥ 2 years preserved or regained the ability to become pregnant or father children[54]. It is estimated that in the current regimen the risk of infertility will be 50% or less. There is a small risk (<5%) for a malignancy and potential for late toxicity includes infertility.

Discussion of Alternatives to participation in the study: Patients and parents will be clearly apprised of alternatives to participation on the study. We will discuss risks and benefits and current outcomes of alternate modalities of treatment such as conventional myeloablative HCT, chronic transfusion, HU or supportive care.

7.1.2. Protection Against Risks

Adequacy of Protection Against Risks: Subjects for this study will be recruited from among patients followed at or referred to the clinical sites. The study will be discussed with the patient or family members by one of the investigators and informed consent obtained. All patients will sign informed consent according to guidelines of the local Institutional Review Board. Patients will be initially evaluated and their Hematologist will initiate the consent process in the context of ongoing medical care. A trusted non-medical member of the team such as an educator or social worker will interview patients to confirm patients and families have explicit consent of further evaluation for HCT.

Protection against Risk: This protocol will be reviewed and approved by the IRB at Emory University as defined by FDA regulations (21CFR Part 56). IRB approval of any future modifications of the protocol or consent form for this study will be given in writing. Unanticipated adverse effects will be reported to the IRB. The IRB will receive notification of the completion of the study. Investigators will maintain an accurate and complete record of all submissions made to the IRB, including a list of all reports and documents submitted.

Risk Management Procedures: Procedures for minimizing specific risks associated with study procedures are outlined above. Patients will be admitted to the HCT inpatient unit and will receive standard care. Once engraftment has been demonstrated, the patients will be followed closely in the outpatient clinic. In addition, one of the investigators will be available by telephone and pager 24 hours per day to address any concerns from patients. Confidentiality will be maintained throughout the study. Subjects will be identified by research study numbers that will be the only identifying information to appear on data and documents used for evaluation or statistical analysis. No verbal or written information concerning any subject will be released without the written consent of the subject. Records will be maintained only in anonymous research files, kept

in locked quarters and made available only to qualified research personnel. There will be regular meetings of the investigators to review the status of the study and to ensure that confidentiality is being maintained.

7.1.3. Potential Benefits of the proposed research to the subjects and others

Potential benefits to patients include cure of the severe SCD and alleviation of chronic morbidity and possibly reversal of organ damage.

7.1.4. Importance of the knowledge to be gained

It is anticipated that a safe and effective haploidentical approach to HCT for severe SCD will greatly enhance the applicability of this modality of treatment to this group of patients. It is anticipated that an understanding of the impact of donor derived hematopoiesis on organ function will enhance our understanding of the impact of HCT on SCD as well as impact on timing of intervention with HCT in SCD patients.

7.1.5. Donor Human Subject Protection

Physicians will carefully evaluate prospective donors for potential risk factors and explain all potential risk to donors. Donors will be evaluated for eligibility by an independent physician separate from the research team. This independent physician could be a general practitioner, pediatrician, family practice physician, or internist. This physician must document that the donor does not have any of the exclusion criteria and that the donor is in good health. This physician will not participate in the consenting process. Donor consent will be conducted through the standard operating procedures of the transplant center.

The donor advocate, with the assistance of the independent physician, will be authorized to postpone or cancel the marrow harvest if the harvest is judged to carry a high risk of a serious and sustained long-term adverse impact on the donor. The transplant recipient will not be enrolled in the study until the donor has been cleared by a medical assessment and has been approved by the independent physician. In this manner, we believe that we can ensure donor safety and informed consent.

If the advocate and independent physician feel that it is not in the interest of the donor to serve as a bone marrow donor, he/she will not be used as a donor. All donors will undergo screening procedures including blood tests (approximately 10-15 ml), a urinalysis, pregnancy test (for female donors of child bearing potential) and any tests clinically indicated for the safety of the donor during general anesthesia. The risks involved in the screening procedures include discomfort from phlebotomy. Since the bone marrow donation occurs under general anesthesia, there is some risk associated with anesthesia. The most common side effects related to the collection of bone marrow are: pain in the hip bones, back stiffness and bruising and bleeding in the skin where the needles were placed. Rare complications include infections in the skin and bone or major bleeding problem. It is possible that a blood transfusion will be necessary so that the donor is not too anemic after the bone marrow collection. In some cases, it will be possible to collect blood in advance from the donor and store it so that it can be given after the bone marrow collection. There is also a potential for psychological distress to the donor in case of serious HCT related complications to the recipient.

Measures that will be taken to minimize the risks of procedures:

1. Every effort will be made to use aseptic technique as well as measures to minimize the discomfort from phlebotomy, including the use of topical analgesia.
2. Pregnancy tests will be done prior to any radiological procedure.
3. Adequate pre-, intra- and post-operative analgesia will be ensured in order to minimize the risk of pain.
4. Appropriate aseptic technique will be used to minimize the risk of infections.
5. Appropriate anesthetic technique and monitoring will be ensured to minimize the risk from anesthesia.

6. Where the patient is eligible for autologous blood donation (e.g., donors >16 years of age and weighing > 50 kg), we would recommend that this be done so as to limit the possibility of requiring transfusion from an allogeneic donor.
7. Blood will be placed on hold with the Blood Bank should the patient become anemic and require a transfusion.
8. A psychologist will be available to see the donor prior to donation and will be available subsequently to address any behavioral and/or psychological consequences of bone marrow donation.

Donation of bone marrow has no prospect of direct benefit to individual subjects but represents an opportunity to understand, prevent, and alleviate SCD which is a serious problem affecting the health and welfare of children and adults. Even though the procedures that the possibly minor donors are asked to undertake are considered greater than minimal risk, donors have a strong emotional bond with the recipient and a genuine likelihood that their donation could benefit the recipient significantly, they themselves also stand to benefit psychologically in an important manner. The research will be conducted in accordance with sound ethical principles and adequate provisions will be made for soliciting the assent of children and the permission of their parents/guardians. As such, this meets the requirements of Subpart D §46.405.

7.2. Data and Safety Monitoring Plan

This study will be centrally reviewed by the Data Safety and Monitoring Board (DSMB) of the Aflac Clinical Research Office. The DSMB will meet quarterly to review all serious adverse events (SAEs) and deaths and to determine whether any patient safety problems necessitate protocol modifications or discontinuation of the trial. The DSMB will also meet on an *ad hoc* basis if unexpected safety events occur that may necessitate study suspension or closure. The DSMB will discontinue the review of outcomes when this protocol is closed to accrual. If the DSMB recommends protocol or informed consent changes during the study, the recommendations will be reviewed by the PI and incorporated into the protocol as deemed appropriate.
All SAEs must be submitted to the designated Aflac Clinical Trials Coordinator within 24 hours. The DSMB will appoint an independent safety monitor who will review submitted SAEs immediately as they are reported, with follow-up through resolution. The monitor will also evaluate individual and cumulative participant data, and will provide recommendations to the DSMB regarding the safe continuation of the study. The monitor will notify the DSMB of any findings of a serious and immediate nature including any recommendations to discontinue all or part of the trial. In addition, the monitor will communicate findings, any concerns and recommendations during the quarterly DSMB meetings. Before each regularly scheduled DSMB meeting, the study coordinator will submit a report including tabular summaries of all reported SAEs, and deaths on study to date. The report will also include a brief summary of each previously unreported SAE and death, including an assessment of whether the event was unexpected or related to the study.

7.3. Adverse Events

Definitions:

Adverse event (AE): any untoward medical occurrence regardless of causality assessment. An AE can be an unfavorable and unintended sign (including an abnormal laboratory finding), symptom, syndrome or disease.

Serious adverse event (SAE): when the AE 1) results in death, 2) is considered life-threatening, 3) results in hospitalization or cause the prolongation of hospitalization, 4) results in permanent disability or irreversible impairment, 5) leads to a congenital anomaly, or 6) represents a significant medical condition which, without urgent medical intervention, would lead to one of the above outcomes. Life-threatening means that the AE represented an immediate threat of death without medical intervention. <u>For the purposes of this study, non-engraftment, graft rejection and late graft failure shall always be considered SAEs regardless of their impact on the patient's condition.</u>

Unexpected adverse events: events whose nature, severity, or frequency is inconsistent with what is known about the patient's prior medical history or the possible adverse effects of HCT.

Expected adverse events: AEs that are anticipated to occur due to the patient's disease or due to HCT. Expected adverse events usually considered to be related to intensive pre-transplant conditioning, allogeneic grafts and GVHD prophylaxis include serious bacterial and fungal infections, VOD, complications arising from GVHD and its treatment, IPS and complications arising from oral or gastrointestinal mucositis. Such events may be deemed *expected*. Adverse events associated with the previous treatment of a patient's SCD may, likewise, be deemed expected.

Unanticipated problems (UP): include unexpected AEs and also unexpected problems, events, or new information which are not AEs but which indicate that research participants or others are at greater risk of harm than previously believed prior to recognition of the unanticipated problem.

Characterizing an adverse event: Adverse events will be described using event terms and severity grading from the NCI Common Toxicity Criteria for Adverse Events (CTCAE) version 5.0 (November 27. 2017). The expectedness of the event (see above) and the relation of the event to the study drug shall also be characterized.

The relation or attribution of the event to the investigational product may be characterized as follows:
Definitely related, clearly associated with study drug
Probably related, likely associated with study drug
Possibly related, may be associated with study drug
Unlikely to be related, or
Definitely not related to the study drug

Adverse Event Reporting: All SAEs occurring through day 100, whether expected or unexpected, will be reported in an expedited manner to the designated Aflac Clinical Trials Coordinator within 24 hours of knowledge of the event. SAEs occurring after day 100 will be reported in an expedited manner to the study team only if graft failure. If reportable SAEs are fatal, they are required to be reported to the study team within 24 hours of knowledge of the event. If non-fatal, they are required to be reported to the study team within five business days. IREs and UPs are required to be reported to the study team within 5 business days. Local IRBs are required to be notified of reported SAEs, UPs and IREs by institutional PIs in accordance with local IRB guidelines.

Adverse Events of interest will be monitored and recorded using NCI's Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0 (November 27. 2017) at regular intervals (e.g., post-transplant timepoints). Adverse Events of interest include infections, GVHD, CNS toxicities, hospitalizations, or any event considered by the Investigator as clinically significant (i.e. with clinical manifestations or requiring treatment or clinical management).

Monitoring of SAEs, unexpected problems and IREs: The CRO will notify the PI or co-PI and the chair of the DSMB within three business days of receiving a report. The PI or co-PI will review the grading, expectedness, and attribution to ensure the accuracy and completeness of reports.

7.4. Pre-transplant Evaluations (standard evaluations for transplant patients)

The following observations are required pre-transplant (please see **Tables 3A and 3B** for details of timing):

- HLA typing by allele-level methodology at HLA A, B, C and DRB1 loci
- ABO and Rh typing
- Baseline echocardiography for LVEF, LVSF and presence or absence of tricuspid regurgitation (TR). If TR present, measure jet velocity as it is a risk factor for early mortality and pulmonary hypertension.
- Baseline EKG
- Pulmonary function testing: Spirometry and lung volumes, including FEV1, FVC, and DLCO
- Oxygen saturation by pulse oximetry
- In patients receiving chronic RBC transfusions one year or longer and in patients with clinical evidence of iron overload as evidenced by a serum ferritin level of > 1000 ng/ml and a history of multiple RBC transfusions, evaluation of liver fibrosis will be carried out by a combination of MRI elastography, ultrasound elastography, fibroscan or liver biopsy as necessary. Hepatology consult will be requested for evaluation of liver fibrosis in patients with liver iron overload. Ferriscan MRI will be performed in patients with iron overload for estimation of liver iron concentration.
- History, physical examination (including Tanner staging), height, and weight
- Karnofsky/Lansky performance score
- Hb electrophoresis quantification of: HbF, HbS, Hb A2 levels
- CBC with differential and platelet count; complete metabolic panel (including serum creatinine, bilirubin, alkaline phosphatase, ALT, AST, and magnesium); serum ferritin
- Infectious disease titers, including CMV antibody test, hepatitis panel (Hep B sAg, Hep B Core Ab, Hep C Ab), herpes simplex, syphilis, HIV and HTLV1 I/II antibody
- β-HCG serum pregnancy test for females of childbearing potential
- HLA antibody screen
- Adequate Renal function as reflected by Serum creatinine ≤ 1.5 x the upper limit of normal for age and **one** of the following: i. creatinine clearance >70 mL/min calculated using the Cockcroft-Gault calculator, ii. creatinine clearance > 70 mL/min by 24-hour urine (preferred)iii. GFR > 70 mL/min/1.73 m2 by radionuclide GFR.
- Offer Fertility consult, to include gonadal history (including time of menarche), timing of pubertal development, and reproductive history
- LH, FSH, and AM testosterone in males and LH, FSH, AMH, and estradiol in females.
- Lymphocyte subsets (T/B/NK panel)
- Exercise capacity by 6 minute walk distance under standardized procedures as per 2002 American Thoracic Society Guidelines[55]
- Urinalysis, spot urine for albumin and creatinine
- Calculated creatinine clearance
- Liver/spleen scan
- MRA/MRI of the brain
- Neurocognitive battery (CHOA patients only).
- Pain diary twice a day for 7 days, .PROMIS HrQOL profile (PROMIS 29 in adults, PROMIS 25 in children)
-

7.5 Post-Transplant Evaluations (standard evaluations for transplant patients)

The following observations are required post-transplant:

- TNC and $CD34^+$ cell content of the infused marrow product (day 0)
- History and physical exam (including performance score and GVHD assessment) weekly until day 100 post-transplant, then at six months and one-year post-transplant.

- 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 and one-year post-transplant.
- Creatinine, bilirubin, alkaline phosphatase, ALT, and AST twice a week until day 28, then weekly until 12 weeks, and then at six months and one-year post-transplant.
- Peripheral blood for fractionated (sorted) chimerism by VNTR examining the myeloid and lymphoid fractions between day 28, day 100, 6 months, and 1 year
- Lymphocyte subsets (T/B/NK panel) at day 28, 60, 100, and 1-year post-transplant
- ABO and Rh typing day 28- and 1-year post-transplant
- Quantitative hemoglobin electrophoresis (HbF, HbA, HbA2, and HbS) on day 100, 6 months, and one-year post-transplant
- Endocrine assessment (at 6 months and 1 year) to include Tanner staging, menstrual history, LH, FSH and AM testosterone in males and LH, FSH, AMH and estradiol in females.
- Serum ferritin at 1-year post-transplant
- Radionuclide GFR or 24-hour creatinine clearance, urinalysis, spot urine for albumin and creatinine at 1 year.
- Echocardiography for LVSF and presence or absence of TR at 1-year post-transplant. If TR present, measure jet velocity as a measure of pulmonary hypertension.
- Pulmonary function testing with FEV1, FVC, DLCO, and oxygen saturation by pulse oximetry at 1-year post-transplant.
- MRA/MRI of the brain at 1-year post-transplant
- Neurocognitive battery (CHOA patients only) at 1-year post-transplant
- Exercise capacity by 6MWD at 1 year post-transplant[55]
- Liver/Spleen scan at 1-year post-transplant
- Ferriscan will be repeated at 1-year post HCT for evaluation of liver iron overload.
- Pain diary for 7 days, and PROMIS HRQoL profile 1-year post BMT(PROMIS 29 in adults and PROMIS 25 in children).

7.6 Summary of Patient Clinical Assessments

Pre-transplant clinical assessments for patients undergoing HCT are summarized in **Table 3A** (patients not already receiving HU) and **Table 3B** (patients already receiving HU). Post-transplant clinical assessments are summarized in **Table 4**.

Table 3A. Schedule of Pre-Transplant Evaluations for Patients Not Already Receiving HU				
Assessments	**Prior to Enrollment (≤180 days)**	**Prior to Enrollment (≤30 days)**	**Prior to HU (post enrollment)**	**Prior to ATG (≤30 days, unless otherwise indicated)**
HLA typing	X[1]			
ABO and Rh typing	X			
Echocardiogram (LVEF, SF, TR jet velocity) & EKG	X		Prn clinical concern	Prn clinical concern
PFT with spirometry (FEV1, FVC, DLCO)	X		Prn clinical concern	Prn clinical concern
Pulse oximetry	X			X
Liver biopsy (if receiving chronic transfusion > 1 year)	X			
History, physical exam (including Tanner staging)		X		
Performance score		X		X
Hgb electrophoresis		X		
CBC, chemistries, ferritin		X		X
Infectious disease titers		X		
Pregnancy test (females)		X		X
HLA antibody screen		X		X May have to be repeated if graft rejection becomes a concern
24 hr. urine collection for creatinine clearance or radionuclide GFR		X		
Fertility consult			X	
LH, FSH, Estradiol, AMH or AM Testosterone			X	
Lymphocyte subsets			X	X
Exercise capacity (by 6MWD)[55]			X	
Specimen for biorepository			X	
Urinalysis, spot urine for albumin and creatinine				X
Calculated creatinine clearance				X
Liver/Spleen scan				X[1]
Cerebral MRA/MRI				X[2]
PROMIS HR QoL profile				X
Electronic pain diary twice a day for 7 days				X
Neurocognitive Battery				X[1]

[1]Timing may be outside of indicated timepoint as per routine clinical care

[2]For patients with history of CNS involvement, must be within 90 days of ATG; for patients with NO history of CNS involvement, must be within 180 days

Table 3B. Schedule of Pre-Transplant Evaluations for Patients Already Receiving HU			
Assessments	**Prior to Enrollment (≤180 days)**	**Prior to Enrollment (≤30 days)**	**Prior to ATG (≤30 days, unless otherwise indicated)**
HLA typing	X[1]		
ABO and Rh typing	X		
Echocardiogram (LVEF, SF, TR jet velocity) & EKG	X		X
PFT with spirometry (FEV1, FVC, DLCO)	X		X
Pulse oximetry	X		X
Liver biopsy (if receiving chronic transfusion > 1 year)	X		
History, physical exam (including Tanner staging)		X	
Performance score		X	X
Hgb electrophoresis		X	
CBC, chemistries, ferritin		X	X
Infectious disease titers		X	
Pregnancy test (females)		X	X
HLA antibody screen		X	X May have to be repeated if graft rejection becomes a concern
24 hr. urine collection for creatinine clearance or radionuclide GFR		X	
Fertility consult			X[1]
LH, FSH, Estradiol, AMH or AM Testosterone			X
Lymphocyte subsets			X
Exercise capacity (by 6MWD)[55]			X
Specimen for **biorepository**			X
Urinalysis, spot urine for albumin and creatinine			X
Calculated creatinine clearance			X
Liver/Spleen scan			X[1]
Cerebral MRA/MRI			X[2]
PROMIS HR QoL profile			X
Electronic pain diary twice a day for 7 days			X
Neurocognitive Battery			X[1]

[1] Timing may be outside of indicated timepoint as per routine clinical care

[2]For patients with history of CNS involvement, must be within 90 days of ATG; for patients with NO history of CNS involvement, must be within 180 days

Table 4. Schedule of Transplant/Post-Transplant Evaluations (All Patients)								
Assessments	**Day 0**	**Day 7**	**Weekly**	**Day 28**	**Day 60**	**Day 100**	**6 mo.**	**1 yr.**
TNC and CD34⁺ cell content of infused marrow	X							
History, physical exam, performance score			X				X	X
GVHD and morbidity assessments			X				X	X
CBC, chemistries			X				X	X
Sorted peripheral blood chimerism (VNTR)				X May repeat for graft rejection or other clinical concern	X	X	X	X
GVHD biomarker panel day 7 post HCT and at onset of GVHD		X						
Lymphocyte subsets				X	X	X		X
ABO and Rh typing				X				X
Hb electrophoresis						X	X	X
Gonadal history and Tanner staging							X	X
LH, FSH, Estradiol, AMH or AM Testosterone							X	X
Serum ferritin								X
Urinalysis, spot urine for albumin and creatinine								X
24 hr. urine collection for creatinine clearance or radionuclide GFR								X
Echocardiogram (LVEF, SF, TR jet velocity) & EKG								X
PFT with spirometry (FEV1, FVC, DLCO, and oxygen saturation)								X
Cerebral MRA/MRI (additional sequences CHOA only, Appendix B)								X
Neurocognitive Battery (CHOA only)								X
Exercise capacity (by 6MWD)[55]								X
PROMIS HR QoL profile								X
Electronic pain diary twice a day form7 days								X
Liver/Spleen scan								X

Target dates for the post-transplant clinical assessments and Laboratory samples are described in **Table 5**. However, samples should still be collected even if outside target window.

Table 5. Target dates for post-transplant clinical assessments	
Study Visit	**Target Day Post-transplant**
Weekly	± 3 days
Day 28	± 14 days
Day 100	± 14 days
6 months	180 ± 28 days
1 year	365 ± 28 days

7.7 Research Evaluations (Table 6)

The following evaluations are to be done in addition to the above routine clinical evaluations:

- Peripheral blood samples for immune reconstitution at baseline (donor and recipient prior to starting HU), day -9 (prior to starting ATG), and days +21, 30, 60, 100, and 365.

Immune reconstitution will be assessed via flow cytometry, ELISPOT, ELISA, and RNASeq. For flow-based studies, the number (events, percentage) of T lymphocytes ($CD4^+$, $CD8^+$), B lymphocytes, and NK cells will be determined longitudinally, and their activation status will be assessed by flow cytometry and ELISPOT.

Table 6. Blood Draw Volumes (in milliliters) for Research Studies

Test	Tube	Donor	Recipient						
		Baseline	Baseline	Day -9	Day 21	Day 30	Day 60	Day 100	Day 365
Immune reconstitution	Sodium heparin (6 ml tube)	-	12	12	12	12	12	12	12
Immune reconstitution	Cyto chex (5 ml tube)	-	4	4	4	4	4	4	4
Immune reconstitution	Red top clot tube	-	4	4	4	4	4	4	4
RNASeq	Tempus	3	3	3	3	3	3	3	3

8. STATISTICAL CONSIDERATIONS

8.1. Study Design

The primary goal of this phase II study is to determine the feasibility of achieving a high rate of patients being alive with donor erythropoiesis at 1 year post-transplant using a conditioning regimen that consists of the combination of Flu/rat/CY/Thiotepa/TBI (200 cGy) and post-transplant CY in a total of 15 patients with severe SCD.

8.2. Efficacy Endpoint

The rate at which patients are alive with donor erythropoiesis (EFS) at 1 year after transplant is the efficacy endpoint in this study. Death, primary or late graft rejection, or recurrence of disease will be considered events for this endpoint. EFS will be defined as time from stem cell infusion until the first event among primary graft failure, secondary graft failure, disease recurrence, or death. While we assume that graft failure and disease recurrence are linked events, we have permitted the earliest confirmed date for either type of event to determine EFS. All patients without events will be followed for a minimum of one year so that no patients will be censored prior to the 1-year time point; this permits 1-year EFS to be defined as a binary end point.

The goal of the study is to demonstrate a 1-year EFS in the neighborhood of 70%. If the study enrolls 15 patients, we will achieve that goal if 11 or more of 15 patients are alive with donor erythropoiesis at one year. This corresponds to an EFS at one year of 73% or higher. If 11 patients are alive without graft failure or recurrence at one year, the lower 10% exact binomial confidence bound for this result is 54%, suggesting that this finding is consistent with an approach in which more than half of patients would derive clinical benefit.

8.3 Toxicity

Transplant-related mortality is the primary toxicity of concern in this study. We define this to be death in the absence of either loss of donor erythropoiesis or disease recurrence within 6 months of transplant. We recognize that patients eligible for this trial may have already experienced end organ compromise as a result of their underlying disease. We therefore mandate that each death within the first six months of transplant be reviewed by the study DSMB to assess attribution to either transplant or to underlying disease.

8.4 Accrual and Study Duration
It is estimated that three years of accrual will be necessary to enroll the targeted sample size. Patients will be followed for a minimum of one-year post-transplant.

8.5 Randomization
There is no randomization in this trial.

8.6 Primary Endpoint
The primary objective is to assess EFS probability 1-year post-transplant. Death, primary or late graft rejection, or recurrence of disease will be considered events for this endpoint.

8.7 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. EFS will be defined as time from stem cell infusion until the first event among primary graft failure, secondary graft failure, disease recurrence, or death. All patients without events will be followed for a minimum of one year so that no patients will be censored prior to the 1-year time point. In addition, the frequencies of each component of the composite endpoint (primary graft failure, secondary graft failure, and disease recurrence) will be described.

8.8 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 one-year post-transplant for mortality.

Incidence of red cell, neutrophil, and platelet engraftment: To assess the incidence of each type of engraftment from the 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.

Acute GVHD: To assess the incidence of grades II-IV or grades III-IV acute GVHD from the day of transplant, the first day of acute GVHD onset of 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.

Chronic GVHD: To assess the incidence and severity of chronic GVHD from the day of transplant, a cumulative incidence curve will be computed along with a 95% confidence interval at one-year 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 (such as stroke, development of overt nephropathy, change in albuminuria and renal function) will be described using proportions along with 95% confidence intervals.

Exercise Capacity: We will use the 6MWD, testing under standardized procedures, to assess exercise capacity.[55] The ATS guidelines indicate sensitivity of this test to a number of subject characteristics, but we will be focusing on the change between two time points, and this orientation will permit us to make a more straightforward comparison. Our primary endpoint will be absolute change from baseline, with increased distance identified as positive change. We anticipate an improvement of at least 50 m in the transplant subjects on average. We will use a paired t-test to examine the change in distance walked within subjects.

Cardiac Function: We will measure changes in TRJV over time. We will again rely on a t-test as well as on a linear mixed model (Desai) for the rate of change in TRJV while incorporating both transplant status and baseline value. As a planned subset analysis, we will also examine the magnitude of changes in the subset of patients who experience increases over baseline measurements.

Pulmonary Function: Subjects will undergo PFTs at baseline (pre-HCT) and at 1-year post-HCT. We will use both the paired t-test and generalized linear models to assess changes in PFTs, but we have no estimates of variability over time on which to base power calculations at this time. We will also assess the proportion of transplanted subjects in whom we find evidence of restrictive lung disease, defined as TLC below the 5th percentile adjusted for age, gender, race, and height, as is also reported by Field.

Renal Function: We will measure albuminuria. Guasch reports that 61% of SCD subjects aged 18 to 30 had either micro- or macroalbuminuria based on a single measurement. We will examine changes in albuminuria at the two time points using the McNemar test, and we will compare the proportions of subjects with albuminuria at each time point using the Fisher exact test.

Pain
Pain will be assessed using a 1-week multi-dimensional pain diary. The time-points will coincide with collection of other PROs (such as PROMIS). Data entry will occur two times per day, morning and evening and it is anticipated it will take less than 3-5 minutes to fill out at each time point. Electronic reminders will be sent to participants twice daily to report their pain data. Follow up phone calls will be done by study staff if there are missing entries to ensure there are no technical issues (forgotten password, inability to log in, etc.). It is possible that the full week of pain data may not be completed due to scheduling priorities related to transplant and post BMT. In these situations, pain data will be collected for maximum allowable days at each time point, up to 1 week.

HRQoL
To assess HRQoL, Patient Reported Outcome Measure Information System (PROMIS®) PROMIS 25 measures will be used for subjects < 18 years old; while, PROMIS 29 will be used for subjects 18 and over. PROMIS® contains a number of self-report forms that each assess domains of physical and emotional function over a seven-day time interval.

8.9 Stopping rules

8.9.1 Overall mortality by day 100 and at 1 year

Overall mortality by day 100: For the transplanted patients, overall mortality in this trial is projected to be less than 20% at day 100. 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 sequential probability ratio (SPRT). The truncated SPRT is based on contrasting 15% versus 30% 100-day mortality, with nominal type I and type II error rates of 10% and 20%, respectively. The common slope of the parallel lines is 0.219 and the intercept for the upper boundary is 2.344. The stopping rule is summarized in **Table 7**.

Table 7. Stopping rules for overall mortality by day 100 for transplanted patients

Number of evaluable patients enrolled	3	4-7	8-12	13-16
Stop if death occurs in	3	4	5	6

Overall mortality at 1 year: The stopping rule for overall mortality at 1 year will be triggered if there is significant evidence that the 1-year overall mortality rate

Table 8. Stopping rules for overall mortality at 1 year for transplanted patients

Number of evaluable patients enrolled	4	5-8	9-11	12-14	15-17
Stop if death occurs in:	4	5	6	7	8

exceed 25% based on the truncated SPRT. The truncated SPRT is based on contrasting 15% versus 30% and 25% versus 40% 1-year mortality, respectively, with nominal type I and type II error rates of 15% and 20%. The common slope of the parallel lines is 0.219 and the intercept for the upper boundary is 1.887 for patients with MRD and 0.322 and 2.415 for patients with MUD. The stopping rule is summarized in **Table 8**.

8.9.2 Graft rejection by day 100

For the patients undergoing haploidentical HCT, failure to engraft donor cells (defined as <20% donor chimerism) by day 100 should occur in < 30% of the patients. The stopping rule for graft rejection by day 100 will be triggered if there is significant evidence that the day 100 graft rejection rate exceeds 30% based on the truncated SPRT. The truncated SPRT is based on contrasting 30% versus 50% 100-day graft rejection, with nominal type I and type II error rates of 15% and 20%. The common slope of the parallel lines is 0.2397 and the intercept for the upper boundary is 1.98. The stopping rule is summarized in **Table 9**.

Table 9. Stopping rules for graft rejection by day 100

Number of evaluable patients enrolled	5-7	8-10	11-12	13-15
Stop if graft rejection occurs in:	5	6	7	8

8.9.3 Grade III-IV Host Disease by day 100

The stopping rule for Grade **III** -IV graft versus host disease at day 100 will be triggered if there is significant evidence that the grade **III** -IV GVHD rates exceed 30% based on the truncated SPRT. The truncated SPRT is based on contrasting 25% versus 35%, respectively, with nominal type I and type II error rates of 15% and 20%. The common slope of the parallel lines is 0.219 and the intercept for the upper boundary is 1.887 for patients with MRD and 0.322 and 2.415 for patients with MUD. The stopping rule is summarized in **Table 10**.

Table 10. Stopping rules for Grade III -IV GVHD at day 100 for transplanted patients

Number of evaluable patients enrolled	4	5-8	9-11	12-14	15-17
Stop if Grade **III** -IV GVHD occurs in:	4	5	6	7	8

8.9.4 Disease recurrence by day 100

The stopping rule for disease recurrence at day 100 will be triggered if there is significant evidence that the disease recurrence rates exceed 30% based on the truncated SPRT. The truncated SPRT is based on contrasting 25% versus 35%, respectively, with nominal type I and type II error rates of 15% and 20%. The common slope of the parallel lines is 0.219 and the intercept for the upper boundary is 1.887 for patients with MRD and 0.322 and 2.415 for patients with MUD. The stopping rule is summarized in Table 11.

Table 11. Stopping rules for disease recurrence at day 100 for transplanted patients

Number of evaluable patients enrolled	4	5-8	9-11	12-14	15-17
Stop if disease recurrence occurs:	4	5	6	7	8

8.9.5. Composite stopping rule for mortality, graft failure, disease recurrence and GVHD grade III-IV

Since graft failure, mortality and GVHD grade III-IV are all unacceptable outcomes we propose a composite of any of these events occurring in patients during the first 100 days after transplant. We rank the four kinds of AEs according to the severity, i.e., disease recurrent < grade III-IV host disease <graft rejection <overall mortality. We treat a high-level event of AE as a lower-level event, then count the number of events during the study and check the stopping rules regarding Table 11, Table 10, Table 9, and Table 7 to see whether terminate the trial. For example, 2 of 9 patients will be dead by day 100, and 3 of 9 patients will be graft rejection by day 100, 1 of 9 patients will be disease recurrence by day 100. In this case, the number of events is 2+3+1=6. We should stop the clinal trial according to Table 11.

8.9.6 Spacing patients for observation of toxicity

In order to allow sufficient time between transplants, we will space patients so that once a patient is transplanted, this patient will need to reach Day 100 before proceeding to the next transplant. This will stay in effect for one year. If for health reasons a patient needs to be transplanted before the 100 day wait window, the PI may appeal for an exception to this rule.

8.9.7. SAE reporting

All SAEs must be submitted to the designated Aflac Clinical Trials Coordinator within 24 hours. The DSMB will appoint an independent safety monitor who will review submitted SAEs immediately as they are reported, with follow-up through resolution. The monitor will also evaluate individual and cumulative participant data, and will provide recommendations to the DSMB regarding the safe continuation of the study. The monitor will notify the DSMB of any findings of a serious and immediate nature including any recommendations to discontinue all or part of the trial. In addition, the monitor will communicate findings, any concerns and recommendations during the quarterly DSMB meetings.

8.9.8. Modification of conditioning regimen

If a decision is made to stop accrual under the initial GVHD regimen, due to excessive GVHD then we will stop accrual. If the DSMB approves we will consider modifying the GVHD prophylaxis with the substitution of Tacrolimus for sirolimus.

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Appendix A: NIH Consensus Criteria for Grading of Severity of Acute GVHD

Clinical Stage of aGVHD According to Organ System			
Grade	**Skin**	**Liver**	**Gut**
I	1-2	0	0
II	3 or	1 or	1
III	-	2-3 or	2-4
IV	4 or	4 or	-

Overall Clinical Grading of Severity of aGVHD			
Stage	**Skin**	**Liver**	**Intestine**
+	Maculopapular rash <25% of body surface	Bilirubin 2.1-3 mg/dl	>501-1000 ml (280-555 ml/m^2) diarrhea per day or nausea, anorexia or vomiting with biopsy (EGD) confirmation of upper GI GVHD
++	Maculopapular rash 25-50% of body surface	Bilirubin 3.1-6 mg/dl	>1001-1500 ml (556-833 ml/m^2) diarrhea per day
+++	Maculopapular rash >50% of body surface area or generalized erythroderma	Bilirubin 6.1-15 mg/dl	>1500 ml (833 ml/m^2) diarrhea
++++	Generalized erythroderma with bullous formation and desquamation	Bilirubin >15 mg/dl	Large volume stool with severe abdominal pain with or without Ileus or stool with frank blood or melena