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## The natural history of overt or silent strokes in individuals with sickle cell disease treated with blood transfusion or hydroxyurea therapy

### 1.0 ABSTRACT

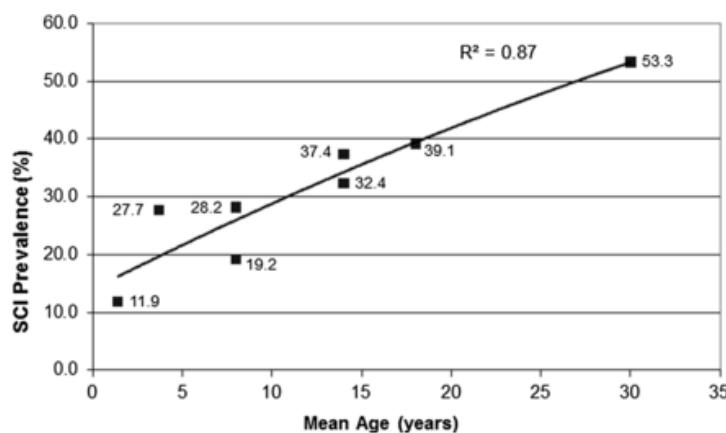
Overt strokes and silent cerebral infarcts (SCI), also referred to as silent strokes, contribute significantly to morbidity in children and adults with sickle cell disease (SCD), resulting in functional impairment, challenges with school and job performance, and premature death. While five NIH-funded randomized controlled trials have identified therapies for primary and secondary overt stroke or SCI prevention in children ( $\leq 17$  years old) with SCD, no prospective multi-center cohort study has systematically evaluated therapeutic options for secondary cerebral infarct prevention in adults. The optimal evidence based therapeutic option for primary and secondary stroke prevention in children with SCD is burdensome monthly blood transfusion therapy, eventually requiring iron chelation therapy. The less burdensome treatment is hydroxyurea therapy, though overall efficacy of this approach is still being evaluated, and some families choose observation alone. These competing choices between optimal blood transfusion therapy, hydroxyurea therapy or observation highlight the challenges of developing acceptable therapeutic strategies, other than blood transfusion, for preventing cerebral infarct recurrence in individuals with SCD. Preliminary data from our cohort suggest that among children with SCI, despite treatment with regular blood transfusions, the presence of magnetic resonance angiogram (MRA) defined cerebral vasculopathy at baseline is a significant risk factor for cerebral infarct recurrence (defined as overt stroke, new or enlarging SCI) compared to those without vasculopathy, 31% (5 of 16) versus 12% (11 of 89), respectively ( $p= 0.053$ ). In seven sickle cell disease (SCD) centers, we propose a retrospective and prospective cohort study describing the natural history of overt strokes and SCI in individuals  $\geq 1$  year old with SCD receiving uninterrupted disease modifying therapy (regular blood transfusions, hydroxyurea or both) versus interrupted therapy or no treatment for secondary cerebral infarct prevention. Inclusion criteria will include: a history of prior cerebral infarct identified on entry magnetic resonance imaging (MRI)/MRA of the brain with a least a 1.5 tesla magnet strength. Exit MRI/MRA will be completed with a minimum and average of 3.5 and 4.5 years of follow-up between imaging studies, respectively. Specifically, based on evidence from the pediatric literature, we will test the hypotheses that, among individuals with SCD, those with baseline vasculopathy will have a higher incidence of stroke recurrence (overt or silent) compared with those without baseline vasculopathy, and those with progressive vasculopathy will have a higher incidence of overt stroke recurrence compared with those without progressive vasculopathy. The aims include: 1) compare the incidence of cerebral infarct recurrence (overt strokes, including intracranial hemorrhage, and SCI) in individuals ( $\geq 1$  year of age) with SCD and evidence of prior overt stroke ( $n=44$ ) or SCI ( $n=180$ ) treated with disease-modifying therapy or interrupted or no therapy for prevention of overt stroke or SCI; 2) determine whether baseline MRA vasculopathy predicts cerebral infarct recurrence in individuals with prior overt stroke or SCI. Critical data obtained from this first multi-center natural history cohort study of individuals

with SCD will facilitate the development of new therapies, other than arduous regular blood transfusions, for secondary prevention of overt stroke and SCI in SCD.

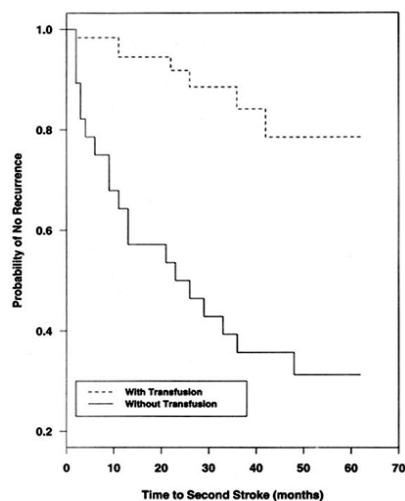
## 2.0 INTRODUCTION

### Untreated silent cerebral infarcts are common and progressive in young adults with sickle cell disease

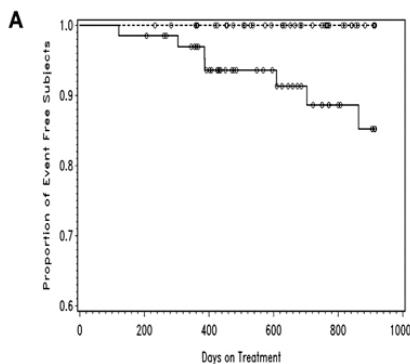
The natural history of overt strokes (also referred to as strokes with associated neurological symptoms) or silent cerebral infarcts (SCI, also referred to as silent strokes without associated neurological symptoms), in adults ( $\geq 18$  years of age) with sickle cell disease (SCD), receiving disease-modifying therapy for secondary stroke prevention (regular blood transfusions, hydroxyurea or both) is unknown. All five NIH-sponsored randomized controlled trials (RCT) for primary and secondary stroke prevention in SCD included only children  $\leq 18$  years of age(1–5). Untreated SCIs are progressive, with a prevalence of  $\sim 37\%$  and  $\sim 50\%$  in children and adults, respectively, Figure 1 (6–13). Hence, both children and adults with untreated SCI or strokes have a high risk of infarct recurrence, although only in children has a RCT been completed demonstrating that regular blood transfusion therapy for SCI is associated with a relative risk reduction of infarct recurrence of 58% when compared to observation alone (8), Figure 2. Some individuals elect to take the less burdensome, yet less effective hydroxyurea therapy (3), Figure 3, instead of regular blood transfusion therapy, while others elect for no treatment. The absolute numbers of adults with cerebral infarcts will continue to increase because 98% of adolescents with SCD are surviving to adulthood(9).



**Figure 1.** Prevalence of SCI in children and young adults with SCD. The figure displays the cumulative prevalence of SCI in children and young adults based on 5 cross-sectional studies and 1 longitudinal study. The cumulative prevalence of SCI suggests that the incidence of SCI does not plateau in young adulthood to  $\geq 30$  years of age. [Kassim et al. Blood. 2016 Apr 21;127(16):2038-40].



**Figure 2.** Kaplan-Meier estimates of the probability of stroke recurrence with chronic blood transfusions compared with historical controls. Chronic blood transfusion significantly decreases the risk of stroke recurrence [Pegelow et al. J Pediatr 1995;126(6):896-899].



**Figure 3.** Event-free (Kaplan-Meier) plot of adjudicated stroke events for the SWiTCH trial, demonstrating the inferiority of hydroxyurea for stroke prevention compared to blood transfusion therapy. The standard treatment arm (blood transfusion therapy) is indicated by dashes, while the alternative treatment arm (hydroxyurea) is indicated by the solid line,  $p < .05$ . [Ware et al. Blood. 2012 Apr 26;119(17):3925-32].

**We have preliminary data to support the development of an international consortium of hematologists to systematically define the natural history of recurrent strokes in individuals with sickle cell disease**

To address the lack of knowledge for optimal management of overt strokes and SCI in the growing population of individuals with SCD, we have identified 420 potential participants with either overt strokes or SCI followed at seven combined pediatric and adult SCD centers. Each pediatric and adult hematologist has agreed to a standard care protocol for management of overt strokes and SCI in patients with SCD. Standard care will include surveillance MRI/MRA of

the brain at study entry and exit. Participants will include those with uninterrupted disease-modifying therapy (regular blood transfusion, hydroxyurea, or both) versus no therapy or interrupted therapy (defined as disease-modifying therapy which is not given continuously over the study period). Among the 420 potential participants that we have identified, we will enroll a total of 224 individuals in this study with overt strokes (n=44, including those with intracranial hemorrhage) or silent cerebral infarcts (n=180) who have already been receiving either uninterrupted disease-modifying therapy, interrupted therapy or no therapy for secondary stroke prevention prior to enrollment in the study. For each group (overt strokes and silent cerebral infarcts), we anticipate that approximately half of the patients will already be receiving uninterrupted disease-modifying therapy for secondary stroke prevention, and the other half will be receiving either no therapy or interrupted therapy prior to enrollment. The decision to receive or not to receive any disease modifying therapy for secondary stroke prevention will be left at the discretion of both the study participants and their treating hematologists. As this is a natural history study, there will be no randomization to any interventions, and all treatment received will be per standard care. We anticipate the following four groups of participants:

- a. At least 90 individuals with SCI treated with uninterrupted disease-modifying therapy
- b. At least 90 individuals with SCI receiving either no treatment or interrupted disease-modifying therapy
- c. At least 22 individuals with overt strokes treated with uninterrupted disease-modifying therapy
- d. At least 22 individuals with strokes receiving either no treatment or interrupted disease-modifying therapy

### 3.0 SPECIFIC AIMS

**Aim 1: Determine the incidence of infarct recurrence in individuals ( $\geq 1$  year of age) with SCD and prior overt stroke or SCI treated with disease-modifying therapy.** Entry criterion will include: a history of prior cerebral infarct and evidence on prior or entry MRI/MRA of the brain with a least a 1.5 tesla magnet strength. Exit MRI/MRA will be completed with a minimum and average of 3.5 and 4.5 years of follow-up between imaging studies, respectively, for 112 individuals with either overt stroke or SCI receiving uninterrupted disease-modifying therapy (regular blood transfusions, hydroxyurea or both) and 112 individuals receiving either no treatment or interrupted disease-modifying therapy. Primary outcome measures are adjudicated stroke, SCI recurrence or transient ischemic attack (TIA). Imaging and clinical data collected as part of standard care will be used for secondary analysis. Combining retrospective and prospective cohorts will enable us to potentially recruit a higher proportion of subjects as it includes those already in treatment. We are able to include combined retrospective and prospective cohort data for the participants because all of the participating sites have already been practicing the standard care for secondary stroke prevention.

**Aim 2: Determine whether baseline MRA-defined cerebral arterial vasculopathy (vasculopathy) predicts cerebral infarct recurrence in individuals with overt stroke or SCI (n=224).** Vasculopathy as visualized on entry MRA will predict cerebral infarct recurrence (overt strokes, including intracranial hemorrhage, and SCI) and progression of vasculopathy between entry and follow up MRI/MRA of the brain.

Critical data obtained from this first international, multi-center natural history cohort study of individuals with SCD will facilitate the development of new therapies, other than arduous blood transfusion therapy, for primary and secondary prevention of overt strokes and SCI in individuals with SCD.

#### **4.0 RATIONALE FOR SURVEILLANCE MRI AND MRA IN INDIVIDUALS WITH PRE-EXISTING SILENT CEREBRAL INFARCTS OR OVERT STROKES**

In children and adults with pre-existing strokes or SCI, routine MRI/MRA of the brain are required to determine if new or progressive cerebral infarcts or cerebral vasculopathy have occurred. The presence of progressive or new central nervous system disease informs the patient, family and health care provider as to whether more intensive interventions should be considered to prevent further progression. More aggressive care could include: increasing the frequency of blood transfusion therapy, optimizing hydroxyurea therapy to maximum tolerated dose (MTD), adding hydroxyurea therapy to regular blood transfusion, indirect re-vascularization procedures or hematopoietic stem cell transplant (HSCT).

#### **5.0 BACKGROUND AND SIGNIFICANCE**

##### **For Aim 1:**

***SCI is defined and can only be detected with MRI of the brain.*** The Cooperative Study for Sickle Cell Disease (CSSCD) included an observational study of children with sickle cell disease who were identified as infants and followed closely over the subsequent decade. This study used surveillance MRI of the brain to detect abnormally increased T2-weighted signal intensity on multiple MRI views in children without corresponding focal neurological deficits as assessed by a hematologist. Subsequently our group, The SIT (Silent Cerebral Infarct Transfusion Multi-Center Clinical Trial) team, expanded on the previously validated definition of SCI to include an MRI lesion measuring at least 3 mm in greatest linear dimension, and visible on at least two planes of T2-weighted images(10–12).

**There is a paucity of data regarding optimal strategies for secondary stroke prevention in individuals with sickle cell disease**

Central nervous system complications contribute significantly to morbidity and mortality in adults with sickle cell disease (SCD). The most complete definition of a silent or overt cerebral infarct includes neuroimaging and a thorough examination by a neurologist(4). Since the 1990's, five NIH-funded randomized clinical trials (RCT) have been completed, providing evidence-based

guidelines for the primary and secondary prevention of strokes in children with SCD living in high income countries(2–6). However, there are unfortunately no evidence-based guidelines for primary and secondary stroke prevention for adults with SCD. Multiple risk factors have been identified for the development of cerebral ischemic infarcts in SCD, including low cerebral oxygen content(7–9), cerebral vasculopathy(13), fever(14), presence of a prior cerebral infarct(8,15), and rapid increases in hemoglobin levels(16,17). Additionally, acute stroke in SCD has been associated with a number of comorbid conditions that are known risk factors for stroke within the general population, such as hypertension in children, and hypertension, diabetes mellitus, hyperlipidemia, atrial fibrillation and renal disease in adults. Most acute strokes (75%) and in-hospital deaths from stroke (91%) occur in adults(18). The rate of stroke in SCD has been shown to peak in older adults and found to be three-fold higher than rates previously reported in African-Americans of similar age range (35-64 years) without SCD(15). Silent and overt cerebral infarcts in patients with SCD are associated with increased risk of future infarcts and a drop of five IQ points on average(19). Hence, secondary prevention of strokes in this high risk population is essential for reduction of morbidity and mortality. Studies for the primary and secondary prevention of strokes in individuals with SCD should include investigation for traditional risk factors for strokes in adults and are urgently needed.

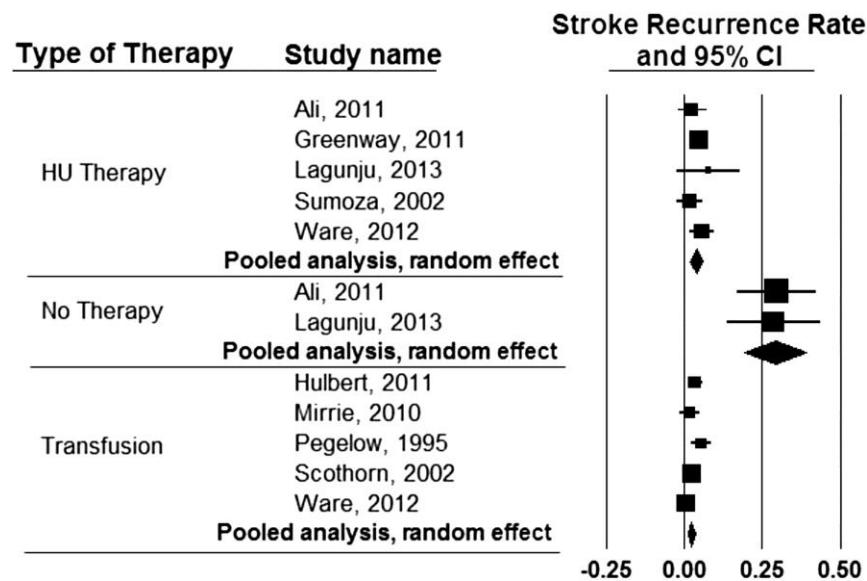
### **Despite compliance with regular blood transfusion therapy, some patients continue to have progressive cerebral infarcts**

The progressive nature of strokes despite regular blood transfusion was also demonstrated in a retrospective cohort study of 137 children with SCD and strokes who were followed for a median of 10 years. Over the course of a decade, approximately 22% had a second stroke despite regular blood transfusion therapy, and among those with a second stroke, approximately 30% had a third stroke(20). In both the retrospective (10) and prospective cohorts (18), participants had strokes when their hemoglobin S levels were 1%, 9%, 22%, 25%, and 26%. The hemoglobin S levels at the time of stroke recurrence were all < 30%, the maximum target percent for hemoglobin S concentration. Based on the strong results from these studies and other large retrospective observational studies(8,20), regular blood transfusion therapy has limited effectiveness for secondary prevention of cerebral infarcts (overt strokes and SCI) over the long term and has to be continued indefinitely in order to reduce the lifetime risk of recurrent cerebral infarcts.

### **Hydroxyurea, while less effective than regular blood transfusion therapy, is better than observation alone for secondary stroke prevention in individuals with SCD**

The SWiTCH trial was designed to test the hypothesis that hydroxyurea therapy with phlebotomy (alternative therapy) was non-inferior to regular blood transfusion therapy and chelation (standard therapy) for secondary stroke prevention(3). As part of the interim analysis, the trial was stopped prematurely based on futility assessment for decreasing iron stores in the alternative therapy group. However, the rates of strokes in the alternative and standard therapy group were 10.5% (7 of 67) and 0% (0 of 66) respectively,  $p < .05$ , Figure 3, suggesting that

blood transfusion therapy is more effective for secondary stroke prevention than hydroxyurea. While the SWiTCH trial did not demonstrate any advantage of hydroxyurea compared to regular blood transfusion therapy, in a cohort of children with SCD, hydroxyurea decreased the rate of stroke recurrence compared with no therapy(21). Additionally, three observational studies found decreased stroke rates in children treated with hydroxyurea compared to observation alone(21–23), and children receiving hydroxyurea were shown to have reduced transcranial Doppler (TCD) velocities(23,24), suggesting a benefit of hydroxyurea for secondary stroke prevention compared to observation alone. A recent published pooled analyses from multiple studies of secondary stroke prevention in individuals with SCD showed a significantly higher rate of stroke recurrence in individuals receiving no therapy compared to those receiving either regular blood transfusions or hydroxyurea(25), Figure 4.



**Figure 4. Incidence rates of recurrent stroke in individuals with SCD receiving either hydroxyurea therapy, blood transfusion therapy, or no therapy for secondary stroke prevention in published studies since 1995.** The expected incidence rates of stroke recurrence while on regular blood transfusion therapy, hydroxyurea therapy, or no therapy were found to be 1.9 (95% CI, 1.0-2.9), 3.8 (95% CI, 1.9-5.7), and 29.1 (95% CI, 19.2-38.9) events per 100 patient years, respectively.

**Understanding the natural history of progressive cerebral infarcts in individuals with SCD may help identify higher-risk subgroups of patients who would benefit from novel, risk-based secondary stroke preventions strategies, such as hematopoietic stem cell transplant and experimental disease modifying therapies**

In summary, all large multi-center cohort studies describing the natural history of strokes have been conducted in children. However, given the increased lifespan of adolescents with SCD, with an expected 98% surviving into adulthood(29), understanding the natural history of pre-existing strokes or SCI, or even new strokes in adults has taken on an urgent importance. For

adults with SCD with either pre-existing or new cerebral infarcts, there is a paucity of age appropriate data to guide management and to develop new therapeutic pathways. The evidence provided from this natural history study is required to optimally select current therapeutic options (regular blood transfusion, hydroxyurea or both) for secondary stroke prevention. Furthermore, the natural history data are required to establish the relative merits of new treatment options such as re-vascularization procedures(30), HSCT(31), or even new targeted agents that may decrease future CNS events such as GBT440, a novel small molecular hemoglobin modifier which increases hemoglobin oxygen affinity and decreases hemolysis(32).

### For Aim 2: Baseline vasculopathy in individuals with SCI may predict future SCI.

MRA-defined cerebral arterial vasculopathy (vasculopathy) in children with sickle cell disease is associated with subclinical stroke(33) and recurrent stroke(34). Within the pediatric literature, there is evidence to suggest that the presence of cerebral vessel abnormalities may predict the risk for future SCI. Specifically, children with SCD and SCI are more likely to have vasculopathy compared to those without SCI; 15.9% and 6.3%, respectively ( $P < 0.001$ )(35). In children with SCD and overt strokes, progressive vasculopathy is associated with infarct recurrence in 100%(13). Our lab (DeBaun et al.) has preliminary data from a longitudinal secondary analysis of participants from the Silent Cerebral Infarct Multi-Center Clinical Trial (SIT) trial with SCD and SCI who were followed for 3 years, showing an overall higher incidence of cerebral infarct recurrence in children with vasculopathy, regardless of transfusion ( $n=16$ ). Those with vasculopathy had a higher rate of CNS events (stroke, new or enlarging SCI) compared to those without; 31% (95% CI, 8.34% to 53.66%) versus 12% (95% CI, 5.25% to 18.75%), respectively (95% CI, -0.0016 to 0.4381;  $p= 0.053$ ). Additionally, we have preliminary data from a prospective study of children with SCD and prior SCI to suggest that the prevalence of cerebral blood vessel abnormalities detected on MRA is predictive of progressive cerebral infarcts in children with SCD, Figure 5.

	MRA-defined cerebral vasculopathy ABSENT	MRA-defined cerebral vasculopathy PRESENT	Total	Percentage of patients with MRA-defined cerebral vasculopathy
ABSENCE of SCI progression on MRI brain	43	8	51	15.7%
PRESENCE of SCI progression on MRI brain	1	4	5	80.0%

**Figure 5.** Preliminary data [DeBaun et al.] shows an increased rate of cerebral infarct progression among patients with SCD and prior SCI who have cerebral vasculopathy detected on MRA compared to those without cerebral vasculopathy.

While there is evidence to support the use of MRA as a predictor of future cerebral infarcts in children with SCD, the data in adults is lacking. This study will provide evidence of the usefulness of MRA to follow vasculopathy in adults with prior SCI or overt strokes and establish that vasculopathy predicts new stroke or TIA. MRA is already being used clinically for this purpose in the pediatric population, in light of the evidence that the presence of baseline vasculopathy detected on MRA is associated with an increased risk of silent cerebral infarcts in children with SCD ( $P = 0.0007$ , odds ratio (OR) 2.84; 95% CI = 1.55-5.21)(35).

While regular blood transfusion therapy is accepted as standard care management for primary and secondary stroke prevention in children with SCD, Bishop et al. showed that children with a history of stroke experience progression of vasculopathy despite uninterrupted blood transfusion therapy(36), suggesting the need for augmented and alternative therapies for this high-risk population. To date, no prospective studies have longitudinally quantified the risk of stroke in adults with SCD and MRA-defined cerebral vasculopathy. **This will be the first prospective and retrospective study of adults with SCD to validate the findings of pediatric studies that vasculopathy is a significant risk factor for cerebral infarct progression.** Identification of a subset of high-risk adults with baseline vasculopathy could lead to earlier augmentation of current secondary stroke prevention strategies or alternatively, justify the use of novel therapeutic approaches for secondary stroke prevention, such as HSCT and experimental disease-modifying therapies. In addition, addressing other comorbid conditions that are known risk factors for stroke within the general population, such as hypertension, diabetes mellitus, hyperlipidemia, atrial fibrillation, and renal disease in adults early on may lead to further stroke risk reduction.

## 6.0 METHODS

**6.1 Study Design:** Multi-center, retrospective-prospective observational cohort study

### 6.2 Study Outcomes

**Primary outcome:** Radiographic and/or neurological evidence of recurrent cerebral infarction (overt strokes, including intracranial hemorrhage, and SCI) in individuals with SCD and prior overt stroke or SCI.

**Secondary outcome:** Mortality

### 6.3 Centers and settings

This study is an observational, retrospective and prospective cohort study. Patients who meet eligibility criteria will be recruited from multiple participating sites that have tentatively agreed to participate. Site selection was based on the following criteria 1.) estimated number of patients at

each site with a history of SCD and prior overt stroke and SCI, 2.) presence of a seamless transition between pediatric and adult sickle cell disease clinics at each site, and 3.) prior experience of each site in conducting clinical trials involving patients with sickle cell anemia.

The seven participating sites include:

1. Vanderbilt University Medical Center (Nashville, TN)
2. Washington University Medical Center (St. Louis, MO)
3. Children's Hospital Oakland (Oakland, CA)
4. Medical University of South Carolina (Charleston, SC)
5. London consortium consisting of 2 sites: Guy's and St. Thomas' (London, UK) and Royal London Hospital (London, UK)
6. University of Alabama (Birmingham, AL)
7. St. Jude-Methodist Sickle Cell Disease Transition Clinic (Memphis, TN)

Informed consent will be obtained from all study participants. A log to identify all individuals who were approached will be kept as part of this study and will be maintained at each site. A one-sentence reason will be recorded as to why eligible participants did not agree to participate.

Every attempt will be made by the Study Team to contact participants regularly and keep them from becoming lost to follow-up. Identifying a stable contact person (such as a grandparent, neighbor, teacher, friend), not living with the study participants' family but who will always know the family's whereabouts, will assist in tracking participants and decrease the number lost to follow-up.

A maximum of 224 participants ( $\geq 1$  years of age) with sickle cell disease of any genotype and prior overt strokes ( $n=44$ ) or silent cerebral infarcts ( $n=180$ ) who have received either uninterrupted disease-modifying therapy (regular blood transfusion, hydroxyurea or both) or no treatment or intermittent therapy for secondary stroke prevention will be recruited and enrolled in the study. Among the 224 study participants, we will include the following 4 groups of patients who are receiving uninterrupted disease-modifying therapy for secondary stroke prevention versus interrupted therapy or observation alone prior to enrollment:

- a. At least 90 individuals with SCI receiving uninterrupted disease-modifying therapy
- b. At least 90 individuals with SCI receiving either no treatment or interrupted disease-modifying therapy
- c. At least 22 individuals with overt strokes receiving uninterrupted disease-modifying therapy
- d. At least 22 individuals with strokes receiving either no treatment or interrupted disease-modifying therapy

Each participant will be prospectively followed for a minimum of 3.5 years between the time of the entry and exit MRI/MRA of the brain. We will collect and record all acute neurological events (stroke and TIA; see table 2 for definitions) requiring hospitalization or ED visits during the

study. Imaging and clinical data collected as part of standard care will be used for secondary analysis.

#### **6.4 During the initial visit**

Informed consent, assent or both will be obtained if required. All information, including labs and neuroimaging studies, will be collected as standard care. Annual screening for snoring, and other sleep disordered breathing will be performed using the previously validated Pittsburgh Sleep Quality Index (49) given the impact on cognition as well as cerebral flow (38). A stroke free questionnaire will also be administered to children and adult participants at the initial visit and at 6 month intervals at routine follow-up visits to assess for any new focal neurological deficits which may have occurred, in addition to a standard care neurological examination at that follow-up visit.

Minimum eligibility criterion includes an MR of the brain with at least a 1.5 tesla magnet describing the presence of the cerebral infarct. The image must be archived and available to be evaluated by the neuroradiology team for central adjudication.

#### Follow-up visits:

Any of the regular office visits will serve as research study follow-up visits. Follow-up visits will occur as per standard care. However, data entry will occur every 6 months. The following information will be collected during follow-up visits:

- The results of any MRI or MRA of the head, performed routinely as a part of the office visit per standard care, will be collected. At minimum, MRI/MRA of the head will occur at the time of study entry and exit as part of standard care management for individuals with SCD and prior overt strokes or SCI. Crucial to the study is that this will be a retrospective and prospective cohort study. Thus, individuals who have had a stroke or SCI prior to 2017 (expected funding year), with at least a 1.5 tesla MRI and MRA, are eligible to participate. Thus, some participants may be eligible for the protocol who were detected to have a stroke or SCI as early as 2010. Inclusion of the past MRI of the brain prior to signing the informed consent, as part of the pre-existing medical data allows us to capture the baseline events and increases the average number of patient years that each person contributes to the cohort. This strategy will prove most efficient in determining the natural history of strokes and SCIs in individuals with SCD.
- Clinical information such as height, weight will be collected and vital signs including blood pressure, temperature and respiratory rate as part of the routine medical care. Data regarding other co-morbid medical conditions and treatment and results of diagnostic lab testing will also be collected. We will collect and record all acute neurological events (stroke and TIA) requiring hospitalization or ED visits during the study. Imaging and clinical data collected as part of standard care will be used for secondary analysis. Study participants receiving interrupted disease-modifying therapy and no therapy for secondary stroke prevention will be analyzed in the same group.

- The participant's primary hematologist will have the option of consulting a neurologist to assess for any interval changes in neurological examination at his or her clinical discretion.

### **Assuring uniformity of treatment guidelines and compliance with disease-modifying therapies:**

Regular blood transfusion therapy defined as scheduled will occur at least every 6 weeks, to maintain HbS concentrations less than 30% for at least the first 2 years after the initial overt stroke or SCI and less than 50% thereafter for asymptomatic patients. In the event of a recurrent stroke or TIA, the hematologist treating the participant will be permitted to individualize the transfusion regimen with the goal to lower HbS concentration. The specific type of regular blood transfusion therapy used for secondary stroke prevention (simple transfusion, exchange transfusion or pheresis) will be recorded in the case report forms (CRF's) and compared for efficacy in the secondary analysis. For patients receiving hydroxyurea for secondary stroke prevention, all investigators will titrate the hydroxyurea to either a dose of 20 mg/kg/day or to the maximum tolerated dose (MTD). Adherence to hydroxyurea will be defined as an MCV  $\geq$  10 ficoliters above baseline. Participants who are not receiving continuous disease-modifying therapy for secondary stroke prevention (hydroxyurea, regular blood transfusion, or both) over the study period will be included in the no treatment group.

### **6.5 Chart Review and completion of case report forms**

Through chart review of standard of care procedures, the clinical information recorded will include but will not be limited to:

- First neuroimaging study demonstrating a cerebral infarct with a minimum 1.5 tesla MRI and MRA
- Completion of case report form (CRF) for hospitalization visit or emergency department visit associated with an acute neurological event, defined as overt stroke or TIA (see table 2 for definitions)
- Standard care CRFs will be completed every six months. The data will include: pre-transfusion hemoglobin S levels and hemoglobin levels, neurological examination forms (optional and preferably to be completed by a neurologist)
- Baseline CRFs focused on co-morbidities and clinical demographic information to be completed annually
- CRFs related to SAE forms (limited to death, life threatening events, suspected and confirmed CNS events)
- CRFs related to AEs to be completed annually, focused on reasons for hospitalizations only

All of this information will be included in our secondary analysis to assess their impact on recurrent stroke and SCI. There will be no interventions in this study that are not part of standard care management for individuals with SCD who have had prior strokes or SCI.

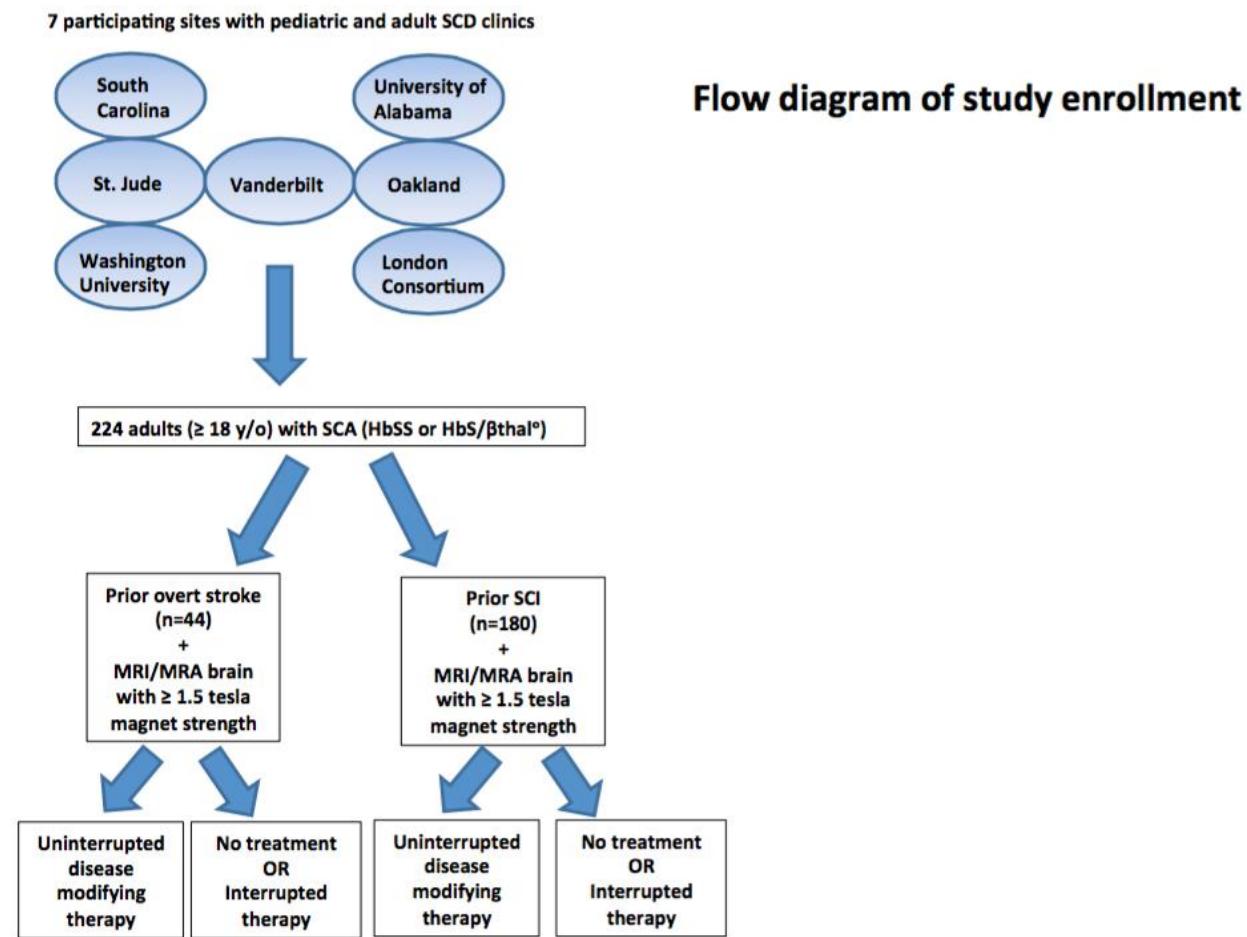
**6.6 Schedule of study tests:** The table below shows the tests that will be performed at baseline and at interval follow-up visits. All testing will be part of standard care management.

	Baseline	Every 6 months data entry	Every 12 months data entry	Prior to study exit
<b>Physical examination and vitals</b>	X	X		
<b>Review of medical record, demographic factors + comorbidities, &amp; hospitalizations</b>	X	X		
<b>Assessment for snoring &amp; sleep disordered breathing</b>	X		X	
<b>Stroke free questionnaire</b>	X	X		
<b>MRI/MRA of the brain *</b>	X			X
<b>Neurological examination **</b>	X		X	
<b>Laboratory testing (CBC with differential, HbF, HbS, reticulocyte indices)</b>	X	X		

\* For the purpose of the primary analysis, only the first and last MRI in the cohort will be evaluated to determine progression based on imaging of the brain.

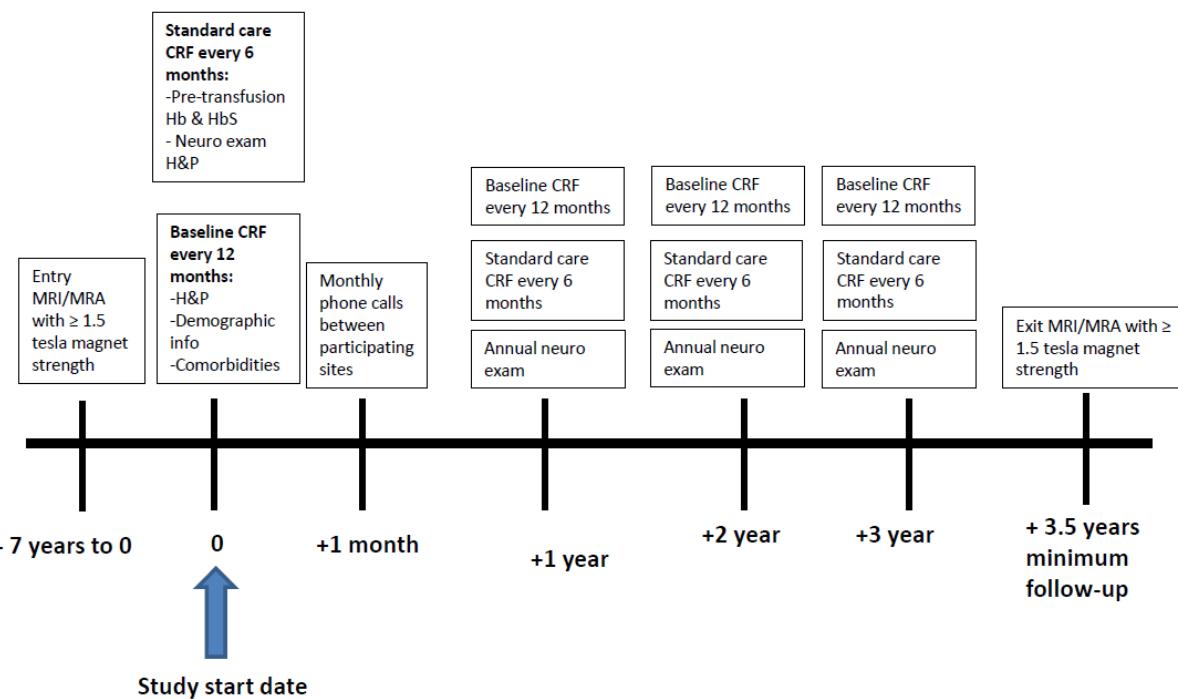
\*\* Neurological exams will be performed by the participant's hematologist. Involvement of a neurologist to perform neurological exams will be optional.

**Table 1.** Schedule of tests that will be performed at baseline and at interval follow-up visits per standard care management.



**Figure 6.** Flow diagram of study enrollment.

## Timeline for this retrospective-prospective study design



**Figure 7.** Study timeline outlined above.

### Inclusion Criteria

- 1 Participants with sickle cell disease of any genotype confirmed on hemoglobin analysis and/or other confirmatory documentation of phenotype
2. Patients  $\geq 1$  year of age
3. Patients followed regularly (at least 3 visits per year) in the hematology clinics and who have demonstrated adherence with follow-up visits for  $\geq 3$  years
4. Patients willing to be followed prospectively for a minimum of 3.5 years and agree to a standard care exit MRI/MRA of the brain.
5. Evidence of a prior cerebral infarction based on MRI with  $\geq 1.5$  m Tesla strength
6. Willingness to comply with study protocol, routine clinic visits

### Exclusion Criteria

- 1) Participants judged not to be non-compliant by the hematologist based on previous experience in terms of clinic appointments and following advice
- 2) Participants with any contraindications to MRI, including individuals with foreign metal objects and claustrophobia
- 3) Participants with cirrhosis of the liver
- 4) Participants with known seropositivity for HIV
- 5) Participants with end-stage renal disease (ESRD)
- 6) Participants with a baseline oxygen requirement to oxygen saturation < 90% due to chronic pulmonary disease
- 7) Participants with any baseline rheumatologic disease associated with end-organ disease
- 8) Participants who do not meet study criteria for having a history of overt strokes or SCI

## **6.7 Study Procedures**

### **6.7.1 Patient Recruitment**

Patients will be recruited starting September 2017 through September 2018. A screening history and physical examination, MRI/MRA with  $\geq 1.5$  m Tesla strength and neurological examination will be completed prior to enrollment of participants to confirm their eligibility for the study. Additionally, individuals who have had a stroke or SCI prior to 2017 (expected funding year), and have had at least a 1.5 m Tesla MRI and MRA are also eligible to participate. After obtaining informed consent, patients will undergo MRI/MRA of the brain at minimum at the time of study entry and exit as per standard care management for individuals with SCD and a history of strokes or SCI. If participants have had a prior brain MRI and MRA of  $\geq 1.5$  m Tesla strength after 2010, this can be used as the initial entry MRA and MRI. Participants will not be randomized to any specific interventions for secondary stroke prevention during the course of this trial. Standard of care labs, including CBC with differential, reticulocyte count, HbS and HbF levels will be ordered at a minimum of every 6 months. Patients will be followed retrospectively and prospectively for a minimum of 3.5 years (refer to section 6.6 schedule of study testing).

### **6.7.2 Data Collection**

Research information, including consent forms and questionnaires will be maintained in a secure fashion in research charts in a locked file room or locked file cabinet. Only the study personnel will have access to the research charts, the database, and to any identifying information on participants. All medical information collected prior to signing the informed may be reviewed and included in the data set.

To maintain the confidentiality of research participants, each participant will be identified with a unique identifier number in REDCap. Any data shared with our collaborators will be provided without participant identifiers (i.e. in coded form only). A de-identified study code for each participant will be generated by an independent statistician.

Annual screening for snoring, and other sleep disordered breathing will be performed using the previously validated Pittsburgh Sleep Quality Index (49) given the impact on cognition as well as cerebral flow (38). At baseline and every 6 months, a stroke free questionnaire will be administered to assess for the possibility of any new focal neurological deficits which may have developed in the interim. We will also record specific type of transfusion strategy employed (simple transfusion, exchange transfusion or pheresis) and any medications received during the course of the study that could have an impact on stroke or SCI recurrence (such as aspirin, warfarin and other anticoagulants). We will record any encephaloduroarteriosynangiosis (EDAS) procedures, which are performed to improve cerebral blood flow by transposing scalp arteries onto the surface of the brain, or administration of tPA into RedCap that occur during the course of this study. All of this information will be included in our secondary analysis to assess their impact on recurrent stroke and SCI.

This information will be recorded for all participants prior to study entry, in addition to their other medical comorbidities, and updated every 12 months. One investigator will review all participants' inpatient and outpatient records to capture critical clinical data. Information extracted will include clinical events at the time of the first stroke, initial stroke symptoms and treatment, occurrence of second or subsequent strokes, HbS concentration at the time of the second stroke, and other neurologic events, such as TIAs or cerebrovascular surgery. As part of standard care, the MRI and MRA scans of the brain will be sent to the imaging reading center. Typically the provider will order the scan at least once every year to once every two years. We will also review medical records to identify prior or concurrent risk factors for strokes or SCI. The information may be included in the prior medical records. Regardless of funding, 6 of 7 sites have agreed to follow the cohort for at least 5 years and will plan to follow the participants through 2027. Results of annual neurological examinations and neuroimaging (MRI/MRA of the brain) will also be entered into RedCap. Data entry will be double-checked by a second investigator to maximize accuracy of data entry.

### **6.7.3 Data Management**

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

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

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

#### **6.7.4 Sample Size**

##### **Sample Size and Power for Aim 1:**

Potential participants will be recruited from seven sites and may include patients followed prior to opening the study. We will aim to include a minimum of 224 patients, of which we expect approximately 44 to have overt stroke and 180 to have SCI. Based on prior results in the children with SCD, the primary hypothesis tested is that the incidence of cerebral infarct recurrence in adults with overt strokes or SCI receiving disease-modifying therapy (transfusion or hydroxyurea) will be lower than the incidence in adults not receiving disease-modifying therapy (or receiving interrupted treatment).

The primary hypotheses will be tested independently within the overt stroke and SCI groups. Based on data from children with SCD, we anticipate that the incidence of stroke recurrence will be at least 8 events per 100 patient-years in treated patients with prior overt stroke and at least 29 events per 100 patient-years in untreated patients with prior overt stroke. For patients with prior SCI, we anticipate rates of at least 2 events per 100 patient-years for treated patients and 5 events per 100 patient-years for untreated patients. We calculated statistical power for detecting the difference between the treated and untreated groups assuming a constant hazard rate; an alpha of .05; a two-sided test; an average loss to follow-up of 10% per year over the course of 3 years in all groups, and an average follow-up time of 4.5 years. For SCI, 90 patients receiving treatment and 90 receiving standard care results in a power of 81.0% to detect a difference in recurrence rates of 2 per 100 patient years versus 5 per 100 patient years. For overt stroke, 22 patients receiving treatment and 22 receiving standard care results in a power of 88.4% to detect a difference in recurrence rates of 8 per 100 patient years versus 29 per 100 patient years.

##### **Sample size calculations for aim 2:**

We calculated our sample size needs based on aim 1, given that aim 1 is the primary aim. Nevertheless, power calculations for aim 2 are informative. Based on prior literature, we anticipate that among the group with overt strokes, approximately 38% will experience progressive vasculopathy, with a relative risk of 12.0 of recurrence for those with progressive vasculopathy compared to those without progressive vasculopathy, over 3.5 years of follow-up. A sample size of 44 individuals with overt strokes gives us a power of 80.9% to detect a difference this large in relative risk between the two groups, assuming an alpha level of 0.05 and a two-sided Mantel-Haenzel test.

For those with SCI, we base the analysis on baseline cerebral vasculopathy since progression of vasculopathy occurred in only a very small proportion in prior pediatric studies. Based on data from children with SCD, we anticipate a baseline prevalence of vasculopathy of 16% of those with SCI, an odds ratio for stroke recurrence of 6.26 comparing those with baseline vasculopathy to those without, and a cumulative incidence of stroke recurrence of 10.8% in those without baseline cerebral vasculopathy over 3.5 years of follow-up. A sample size of 180 individuals with SCI gives us a power of 93.9% to detect a difference this large in relative odds between the two groups, assuming an alpha level of 0.05 and a two-sided Mantel-Haenzel test.

### **6.8 Value of natural history data for stroke recurrence for designing a phase III trial to prevent cerebral infarct recurrence.**

We have included sample size calculations for the purpose of illustrating how data collected in this proposal will be useful for a planned phase III randomized controlled trial (or non-randomized parallel comparison trial) comparing standard medical care (regular blood transfusion and/or hydroxyurea therapy) to hematopoietic stem cell transplant (HSCT). In an entirely separate study protocol from this, participants will be randomly allocated to either receive blood transfusion therapy or HSCT (experimental therapy). Participants will be followed for a minimum of 3.5 years with the following assumptions: an alpha of 0.05, two-sided is used, lost to-follow up rate of 3% per year, and hazard rates with an exponential distribution. The standard care arm (blood transfusion and/or hydroxyurea) will have 100 participants and the experimental arm (HSCT) will have 50 participants with an expected infarct recurrence incidence of 8.1(39) and 1.0 events(6,40), respectively. Based on these assumptions with an alpha level of 0.05, the power will be 80%. Clearly without the ability to collect the natural history data for stroke recurrence in individuals with SCD, we will not have sufficient information to design a phase III trial for HSCT or for that matter other promising therapies that may prevent infarct recurrence in this population.

## **7.0 STATISTICAL ANALYSIS**

**Statistical analysis for aim 1 and 2:** The same descriptive statistics will be used for both aims 1 and 2. Differences in the distribution of relevant demographic and clinical factors will be compared between groups using two-tailed chi-square tests for categorical variables, t-tests for continuous variables, and negative binomial regression for count variables. For inferential aims, we will use multivariate Cox proportional hazards regression. The first multivariate model will use all covariates potentially associated with stroke recurrence, TIA, or death (primary endpoints) in one block (forced). A subsequent model will include only those covariates that were nominally significant predictors ( $p < 0.20$ ) from the first model. These covariates will include, but will not be limited to, age, gender, treatment, length of treatment, baseline MRA-defined vasculopathy, and progressive vasculopathy.

As a secondary analysis, we will also explore the relationship between the presence of standard risk factors for strokes (hypertension, diabetes mellitus, hyperlipidemia, atrial fibrillation, renal disease) and primary endpoints. We will have a sub-analysis focused on: 1) the efficacy of the

specific blood transfusion therapy method (simple blood transfusion, exchange blood transfusion or pheresis) for secondary stroke prevention, 2) hemoglobin S and hemoglobin levels at the time of stroke as well as the mean hemoglobin S and hemoglobin level while being transfused or receiving hydroxyurea therapy; and 3) the role of excessive iron storage at the time of stroke recurrence based on the most recent liver iron content assessment via MRI of the liver. Given that the associated morbidity of 5 mm cerebral infarcts has not been uniformly assessed, this will be included as part of our secondary analysis. We will record any medications taken during the course of the study (such as aspirin, warfarin and other anticoagulants) or any interventional procedures (such as EDAS) that could potentially have an impact on stroke or silent strokes recurrence, as well any tPA administration during this study. This information will be included as part of a post-hoc analysis to estimate their impact on recurrent overt strokes and SCI. Statistical analysis will be completed by Dr. Mark Rogehier, Ph.D., an independent statistician based in Chicago, IL. For the past 15 years, Dr. Rogehier has been the dedicated statistician for Dr. DeBaun's research team and he currently has 33 peer-reviewed publications.

## **8.0 STUDY ORGANIZATION STRUCTURE**

To accomplish the aims of the study, we will employ the same successful methods of operation and procedures from previous stroke prevention trials for children with SCD.

**Executive Committee:** The executive committee will review enrollment, protocol conduct, scientific merit of proposed ancillary studies or publications, and issues raised by the clinical site. Dr. DeBaun will chair the committee, and at least one member from each participating site will serve as a member of the executive committee. The committee will meet via 1-hour weekly Skype conference calls. Other investigators on the committee will include multi-PI Lori Jordan, MD, PhD (Vanderbilt), Bob McKinstry MD, PhD (Washington University), Adetola Kassim, MD, MS (Vanderbilt); and Fenella Kirkham, MD (University College London), an international expert in pediatric strokes, with >10 years of experience working with Dr. DeBaun on NIH-funded studies and > 20 years of experience in training teams in the UK and Africa on the use of TCD. Other investigators on the committee will include multi-PIs: Lori Jordan, MD, PhD (Vascular Neurologist), Robert McKinstry MD, PhD (Neuroradiologist), Adetola Kassim, MD, MS, (Hematologist); and Andria Ford, MD (Vascular Neurologist).

At least one member of the executive team will visit each site at least once to review research governance integrity and to inspect the quality of the source document that was entered into REDCap.

**Neurology Committee:** The role of the committee will be to assess and adjudicate patient eligibility and endpoint determinations (overt stroke, SCI, TIA) through central review of clinical history, neurological examinations and neuroimaging studies when available.

**Neuroradiology Committee:** The Washington University neuroradiology committee, led by Robert McKinstry, will form the adjudication committee and will review initial and exit MRI/MRAs of the brain. The MRIs/MRAs will be used to assess interval changes when compared to baseline neuroimaging studies.

There will be no funding for standard care neuroimaging studies (MRI/MRA) and neurology examinations. We will have phone conferences monthly to discuss progress and will meet annually as well.

## 9.0 STROKE ADJUDICATIONS

The stroke adjudication process will provide inclusive and systematic evaluation of all new acute neurologic events by treatment-masked neurologists or hematologists and neuroradiologists. Neurological examinations can be performed either by the participant's hematologist or a neurologist.

## 10.0 DEFINITIONS

**Table 2. Definition of Neurological Events**

Diagnosis = Transient ischemic attack (TIA)	Diagnosis = Clinical Stroke	Diagnosis = Clinical Stroke	Diagnosis = Neurological Deficit Due to Other Cause
< 24 hours Neurological Deficit*	< 24 hours Neurological Deficit*	> 24 hours Neurological Deficit*	> 24 hours Neurological Deficit*
Negative Head CT or MRI or no Head CT or MRI obtained	Positive Head CT or MRI if obtained for clinical indications  (abnormality on brain imaging which could explain deficit)	Positive Head CT or MRI if obtained for clinical indications  (abnormality on brain imaging which could explain deficit)	Based on investigations, physicians believe deficits are consistent with complex migraine, seizure or other non-stroke etiology

\*Neurological deficit in this setting means an abnormality consistent with a stroke. Usually, a stroke is associated with weakness of face, arm, and/or leg.

\*\*The influence of blood transfusion on acute symptoms of stroke has not been well defined in SCD; some neurologic symptoms may last less than 24 hours after the treatment with blood transfusion therapy obscuring the diagnosis of stroke. Transfusion therapy may decrease the persistence of neurological findings. Neurologic deficit in this setting means an abnormality consistent with a stroke.

## **10.1 Definitions of acute neurological events**

### **Overt Strokes**

First and subsequent overt strokes will be defined as new focal neurologic deficits attributable to stroke lasting greater than 24 hours. If symptoms resolved within 24 hours, the event will be considered a stroke if the patient has had an imaging study demonstrating acute cerebral ischemia, based on restricted diffusion on diffusion-weighted MRI, in a region of the brain

corresponding with the symptoms if the MRI is done within 7 days of symptom onset. If done more than 7 days after symptom onset, a cerebral infarction may only show increased signal intensity on T2-weighted MRI as defined below. Intracranial hemorrhage will be counted as an overt stroke and will be recorded as a severe adverse event.

#### Transient ischemic attacks

Transient (lasting < 24 hours) weakness or sensory deficits without acute cerebral infarcts on MRI of the brain will be considered TIA's.

#### New cerebral infarctions

New cerebral infarctions, defined as new 3 mm or greater lesions of increased intensity on MRI sequences visible in at least 2 views, will be denoted as present or absent.

#### Enlarging cerebral infarctions

Enlarging infarctions will be defined as an increase in a previously existing area of signal hyperintensity of at least 3 mm in one dimension.

#### Silent cerebral infarctions

New or enlarging cerebral infarctions on imaging without co-localizing clinical signs or symptoms will be defined as silent cerebral infarctions. A silent cerebral infarction could be either an enlargement of an existing infarct lesion or a new cerebral infarct, as long as there is no new focal neurologic deficit that was called an overt stroke by the treating hematologist.

#### Time to first recurrent cerebral infarction

Time to the first recurrent overt stroke or silent cerebral infarct will be noted as the time to the progression of the cerebral infarct after the first stroke. The date for progression of a silent infarction will be defined as the date of the MRI on which the new lesion was noted, whereas the date for second overt strokes will be defined as the date of symptom onset or recognition.

### **10.2 Radiographic definitions of cerebral infarct progression and arterial stenosis**

#### MRI classification

Each MRI will be independently reviewed by the study neuroradiologists and subsequently, consensus will be achieved. The scan will be judged as 1) no infarct or 2) infarct if there is an ischemic lesion  $\geq$  3 mm in diameter visible in two planes (axial and coronal) using established criteria(10). The radiologists will record the total infarct count. The MRI scan will last approximately 30 minutes.

#### Arterial stenosis

The study radiologists will also grade the MRA brain for vasculopathy. In the major intracranial vessels, the first segments of the anterior, middle, and posterior cerebral arteries bilaterally (M1, A1, P1) and the intracranial vertebral arteries will be examined. Brain MRA examinations will be

reviewed for vessel stenoses in both the anterior and posterior circulations. A standardized scale for vessel stenosis will provide a means of accurately assessing the location, extent, and severity of vascular disease (50). Four segments of each internal carotid artery (ICA), and 3 segments of each anterior cerebral artery (ACA) and middle cerebral artery (MCA) will be examined, with results recorded for a total of 20 vessel segments per subject (10 per cerebral hemisphere). Vessel stenosis will be recorded by vascular location, frequency (number of stenoses), length (millimeters), and severity (percentage vessel occlusion). Severity of vessel segment occlusion will be graded as mild (25-49%), moderate (50-74%), severe (75-99%), or occlusion (>99%). These definitions are per prior pediatric SCD and vasculopathy literature where stenosis >50% was considered significant cerebral vasculopathy(35,39). The MRA scan will last approximately 20 minutes.

### **10.3 Rationale for Utilization of National Institutes of Health Stroke Scale:**

The National Institutes of Health Stroke Scale (NIHSS) is a validated, standardized neurological examination that will be used for this study to perform neurological examinations. The neurological examination has been standardized in order to minimize variability that could be created by having multiple examiners at various sites. The rapid, standardized NIHSS examination is efficiently performed and has been validated for use (44-48). The NIHSS is a quantitative neurological examination developed for use in following strokes of various etiologies. Specifically, it is easily performed by non-neurologists, detects changes in neurological status well, and has excellent inter rater reliability, which can be further improved through the use of standardized video training. The scale consists of 11 items. Normal performance in any area receives a score of zero; hence a completely normal patient will have a cumulative score of 0. The involvement of a neurologist to complete neurological examinations for the purposes of this study will remain optional and left to the discretion of the treating hematologist. All study personnel responsible for neurological evaluations will be required to provide proof of certification on the NIHSS to the Coordinating Center (CC). For subjects under 18 years of age, the previously validated pediatric NIH Stroke Scale and Modified Rankin Scale for Children will be used to record the presence of any focal neurological deficits on examination (51,52).

## **11.0 STUDY RESPONSIBILITIES**

### **11.1 Coordinating Center**

The Coordinating Center (CC) is directly responsible for the monitoring and oversight of all fiscal, regulatory, clinical and other general administration needed to centralize and coordinate the research endeavors of all sites participating in the trial. The CCC will be located at Vanderbilt University Medical Center and will be led by the Principal Investigator, Michael R. DeBaun, MD, MPH. Two project managers, with overlapping responsibilities, will individually be

responsible for the clinical and fiscal duties. The project manager will also plan and coordinate all meetings and travel related to the study.

The responsibilities of the CCC will include:

- protocol development and amendments
- facilitating and monitoring protocol conduct at the participating sites
- overseeing regulatory compliance
- monitoring of adverse effects and events
- assuring quality control via site audits
- providing annual and quarterly reports on the progress of the study
- participating in the analysis and interpretation of data
- manuscript preparation and prioritization
- maintaining ongoing communication with all participating study sites
- plan and organize investigator meetings
- coordinate all NIH requirements related to the grant

## **12.0 PRIVACY AND CONFIDENTIALITY ISSUES**

All key personnel involved in the design or conduct of research involving the human subjects will receive the required education on the protection of human research participants prior to funding of this project. The patients will not be individually identifiable. Confidential data, including name, address, employer, and name and address of relatives, are collected to aid in maintaining patient follow-up. However such information is kept separate from study forms in a secure file.

Patients' risks of participating in research are kept to a minimum with measures to protect confidentiality and planned interim analyses for safety monitoring and early termination. Steps to protect privacy will include assignment of study codes to all records, without patient names, with the key for connecting patient names and study codes kept in a secure site separate from the study records and at the local site only. All study records will be kept in a secure location that will be locked to prevent unauthorized access.

## **13.0 HUMAN SUBJECTS RESEARCH**

### **13.1 IRB Review**

The protocol, informed consent documents, and all types of participant educational information must be submitted to the IRB for review and must be approved before the study is initiated. Any amendments to the protocol must also be approved by the IRB prior to implementing any changes in the study.

The investigator is responsible for keeping the IRB updated of the progress of the study and of any changes made to the protocol as deemed appropriate, but in any case at least once a year. The investigator must also keep the IRB informed of any significant adverse events.

The study may be discontinued for administrative reasons and/or IRB.

If for any reason the study is prematurely terminated, the Investigator should promptly inform the study participant and the IRB.

All key study staff regularly undergo training in human subjects issues.

### **13.2 Consent Process**

Written informed consent will be obtained from all study participants, without exception, before their enrollment into this study. A waiver of consent will not be sought for this study. The study investigator or coordinator will review all portions of the consent in detail. Any questions will be addressed prior to asking for a signature. Study staff will emphasize that participants may continue to ask questions at any time during the study and they may withdraw consent at any time.

A copy of the consent will be given to the participant before participation in the study begins. The original signed consent and will be available for review at any time.

Patients with a history of stroke and significant associated cognitive deficit(s) precluding their ability to provide informed consent will not be excluded if they have a family member or significant other with Power of Attorney to consent on their behalf.

Members of the Data Safety Monitoring Committee (DSMC) will be selected to reflect a mix of appropriate clinical expertise in sickle cell disease and knowledge of the design, monitoring, analysis and ethical issues of clinical research to protect the participants' safety. The DSMC will monitor study quality, safety of participants, and efficacy.

The DSMC will monitor the study performance by reviewing:

- participant recruitment
- flow of forms
- quality control of the data

- adequacy of medical monitoring
- adverse event reporting
- adherence to protocol
- appropriateness of protocol changes with regard to scientific integrity

The DSMB will monitor the safety of participants by reviewing:

- risk of harm inherent in participating in the study
- adverse events (type, incidence, and severity)
- 
- effect of protocol changes on risk

The DSMC will monitor the efficacy of the study by reviewing:

- data
- planned and/or unplanned interim analyses
- stopping rules, their implementation, and resulting decisions
- results and conclusions

### **13.3 Statistical Coordinating Center**

The Statistical Coordinating Center (SCC) will reside at Vanderbilt University Medical Center. The data will be developed, managed and entered using REDCap, a secure, web-based application designed exclusively to support data capture for research studies. The SCC will coordinate the collection and analysis of data generated for the study.

### **13.4 Preparation of study documents**

SCC staff will collaborate with staff from the CCC and other investigators to develop: protocol, Manual of Operating Procedures (MOP), case report forms (CRFs), and Question by Question guidelines (QxQs), which give the details on answering the questions on every CRF.

### **13.5 Training site personnel**

SCC staff will train site personnel in all aspects of the study related to: details of the study protocol, data collection procedures, screening procedures, submitting forms to the SCC and responding to queries from the SCC. SCC staff will also monitor each site to assure that the site coordinators have completed the required training.

### **13.6 Receipt and processing of study forms at the SCC**

SCC staff will design and implement the data management system for the study, including: electronic data entry, comprehensive editing of the data and preparation of protocol adherence aids, and data extraction for analyses related to research objectives. Data are submitted to the SCC through REDCap, a web-based data entry system. Data are comprehensively edited once received at the SCC, prior to insertion into the main study database. Edit queries will be resolved within a few days with the site coordinators. Protocol adherence aids, such as delinquent forms listings and patient schedules, are produced by the data management system to help the site coordinators keep the patient visits on schedule with the appropriate forms submitted. SCC staff will also enforce patient confidentiality and privacy rules so that patient health information (PHI) data are not shared inappropriately and so that data are protected by multiple layers of security.

### **13.7 Monitoring study progress and data quality**

SCC staff will ensure that clinic site staff is properly trained, that performance of the required procedures is monitored, and that deviations from the protocol or from study norms are investigated. Routine site monitoring reports, including patient recruitment, performance of follow-up visits, and protocol compliance, will be produced at regular intervals.

SCC and CCC staff will develop and implement quality assurance procedures and programs will be developed and implemented for central reading centers, data collection, and data entry.

### **13.8 Quality Assurance Procedures**

The study investigators and study personnel will conduct quality assurance site visits throughout the study as a means of building rapport. In addition, quality assurance procedures and programs will be developed and implemented for data collection and for data entry by the Statistical Coordinating Center and Coordinating Center. At least one site visit will occur by members of the executive team to each site to review the research governance documents and quality of the collected data, including sources documents.

### **13.9 Collaboration in Study Publications**

SCC staff will collaborate in all study publications, providing statistical analysis, preparing the statistical methods and results sections of manuscripts. SCC staff will also participate in the development and review of abstracts for presentations and in the review of the statistical analysis for ancillary publications.

## **14.0 ADVERSE EVENT REPORTING**

For the purposes of this study an adverse event (AE) will be defined as any unfavorable and unintended sign, symptom or disease associated with a subject's participation in this study. As this is an observational study, reportable adverse events are only those directly related to an individual's participation in this study

Although adverse events are not anticipated from the nature of this study, all participants will be under the care of experienced study staff who will be available to provide necessary care to participants who may experience an adverse event during the study.

The investigator or designate is responsible for the detection and documentation of AE and serious adverse events (SAE) in persons participating in this study. At each clinical evaluation during the study, the investigator or site personnel should document any AEs or SAEs, as detailed in this protocol. AEs and SAEs should be reported to the IRBs.

All adverse events (AE), regardless of causal relationship, will be monitored and reported on a form that includes the description of the event, onset date, stop date, and outcome. The Investigator will determine the intensity of any AE according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) Version 4.03 (see <http://ctep.info.nih.gov>) and their causal relationship. Adverse events will be reported to the institutional review board at the time of study renewal or final report submission. All SAE will be reported to the DSMC and IRB within 24 hours of awareness of the SAE. The DSMC will review the progress and safety of the study every 3 months. Recruitment, follow-up rates, compliance with the study protocol and safety will be reviewed.

This study is currently not federally funded. However, if this research study becomes federally funded in the future, any action resulting in a temporary or permanent suspension of this project will be reported to the grant program director within 48 hours in writing.

Each individual participating site will be responsible for completing case report forms (CRFs) related to serious adverse events (SAE), limited to death, life threatening events, suspected and confirmed CNS events. Each participating site will also be responsible for completing CRFs related to adverse events (AEs) annually, focused on reasons for hospitalizations only. Each participating site will be responsible for submitting CRFs for SAE and AE to the coordinating center's project manager. The coordinating center's project manager will be responsible for disseminating the AE and SAE case report forms to other participating centers, the participating site IRBs, data safety monitoring boards and applicable regulatory agencies.

#### **14.1 Potential risks to the study participant**

**MRI/MRA of the brain:** During the MRI scan, the patient lies in a closed area inside the magnetic tube. Some patients may experience claustrophobia during the procedure. Therefore, participants with any history of claustrophobia will be asked to report this to their hematologist so that he or she can make appropriate accommodations for the participant's claustrophobia if necessary. Patients with metal devices in their body or any other contraindications to a magnetic resonance study will be excluded from this study for their own safety (see Exclusion Criteria in Section 6.6 for a full list of contraindications).

**Phlebotomy:** Phlebotomy is often associated with mild pain and occasionally with a small bruise at the site. Rarely, patients feel lightheaded or have syncope. There is also a very small risk of infection at the site of the needle puncture.

**Questionnaires:** The participant may experience emotional discomfort when answering some questions. If a particular question makes the parent or participant uncomfortable, the parent or participant can discuss its importance and the need to answer it with the reviewer. Completing the questionnaires may be inconvenient because of the time it takes to complete them.

**Disclosure of confidential information:** There is a small risk of breaches in confidentiality. This may affect the participant's future ability to obtain employment or medical, disability, or life insurance.

## **15.0 STUDY WITHDRAWAL AND DISCONTINUATION**

The Principal Investigator (PI) may withdraw the patient from the study if considered appropriate. It may be in the best interest of the patient to allow follow-up outside the study. The PI will share any new information that could change how patients feel about continuing in the study. The study may be discontinued by the Investigator, and/or IRB. If the study is prematurely terminated or suspended, the Investigator shall promptly inform the IRB and enrolled patients.

## **16.0 FOLLOW-UP AND RECORD RETENTION**

Patients will be followed for 2 years after the completion of the trial. Records will be kept for 2 years after the completion of the study.

## **17.0 LONG TERM OBJECTIVES**

This will be the first observational cohort study to prospectively and retrospectively describe the natural history of individuals with SCD and prior SCI or overt strokes receiving standard care management (regular blood transfusion therapy and/or hydroxyurea) to attenuate further neurological injury. The discovery of progressive or new central nervous system disease will inform the patient, family and health care provider as to whether more intensive interventions should be considered to prevent further progression of cerebral infarcts. Descriptive outcomes from this study could help guide the development of hypotheses-driven randomized clinical trials to optimize the management of patients with SCD and prior cerebral infarcts.

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