Hydroxyurea to Prevent CNS Complications of Sickle Cell Disease in Children (HU Prevent)

Study Protocol

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Study Leadership and Key Components of the Trial

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Other Participating Institutions and Principal Investigators

A complete list of the participating PIs and institutions is available at https://clinicaltrials.gov/ct2/show/study/NCT01389024

1.0 INTRODUCTION AND HYPOTHESES

1.1 Preliminary Studies

Sickle cell disease (SCD) is one of the most common genetic diseases in childhood, affecting approximately 1 in 2500 births in the United States.[1] The incidence among African Americans is 1 in 300, and there is a tremendous global burden of 250,000 new cases each year.[2,3] Central nervous system (CNS) injury arguably represents the most debilitating frequent complication of SCD, resulting in significant cognitive dysfunction that limits learning and academic achievement, work performance and the ability of individuals with SCD to lead normal lives. Stroke, silent cerebral infarct (SCI), and cognitive impairment are all frequent and highly morbid complications of SCD in children and adults. Among children with HbSS, 11% had a stroke by age 18 years before the implementation of transcranial Doppler (TCD) screening.[4] SCI is the most commonly recognized cause of serious neurological disease in HbSS, affecting approximately 27% of children by their 5th birthday[5] and 37% by age 14.[6] Children who develop SCI have greater cognitive impairment compared with either children with HbSS without SCI or siblings without SCD.[7] A recent study of adults demonstrated significant cognitive dysfunction, even in participants with apparently mild SCD.[8]

Hydroxyurea may have beneficial effects on neurological complications in HbSS[9,10] and reduces the frequency of painful crisis, acute chest syndrome and transfusion. At this point, however, the indications for the use of hydroxyurea in children remain unclear. A recent NIH Consensus Conference concluded that hydroxyurea therapy has not been broadly adopted in young children or adults.[11] There are currently no FDA-approved indications for hydroxyurea in children. Although the recent NHLBI BABY HUG study aimed to establish that hydroxyurea could prevent chronic organ system damage due to HbSS and HbSβ^0, this study failed to achieve either of its primary endpoints (prevention of splenic or renal injury); however, secondary analyses suggested that the intrapatient increase in TCD velocity from entry to exit was less in the hydroxyurea group than in the placebo group. In addition, there were fewer children with very low Bayley developmental scores in the hydroxyurea group (0 vs. 5 in the placebo group).[12] As expected in the very young group of participants in this study (9-48 months of age), the incidence of stroke was very low and no differences were seen between the hydroxyurea and placebo groups. The efficacy of hydroxyurea in preventing SCI was not evaluated. Importantly, the expected statistically significant decrease in painful events, acute chest syndrome and transfusions was seen in this study.

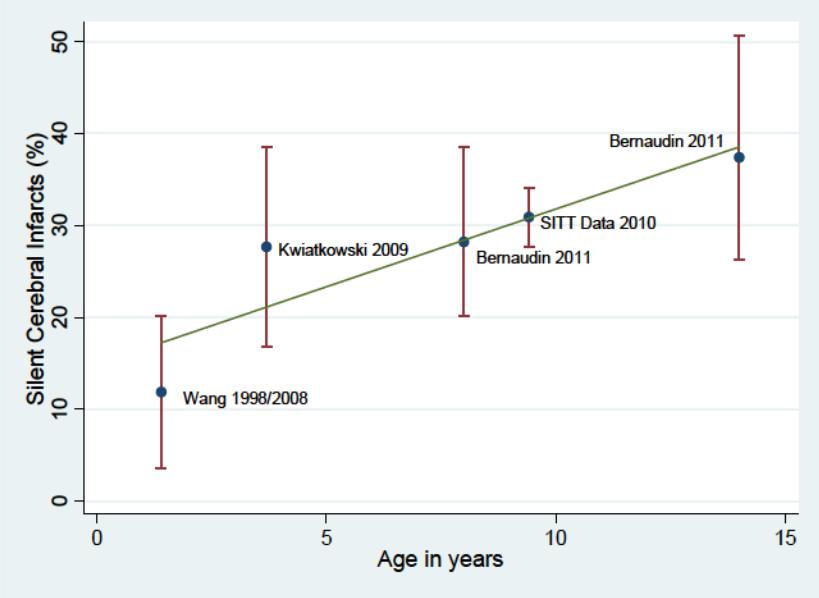
BABY HUG and other studies suggest, but do not prove, that hydroxyurea may prevent abnormal TCD and neurological injury in children with HbSS.[10,13-17] Preliminary expert opinion from the SIT Trial investigators provides support that a 50% reduction in CNS complications would be a compelling reason for most practitioners to use hydroxyurea in all children with HbSS. Thus, the public health implications of a clinical trial that establishes the ability of hydroxyurea to provide true primary prevention of CNS complications and broadens the use of hydroxyurea in this population would be immense. The purpose of this proposal is to lay the groundwork for such a trial by demonstrating the feasibility of recruitment and the safety of study procedures, while completing the planning and other procedures necessary for a definitive phase III trial.

1.2 Current Treatment for Primary Prevention of Overt Stroke and SCI

Based on the highly successful STOP Trial, the current approach for primary prevention of overt stroke in HbSS includes measurement of TCD velocity in the middle cerebral artery or terminal portion of the internal carotid. If individuals with velocities greater than 200 cm/sec are transfused with red blood cells to maintain sickle hemoglobin (Hb S) <30%, there is an 86% relative risk reduction for overt stroke in the first 30 months.[18] Although highly effective, transfusion therapy is associated with significant burden to families and morbidity, including excessive iron stores requiring chelation, risk of sensitization to minor red cell antigens, infection, and is inconvenient and expensive. In addition, even without transfusions, many children with TCD measurements above the threshold for transfusion therapy will never have a stroke; hence, many unnecessary transfusions are given. Current estimates are that approximately 7.5 children need to be transfused for one year to prevent one stroke and that 60% of those transfused would never have had an overt stroke without treatment. Abnormal TCD velocities are now the most common reason for chronic transfusions at most pediatric sickle cell centers, placing a great burden on patients and the health care

system. In several large hematology centers, 50 or more children may be receiving blood transfusion for primary prevention of strokes at a given time. If the number of abnormal TCDs can be lessened through primary prevention with hydroxyurea, the burden of transfusion will be lessened proportionately. As hydroxyurea is an orally administered agent that is relatively inexpensive compared to transfusion, the reduction in cost would also be substantial; in addition, the complications of transfusion would be avoided for many children currently receiving transfusion, the majority of whom do not benefit in terms of stroke prevention. This could facilitate the treatment of a much larger proportion of patients at risk for stroke (possibly including children in resource-poor countries). As an initial estimate of possible cost savings, approximately 10% of an unscreened population of young children with SCD will have abnormal TCD velocities. There are about 14,000 children in the United States between 2 and 16 years of age with HbSS or HBSβ^0 and about 1400 will have abnormal CBFV. Assuming that 1120 (80%) of these children accept transfusions (based on the proportion in the STOP Trial),[18] 50% of these abnormal TCDs could be prevented by hydroxyurea, and a cost of $28,500 per year for transfusions and chelation[19] versus $8500 for hydroxyurea, $11,200,000 could be saved per year. Adding the reduction in other costs of iron overload to these figures would considerably increase the cost savings, not to mention the potential reduction in burden to the patients and their families. This calculation assumes that a reduction of TCD velocities will prevent stroke; this has not been rigorously proven, but is felt to be a reasonable assumption.

Figure 1: Prevalence of Silent Cerebral Infarct by Age



1.3 SCI is morbid and occurs early in life

The highest incidence of SCI is in the first 4 years of life, as the cumulative prevalence increases (Figure 1) from 12% at 15 months[20,21] to 28% at 3.7 years[5], 31% at 9.4 years (DeBaun personal communication), and 37.4% at 14 years.[6] Specific neurological morbidities associated with SCI include decrements in cognition, poor academic attainment, progression to overt stroke, and new or progressive SCI on MRI.[22-25] Chronic transfusion is being evaluated for secondary prevention of SCI in the SIT Trial, but presently there is no primary prevention strategy for SCI. The degree to which HU prevents SCI, a problem that affects nearly a third of children with HbSS, could be a very important determinant of how widely this agent is used in children.

1.4 The Rational Basis for the Use of Hydroxyurea to Treat Neurological Complications in SCD

There is sufficient evidence to suspect that an increase in fetal hemoglobin (HbF) and total Hb could ameliorate the CNS complications of HbSS. Given that hydroxyurea is known to increase HbF, this therapy has been used extensively in adult patients with SCD. The seminal studies of Charache et al., including the Multi-Center Study of Hydroxyurea, demonstrated definitively that hydroxyurea reduced the frequency of crisis, chest syndrome, transfusions and hospitalization in adults.[26] Similar results have been reported in children.[27] The underlying causes of elevated TCD velocities and the high rate of SCI and stroke among young children with HbSS are not completely understood, but may be related in part to anemia[28] and pathological arterial stenosis.[29] Autoregulation, a compensatory vasodilatation of the cerebral vasculature to preserve oxygen delivery to the brain, manifests initially as increased cerebral blood flow.[30,31] Elevated CBFV as assessed by TCD measurement is a risk factor for overt stroke and anemia is a risk factor for SCI.[29,32] Direct benefits of hydroxyurea in children with SCD include an increase in the baseline hemoglobin level and a decrease in CBFV,[10] resulting in an increase in oxygen delivery; we postulate that the sum total of the multiple benefits of hydroxyurea including, but not limited to, decreased CBFV,[29] increased HbF and

NO production[33] and decreased red blood cell adhesiveness to the endothelium[34-36] will decrease the rate of conversion to high TCD measurements and prevent SCI and stroke.

Consistent with this hypothesis, our preliminary results suggest inverse correlations of both total Hb concentration and percentage of HbF with the incidence of SCI. Increases in total Hb concentration may also improve cognitive function, as more severe anemia is associated with lower IQ in both children and adults with SCD.[7,37] Thus, we feel there is compelling reason to investigate further the efficacy of hydroxyurea in preventing neurologic complications in SCD.

1.5 Detection of SCI and Sedation

MRI of the brain is the only presently available method to detect SCI. For children with the greatest incidence of SCI (less than 5 years old), MRI usually requires sedation. No standard approach for sedation of this population exists, limiting accessibility for screening for SCI in the group with the highest risk. At some medical centers, hematologists and anesthesiologists elect to transfuse red blood cells to all patients with SCD prior to any sedation, in an effort to decrease the rate of complications; however, many centers do not administer transfusions for all sedation procedures. In a recent survey of 21 of 25 SIT Trial Center Investigators, the majority indicated that transfusion was not given before all or some MRIs requiring sedation. At Johns Hopkins and Washington Universities, we evaluate such patients on a case by case basis. Based on data from our co-investigator (J. Kwiatowksi at CHOP) supporting the safety of a structured protocol for patient selection and sedation without antecedent transfusion in over 60 children with SCD, we believe that it is reasonable to implement a standardized protocol for sedation at other centers. Demonstrating that MRIs with sedation can be safely obtained without transfusion will greatly increase the feasibility of screening for SCI in the age group with the highest rate of SCI. Early detection of SCI is critical, as additional educational services, even in pre-school, can be mandated by federal law. A successful demonstration that sedation without transfusion using our protocol and procedures is safe would provide necessary information to conduct a definitive phase III trial. It would also provide information that could be applied to other procedures and reduce the use of transfusions and their associated risks.

We are acutely aware of the increased risk of sedation in patients with SCD and that a death occurred during sedation/anesthesia for a research MRI in the NIH-NHLBI-funded BABY HUG study. Expert pediatric anesthesiologists have designed our protocol for sedation to minimize these risks; specifically, children with recent respiratory illnesses and other risk factors will not undergo sedation. Based on this protocol, our study would have excluded the infant who died in the BABY HUG study from our trial. Several additional differences exist when comparing our study to the BABY HUG study: 1) Our sedation team of anesthesiologists have defined a rigorous set of criteria and specific sedation protocols to minimize risks associated with sedation; 2) Each site team will be required to have their sedation protocol reviewed centrally and approved prior to starting enrollment; and 3) The Sedation Committee will continue to review the sedation protocols and records and be available to address any AE or SAE associated with sedation.

1.6 Biomarker Discovery

Proteomic discovery approaches permit the identification of potential biomarkers by an unbiased evaluation of rare plasma proteins. Our group has interest and expertise in this area of research, as well as the unique resources of the Johns Hopkins NHLBI Proteomics Center. This work was funded by U54 HL090515 and R01 HL091759. We have identified elevated levels of GFAP in patients with SCD with evidence of subclinical brain injury.[38] We have also identified thrombospondin and L-selectin as proteins that are elevated in patients with SCD and SCI compared to those with normal MRI. The samples from this study will be used to validate these biomarkers and for further studies.

1.7 Hypotheses:

Hypothesis 1: Hydroxyurea therapy in young children (age 1-4 years) will reduce by 50% or greater the CNS complications of sickle cell disease, including conditional or abnormal TCD, silent cerebral infarction, TIA and overt stroke.

Hypothesis 2: Of 80 screened participants, approximately 40 will accept randomization to hydroxyurea.

Hypothesis 3: The proportion of serious adverse reactions attributed to sedation for the MRI will be <4%.

1.8 Aims of the Trial

A. Primary Objective:
To conduct an internal pilot, a randomized placebo-controlled trial of hydroxyurea to reduce the CNS complications of SCD, in preparation for a NIH-funded multicenter, phase III Trial. The primary endpoint for the pilot and definitive phase III trial will be prevention of conditional or abnormal CBFV, SCI, TIA and stroke. In the pilot trial, from an eligible group of 270, we will screen 80 children 9–48 months of age with HbSS or HbSβ^0 using MRI/MRA of the brain, TCD, and assessment by a pediatric neurologist. Of these 80 participants, we estimate that 40 (50% of those screened after exclusions and refusals) will accept randomization. TCD, MRI/MRA of the brain and measures of cognition will be obtained every 12 months for 36 months. The screening MRI and randomization will not occur until the child reaches the age of 12 months (i.e., has attained their 1st birthday). The results from the pilot will be incorporated into the design of the definitive Phase III trial

B. Secondary Objectives

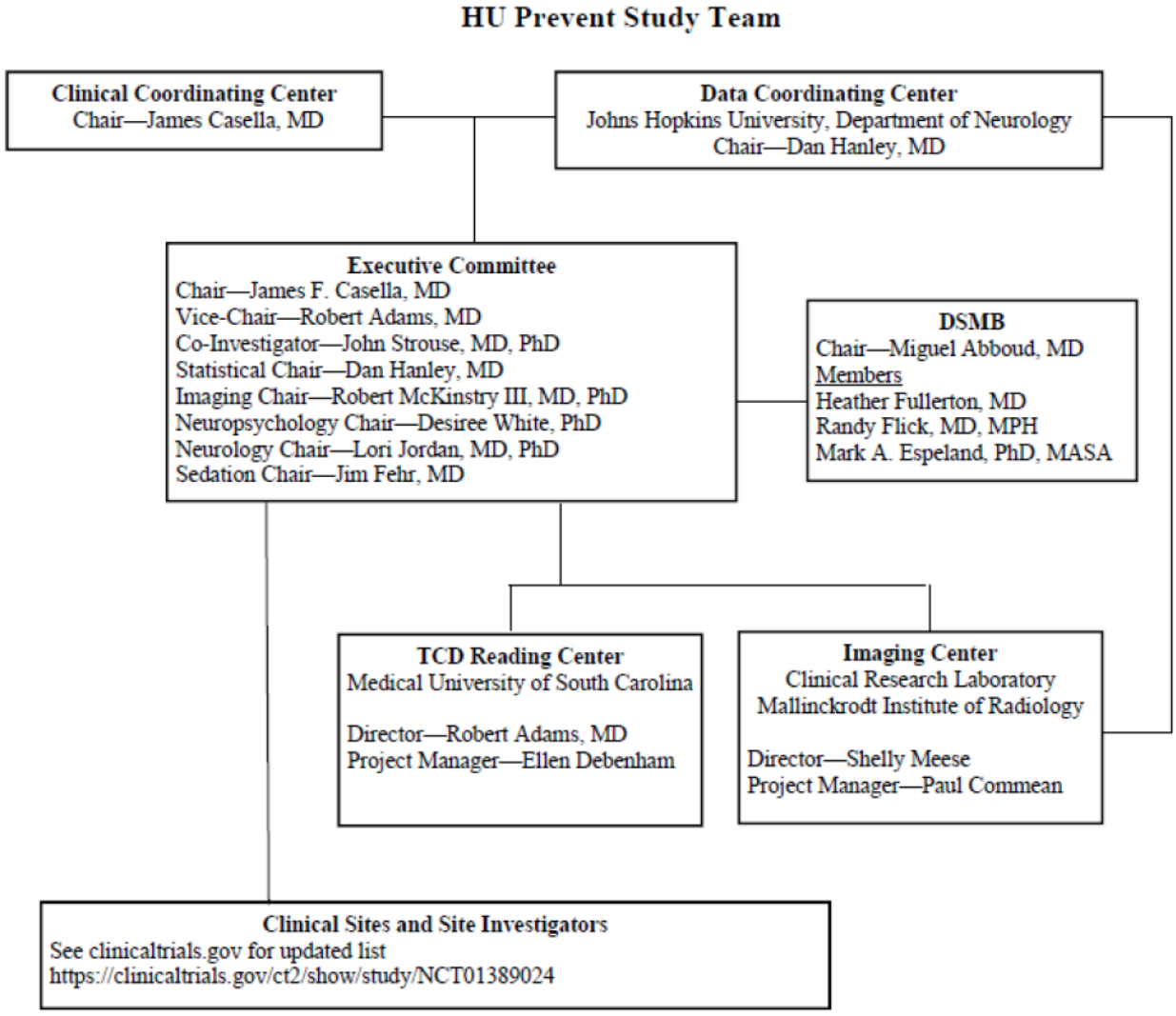
Objective 1:
To determine the acceptability of randomization to hydroxyurea vs. placebo for primary prevention of CNS complications of SCD. We will calculate the proportion of potentially eligible partipants undergoing screening and accepting randomization into the pilot study and use these proportions in the design of the definitive Phase III trial.

Objective 2:
To determine the safety of study procedures using standardized protocols in toddlers and prechool children with SCD.

Objective 3:
To collect plasma specimens to evaluate putative biomarkers of CNS injury as a surrogate outcome of hydroxyurea efficacy in an ongoing proteomics discovery project. We will collect plasma specimens on all screened participants on the day of their MRI and every 4 weeks on children randomized to the study. We will measure GFAP, thrombospondin, and L-selectin by electrochemiluminescence detection (Mesoscale Discovery Gaithersburg, MD) and apolipoprotein A1 by flow cytometry immunoassay (Milliplex MAP, Millipore, St. Charles, MO. Additional potential biomarkers will be assayed and evaluated, as they become available.

Objective 4:
Complete the necessary preparations for a definitive phase III trial. During the course of the current study, we will also prepare a manual of operations and case report forms for the proposed trial, develop sample IRB submission templates and organize all committees, collaborators and study procedures necessary for initiation of a successful, definitive, multicenter trial.

1.9 HU Prevent Trial Organization Chart: Figure 2



2.0 STUDY DESIGN, PHASING, AND ELIGIBILITY

2.1 Study Overview

The study is a randomized, controlled, double-blind, internal pilot of hydroxyurea vs. placebo for the prevention of CNS injury in children 12 to 48 months of age with sickle cell anemia. The study will be analyzed using intention to treat for outcomes, which will begin immediately after randomization and after receipt of the first dose of hydroxyurea or placebo for safety. All procedures are research procedures, except for the annual TCD (beginning at age 2), a history and physical examination every 6 to 12 months and CBC with differential, reticulocyte count every 4 weeks and comprehensive panel every 3 months, which are routine for participants on hydroxyurea. We propose to screen 80 children with a TCD, cognitive testing, a neurological exam by a pediatric neurologist, labs, and MRI/MRA of the brain, to randomize 40 of the approximately 180 children required for the definitive Phase III trial. We will also collect specimens for a biorepository (plasma and lymphocytes to make transformed lymphocytes as a source of DNA) and demographic and clinical information about these participants from the medical record and interviewing their parents/legal guardians. The parents/legal guardians of these children will have an educational session on the brain injury and treatment of sickle cell disease. **The last screening procedure will be the sedated MRI of the brain.** Screening procedures may be done beginning at 9 months of age; but the screening MRI will not be done until the child reaches the age of 12 months. Children without evidence of brain injury will be eligible for randomization at 12 months of age to 3 years of treatment with hydroxyurea or placebo.

2.2 Study Phases

PHASE I – Standardization **08/11– 07/12 (12 months)**

During this phase, each Clinical Center will obtain or will already have obtained IRB approval of the protocol, and consent form and obtain certification for the study procedure. This phase of the study may begin at *different time points for different sites, and the dates for the phases of the study can be adjusted for each site.*

The Study Chair, Vice Chair, Data Coordinating Center Staff and the Executive Committee will work together to complete mutually understood study documents such as the Protocol, Manual of Operations and Study Forms. Before a clinical site is allowed to begin participant recruitment, a clinical site investigator, neurologist, psychologist, neuroradiologist and study coordinator must be certified in each of the procedures of key importance to the IRB-approved study. Training will include the methods for recruitment, obtaining informed consent, collection of protocol specific data, (e.g., TCD, MRI, psychological evaluation, and neurological examinations), completion of the study forms, and use of the data entry (web entry/fax entry) system. All study site neurologists must complete the NIH Stroke Study Scale certification package prior to study center enrolling participants.

The completion of study forms and certain study procedures (e.g., recruitment) are to be performed only by clinical site staff that have been trained and certified to complete these tasks. Certification will be given to staff that pass a simple test concerning the protocol and demonstrate proficiency in required tasks (e.g., completion of forms and use of the web entry/fax entry systems). Re-certification will occur in association with site visits and will be based on proficiency demonstrated in the on-going performance of study activities.

For the original sites who have been involved in study development, certification may be granted at the discretion of the PI, based on participation in Executive and Operation calls and demonstration of adequate knowledge of the protocol and procedures.

PHASE II – Screening and Randomization **02/12 – until randomization completed**

During the screening period up to 80 children from four or more centers will be screened using MRI to identify children with silent cerebral infarcts. We anticipate approximately 40 will agree to participation in the randomization portion of the study. Written informed consent must be obtained from the parents prior to the screening process. A screening log to identify all individuals approached for the trial will be kept up to date and reviewed by the external auditing team. A second log will be kept to identify individuals who began, but did not complete screening. A one-sentence reason will be recorded as to why eligible participants did not proceed to the randomization component of the trial. The goal is to randomize participants within 3 months of signing consent, with a maximum period of 6 months from consent to randomization (unless otherwise approved by the study leadership).

PHASE III – Active Clinical Trial **5/12 – 7/17**

Participants identified on screening MRI without silent cerebral infarcts will be randomly allocated to placebo or hydroxyurea therapy, followed and then discharged from the trial or continued to be followed in the proposed phase III trial, if approved and funded. Participants will receive placebo or hydroxyurea for 36 months, or the duration of the feasibility trial. Twelve months after study entry, cognitive assessment, MRI, TCD and a neurology examination must be done for safety and interim analysis.

All participants will receive yearly evaluations that include an MRI, TCD, cognitive testing, H&P, and neurological evaluation for three years. The exit evaluation includes H&P, MRI, TCD, neurological examination, cognitive and laboratory testing.

PHASE IV - Study Close Out and Data Analysis **8/17-11/17**

During this phase, data analysis and manuscript preparation for the feasibility study will occur; in the event that the phase III study is approved and funded, the results of the feasibility study may be reported at the end of the phase III study, at the discretion of the PI.

2.3 Eligibility

Inclusion Criteria for Screening

1. Participant must have sickle cell anemia (hemoglobin SS) or sickle β°-thalassemia (hemoglobin Sβ°) as confirmed at the local institution by hemoglobin analysis after six months of age or by genotyping at any time.
2. Participant must be 9 to 48 months of age (i.e., must not have attained their 4th birthday). All screening procedures except MRI may be completed between 9 and 12 months of age, with the exception of the MRI, for which the child must have attained their 1st birthday.
3. Informed consent in accordance with the institutional policies (institutional IRB approval) and Federal guidelines (approved by the United States Department of Health and Human Services) must be signed by the participant's legally authorized guardian acknowledging written consent to join the study.

MRI of the brain with sedation

1. The parents or guardians must provide consent for sedation, as well as signing the study consent form.
2. Adequate study TCD with CBFV <170 cm/sec and completion of baseline cognitive testing and neurologist evaluation without evidence of TIA or overt stroke.

Exclusion Criteria for Screening

1. History of a focal neurologic event lasting more than 24 hours with medical documentation or a history of prior overt stroke or silent cerebral infarct.
2. Other neurological problems, such as TIA, neurofibromatosis, lead poisoning, non-febrile seizure disorder, or tuberous sclerosis.
3. Known HIV infection.
4. Treatment with anti-sickling drugs or hydroxyurea within 3 months or anticipate receiving anti sickling drugs or hydroxyurea during the course of the study.
5. Chronic blood transfusion therapy, ongoing or planned.
6. Poor adherence felt to be likely per his/her hematologist and study coordinator, based on previous compliance with clinic appointments and following advice.
7. Presence or planned permanent (or semi-permanent) metallic structures attached to their body. (e.g., braces on teeth, body piercings), which their physicians believe will interfere with the MRI of the head to assess the presence of silent cerebral infarct.
8. History of two or more TCD studies with a TAMMV or TAMX ≥170 cm/sec by the imaging or non-imaging technique, or a TCD that is indeterminate (indeterminate TCDs measurements may be repeated, and if evaluable on repeat, this exclusion is lifted).
9. Significant cytopenias [absolute neutrophil count (ANC) <1500/ul, platelets <150,000/ul, reticulocytes <80,000/ul, unless the Hb is > 9 g/dl]. Cytopenias will be considered transient exclusions.
10. Other significant organ system dysfunction
11. Known allergy or intolerance of hydroxyurea
12. Significant prematurity (gestational age of < 32 weeks)

MRI of the Brain with Sedation

1. Failure to pass MRI screening checklist
2. Obstructive sleep apnea [OSA] and receiving therapy [e.g. CPAP], or being evaluated or followed by a specialist for management of severe OSA
3. Less than 12 months of age.

4. Allergic reactions such as urticaria or bronchospasm or previous adverse reactions to propofol, eggs, or soy products.
5. Known major chromosomal abnormalities
6. Known airway abnormalities that would increase the risk of sedation/anesthesia.

Temporary Exclusions

7. Room air oxygen saturation greater than or equal to 5% below the participant's baseline on the day of the MRI with sedation.
8. Room air oxygen saturation <90% on the day of the MRI with sedation.
9. Hemoglobin <6.5 g/dl (must be measured within 30 days of MRI).
10. Temperature >38˚ C on the day of sedation
11. Upper or lower respiratory infection, active bronchospasm, acute chest syndrome, splenic sequestration or other acute complications of sickle cell disease other than pain in the last 4 weeks (defined by the time from resolution of symptoms to sedation).
12. Pain crisis within two weeks requiring treatment with opiates

Inclusion Criteria for Randomization

1. Participant must have sickle cell anemia (hemoglobin SS) or sickle β-null thalassemia (hemoglobin Sβ°) as confirmed at the local institution by hemoglobin analysis after six months of age.
2. Participant must be 12 to 54 months of age (i.e., must have attained their 1st birthday, but not be > 4.5 years of age).
3. Participant must have successfully completed screening procedures (TCD, MRI of the brain, neurology exam, and cognitive evaluation).

Exclusion Criteria for Randomization

1. Participants whose MRI show a silent or overt cerebral infarct.
2. Participants who have a non-imaging TCD study with a TAMMV ≥ 170 cm/sec verified by the study radiologist or a TCD that is indeterminate (indeterminate TCDs measurements may be repeated, and if evaluable on repeat, this exclusion is lifted).
3. Participants with abnormal kidney function (creatinine > 0.8 mg/dl)
4. Significant cytopenias [absolute neutrophil count (ANC) <1500/ul, platelets <150,000/ul, reticulocytes <80,000/ul, unless the Hb is > 9 g/dl]. Cytopenias will be considered transient exclusions.

2.4 Compliance Retention Strategies

To further increase the likelihood of adherence and retention, we plan to use several evidence-based methods that have been effective in clinical trials. The use of simple, clear, detailed written and verbal instructions regarding therapy, possible side effects of hydroxyurea and tests required for the study increases comprehension of the purpose and importance of the study, and yields better adherence to the trial.[39]

Education Sessions

Given the intensity and duration of this study, we believe that a solid participant-provider relationship needs to be established prior to the informed consent and randomization process. Prior to obtaining consent for the treatment component of the study, each parent/guardian should be educated about the potential sequelae of silent cerebral infarcts and overt stroke and the benefits/risks of hydroxyurea for 36 months. The overall goal of these educational visits is to fully inform parents/guardians and participants about the importance of the trial and the requirements of participation. After parents/guardians have received baseline information about the potential sequelae of stroke and risks/benefits of hydroxyurea therapy, they will be better informed of the risks and benefits of participating in this clinical trial for up to 36 months.

The primary focus of the parent and participant education visit will be to promote participant recruitment, as well as participants' adherence to the protocol and long-term retention in the study. This educational method

has been effective in a previous National Heart, Lung, and Blood Institute (NHLBI) multi-institutional trial, the Childhood Asthma Management Program (CAMP). [40] Although attendance is not an assurance of compliance in clinical trials, non-attendees are frequently unlikely to comply with the trial. [41]

The first educational visit should occur during initial screening. Topics covered should include a parent/participant education curriculum that covers the following subject matters: the need for and risks of sedation to obtain a MRI of the brain and TCD, identification and management of abnormal TCD, silent cerebral infarct and overt stroke; school-related problems in children with sickle cell anemia, silent cerebral infarcts and overt stroke; and strategies to assist parents/guardians in improving the school environment and obtaining additional evaluation and early intervention resources for toddlers and preschool age children. These strategies will include options for decreasing school absenteeism, such as intermittent homebound instruction, guidelines for initiation of an Individual Education Plan (IEP), and information regarding help for children with potential developmental delay or cognitive impairment.

During the second educational visit which should follow a qualifying screening MRI, parents/guardians will be informed of the implications of hydroxyurea therapy, including reversible bone marrow suppression, macrocytosis that may mask folic acid or B12 deficiency, rash and the precautions taken to decrease these adverse events.

Parent/Caregiver Relationship

The staff will contact parents/guardians immediately and reschedule participants' appointments when missed. Closely monitoring the participant visits and need for return appointments, sending reminder cards and making follow-up telephone calls to assure participant attendance for blood tests and receiving hydroxyurea or placebo are key elements in keeping participants from becoming lost to follow-up. Also, participant randomized to receive hydroxyurea or placebo will receive $20.00 for every study visit, and an additional $20.00 for every research visit that includes a sedated MRI, TCD or cognitive testing. If the participant completes all of the study visits (16 visits in the first year, 15 visits in the second year, and 15 visits in the third year) this will total up to $1100 ($920 for the regular visits and $180 for sedated MRI, TCD, and cognitive testing) over 3 years to help defray any study associated costs.

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 families whereabouts, will assist in tracking participants and decrease the number lost to follow up. We will also require the participant's social security number to assist in tracking of the participant long term. The research team will use and share the participant's information until ten years after closure of the study. At that point, the site investigator will remove the identifiers from the participant's information, (kept only at the local site by the local principal investigator) making it impossible to link the participant to the study.

Community Outreach

To promote close relationships with all health providers involved with HU Prevent at each institution, we will recommend that the study staff notify the participants' private physician when participants are entered on the trial. The Site Investigator will keep the pediatric medical community abreast of the progress at grand rounds and/or routinely scheduled educational conferences at each institution, emphasizing the importance of following these participants for a minimum of 36 months.

3.0 CENTRAL NEURORADIOLOGY REVIEW of MRI/TCD

3.1 MRI Review

The Neuroradiology Committee is made up of three members. All three members read every entry scan (MRI-1) and vote independently on two issues: 1) Does the scan meet quality assurance guidelines? and 2) Does the scan reveal a cerebral infarct-like lesion(s)?

Although a majority vote is not changed, the committee makes every attempt to hold a monthly consensus call, to discuss cases where there is no consensus.

In addition to a reading to exclude potential participants from the HU Prevent study, an MRI is also read within 24 hours of uploading into the CRL website for any emergency medical condition that will require follow up at the local site. The emergency read is performed by one assigned neuroradiologist. This has been built into the system to accommodate several sites that are using a research MRI, which means that the scan is not read locally. If an emergency or incidental finding is found, the neuroradiologist E-mails and telephones either Dr. James Casella, Dr. John Strouse, or Dr. Lori Jordan.[42] The critical medical information is relayed, and either the Study PI or Neurology Committee Chair will call and E-mail the local site investigator. This chain of communication is to keep the neuroradiologists 'blind' to any other clinical conditions that may be discussed during the transfer of pertinent clinical information. These findings will be communicated to the participant's physician, parent, and/or guardian as necessary by the local study investigators or personnel, recognizing the choices made by the participant in the consent form.

The committee performs the same process for all MRIs, study close-out scans, and unscheduled scans; however, instead of seeking disqualifying lesions, now the question of interest is, "Does the MRI meet the study defined endpoint?"

3.2 TCD Review

TCDs may be interpreted locally by radiology, neurology, or hematology, in addition to a study interpretation by the staff of the central reading facility. TCDs will be transmitted to the Medical University of South Carolina (MUSC) for quality control and central interpretation by the MUSC Neurology Core. Dr. Robert Adams, Professor of Neurology at the Medical University of South Carolina, is a long-standing consultant to our group and directs the Neurology Core. Dr. Adams will provide training using standardized protocols to assure the validity and reliability of measurements obtained by non-imaging TCD at participating sites. Personnel at each site will participate in a screening training and testing program developed by the TCD Imaging Center for non-imaging TCD assessment (described in detail in Appendix 11). The image analysis quality assurance program includes two components: 1) initial training on-site or via videoconference/ Webex; and 2) intra-observer variability testing. Each research study reader will independently evaluate a set of three readings in the same individual within a 120 minute interval. Intra-observer variability will be assessed and the degree of variability must not exceed a predefined threshold. The Principal Investigator and Imaging Center Director (Dr. Adams) will agree upon the exact parameters to be assessed and the threshold to be set. If the readers exceed the threshold, then re-training will be required.

Dr. Adams and his team will evaluate images from both eligibility screening and from enrolled participants, including participants with a suspected or confirmed primary endpoint, that is, a TCD measurement of CBFV above a pre-defined threshold ($\geq$ 170 cm/sec) in the distal internal carotid or middle cerebral arteries. He will use a reference set showing examples of the various diagnostic criteria developed prior to the start of the study. The MUSC Neurology Core will be responsible for both a qualitative and quantitative assessment of the images. The qualitative assessment will be based on the presence or absence of TCD measurement reaching the endpoint of the trial (1=definitely not, 2=indeterminate, 3=definitely present). Dr. Adams will independently assess and record whether a TCD endpoint has been reached.

In the event of a TCD indicating a CBFV $\geq$ 200 cm/sec and confirmed by the imaging center, a repeat TCD will be obtained and confirmed as quickly as possible, preferably within 48 hrs, but no longer that 1 week.

Conditional TCDs will be repeated within 6-12 weeks, or earlier, at the discretion of the site PI. Two TCDs above 170 cm/sec will be required to determine an endpoint, unless the initial TCD is greater than or equal to 220 cm/sec, in which case a single TCD will suffice. In the event that a child has been started on chronic transfusions by the local treatment team, based on a local read of a TCD > 200 cm/sec, this will be considered an endpoint for the study, even if the TCD is not > 170 cm/sec by the central read. As two patients in the STOPII trial of the 16 reaching endpoints developed stroke before a second TCD could be obtained, we will ask the PIs and coordinators to adhere very strictly to the above time criteria for repeat TCD. In practice, the investigators in the HU Prevent trial feel that repeating abnormal TCDs is the standard of care, making this approach desirable and acceptable.

4 PARTICIPANT EVALUATIONS

Standard Care vs Experimental procedures

Yearly TCDs have been recommended by the NHLBI, beginning at 2 years of age, and will be considered standard of care and billed to the participant's insurance or self-pay. At some sites, MRIs are routinely performed at yearly or every other year intervals; in these cases, they will be considered standard of care and billed to the participant's insurance or self-pay. Monthly CBCs with differentials and reticulocyte counts and metabolic panels every three months are standard care for children being treated with hydroxyurea. All other study procedures are considered experimental. Participants will be counseled by social workers or members of the team regarding the potential expenses incurred by inclusion in the study and encouraged to verify whether payment for standard of care procedures will be acceptable to their insurance companies.

4.1 DNA and Proteomics Sample

We have an exceptional opportunity to expand the existing biological repository of specimens from children with sickle cell disease from the SIT Trial. This resource will be invaluable to those studying the genetics, proteomics and biology of sickle cell disease. The value of the existing repository is in its large size (over 1200 participants) and in the high quality of the information about the participants. All participants included in the repository will have an MRI of the brain and provide (with help from their parents and health care providers) detailed information on their previous complications from sickle cell disease. Lymphocytes from the specimens will undergo EBV transformation and be stored in the Cell Center of the Genetic Resources Core Facility (GCRF) at Johns Hopkins Hospital. In addition, plasma and DNA isolated from the lymphocytes will be stored in the repository. The viability of lymphocytes decreases over time, so it is important that we receive all samples within 48 hours of collection, preferably within 24 hours. In addition, to the collection of genetic samples, we will collect plasma samples under optimal conditions for proteomic experiments. The HU Prevent Study will represent a unique opportunity to study a well-defined cohort of participants who will be followed longitudinally. Please refer to Appendices 14 and 15 for specimen preparation and shipping instructions

4.2 Hydroxyurea

Participants randomized to receive hydroxyurea will begin at 20 mg/kg/day rounded to the nearest 10 mg, with a stable liquid formulation compounded at 100 mg/ml by local research pharmacies using a standardized procedure and Hydrea™ 500 mg capsules (Bristol-Myers Squibb) obtained by the local site research pharmacy (Appendix 17).[43] Dose escalation will occur in 5 mg/kg/day increments every eight weeks, to a maximum of 35 mg/kg/day, if the ANC is > 4.0 x 10^9/L and no other hematological toxicity is present (Hb < 7.0 g/dL when absolute reticulocyte count < 80 x 10^9/L, or platelet count < 80 x 10^9/L). Hydroxyurea will be held for hematological toxicity and a CBC with differential and a reticulocyte count will be obtained weekly, until resolution of the toxicity. Hematological toxicity is defined as an ANC < 1 x 10^9/L, Hb < 7.0 g/dL, when absolute reticulocyte count < 80 x 10^9/L, or platelet count < 80 x 10^9/L. Recovery is defined as an ANC > 1.5 x 10^9/L, Hb ≥ 6.0 g/dL (unless the patients pre-treatment baseline is < 6.0 g/dL, in which case the Hb should be within 0.5 g/dL of baseline), absolute reticulocyte count >80 x 10^9/L and platelet count > 100 x 10^9/L. Hydroxyurea will then be restarted at the same dose, unless hematologic toxicity has previously occurred at this dose; if so, the dose will be reduced by 2.5 mg/kg/day. Further dose escalation will not occur after dose reduction.

4.3 Placebo

Participants randomized to receive sucrose placebo (as in the BABYHUG Study) will begin 0.2 mL/kg rounded to the nearest 0.1 mL of placebo compounded by local research pharmacies. Dose escalation will occur based on local counts as with hydroxyurea in 0.05 mL/kg/day increments to a maximum of 0.35 mL/kg/day, every eight weeks, if the ANC is > 4.0 x 10^9/L and no other hematological toxicity is present (Hb < 7.0 g/dL when absolute reticulocyte count < 80 x 10^9/L, or platelet count < 80 x 10^9/L). Hydroxyurea will be held for hematological toxicity and a CBC with differential and a reticulocyte count will be obtained weekly, until resolution of the toxicity. Hematological toxicity is defined as an ANC < 1 x 10^9/L, Hb < 7.0 g/dL, when

absolute reticulocyte count < 80 x 10^9/L or platelet count < 80 x 10^9/L. Recovery is defined as an ANC > 1.5 x 10^9/L, Hb ≥ 6.0 g/dL (unless the patients pre-treatment baseline is < 6.0 g/dL, in which case the Hb should be within 0.5 g/dL of baseline), absolute reticulocyte count > 80 x 10^9/L and platelet count > 100 x 10^9/L. Study treatment (placebo) will then be restarted at the same dose, unless hematologic toxicity has previously occurred at this dose; if so, the dose will be reduced by 0.025 mL/kg/day. Further dose escalation will not occur after dose reduction.

Simulated dose escalations will occur at the same frequency as typically required in the hydroxyurea arm (1 to 3 per participant, with a mean of 2).

4.4 Monitoring

All participants will have a CBC with differential and reticulocyte count 2 weeks after dose initiation or escalation and every 4 weeks otherwise. An unblinded, local study monitor will review all local counts and an unblinded local data manager will submit these studies electronically to the Data Coordinating Center (DCC) and issue local holds for toxicity and dose escalation. A central study monitor will be responsible for reviewing laboratory results to assure that toxicity and dose escalations are occurring appropriately. The central study monitor will inform sites of random stops and holds for placebo participants as well. Other monitoring for toxicity and the effect of hydroxyurea will include a comprehensive panel, lactate dehydrogenase, hemoglobin F, F-cells, every 3 months and F-reticulocytes annually.

4.5 Safety Reporting and Adverse Events

Safety assessments

Complications of sedation for MRIs will be recorded by the anesthesiologist or other provider performing the sedation under the supervision of the anesthesiologist, using a standard form developed by the Sedation Committee (see appendix 18) and by follow-up telephone call by the study coordinator after 72 hours and follow-up visit or telephone call at 14 days, using a standardized questionnaire (Appendix 19). Participants will be evaluated every two weeks after a change in dose and otherwise every four weeks. An interval history of medical events and potential adverse events will be obtained at these visits. This information will be collected for 4 weeks after the participant has stopped the study medication. Conditional and abnormal TCDs will be repeated within defined intervals (see Section 5.1) to detect progression and the need for transfusions.

Safety information will be sent to the coordinating center led by Daniel Hanley, MD and supported by the Johns Hopkins ICTR. This center will be responsible for data management, data quality assurance, random stop orders and dose escalation orders for hydroxyurea, and providing information on results and analyses to the DSMB.

Adverse events: Data will be collected on adverse events (AE), any abnormal finding (history, physical exam, laboratory, or imaging) of a participant in the study. Unexpected AEs are adverse reactions that are not consistent (nature or severity) with the available information on hydroxyurea or SCD. A full list of expected AEs are below. AEs will be scored using the Common Toxicity Criteria for Adverse Events (CTCAE) 3.0 of the National Cancer Institute and the standard nomenclature for defining the causal relation between the AE and study drug (unrelated, probably not related/remote, possibly related, probably related, definitely related). AEs will be followed until they resolve or stabilize. AEs will be classified as ongoing, resolved without sequelae, resolved with sequelae, or death. The response to the AE will be recorded.

Expected Adverse Events in Children with Sickle Cell Disease

- Acute chest syndrome
- Aplastic crisis
- Anemia
- Bactermia
- Cholelithiasis, cholecystitis, biliary tract obstruction
- Dehydration
- Failure to thrive
- Fever

Gastroenterititis
Headache
Hematuria
Hypothenuira
Hyperbilirubinemia
Leukocytosis
Nocturia
Obstructive sleep apnea
Osteomeylitis
Otitis media
Pain
Pharyngitis
Pneumonia
Priapism
Splenic sequestration
Upper respiratory tract infections
Urinary tract infection
Tachycardia
Tachypnea
Thrombocytosis

Serious adverse event (SAE)
Results in death
Life-threatening (participant at risk of death at the time of the event)
Requires or prolongs hospitalization (unless an expected event).
Intracerebral or subarachnoid hemorrhage
Ischemic stroke
Causes persistent or significant disability.
Results in a congenital anomaly.
Other medical events (in the opinion of the investigator) that may put the participant at risk or require intervention to prevent a serious AE.

Reporting of events
Serious safety issues will be reported to the DSMB and IRB, which will make recommendations to the sponsor (Johns Hopkins and the NHLBI) about the study.

Unexpected SAE that are possibly related, probably related, or definitely related to study medication or procedures will be reported to the DSMB, FDA, and IRB within 7 calendar days, if fatal or life-threatening, and within 15 calendar days if not. All SAE (expected and unexpected) will be provided to the DSMB by the DCC quarterly. All AE (serious and non-serious) will be reported to the DSMB every six months.

4.6 Evaluation of Adherence

Adherence will be ascertained by an interview with a brief questionnaire and measurement of medication dispensed and returned. The parent will be asked to estimate the number of hydroxyurea doses missed, spilled, or otherwise lost since the last visit and to complete the modified Morisky Medication adherence scale.[44,45] In addition, the study coordinator will obtain information, including the dose, dispensing date, and volume of liquid dispensed from the site pharmacist and weight of the bottle before it is given to the family and upon its return. This information will be used to complete the hydroxyurea log. We will evaluate the proportion of participants receiving at least 80% of their prescribed doses of hydroxyurea and calculate 95% CI by exact methods.

4.7 Withdrawal from the study.

As the study is being conducted as intention to treat, participants will only be withdrawn from the study if they withdraw consent or become unable to comply with study procedures. In these instances, all reasonable efforts will be made to collect as much endpoint information as possible prior to withdrawal.

4.8 Discontinuation of hydroxyurea or placebo.

If a participant is begun on regularly scheduled transfusions during the study, hydroxyurea or placebo will discontinued for the period of chronic transfusions. If the participant is taken off of transfusions during the 3 years of intended therapy, the study drug may be resumed. Monitoring for hydroxyurea will be discontinued during the period of the chronic transfusions. If a participant is given transfusions during the study, without the intention of continuing chronic transfusions, hydroxyurea or placebo will be continued for the period of the transfusions; the study drug can be continued for a period of up to 6 months of approximately monthly transfusions, after which it should be discontinued, if the patient remains on transfusions for any reason. All participants will continue to be followed for the duration of the study period (3 years). All study procedures, except for monitoring for toxicity from hydroxyurea, should continue.

If a provider decides to begin hydroxyurea on a participant during the trial, a discussion will be held with the PI and the provider, to review the indications for doing so. We expect these events to be rare, as the study investigators feel that there are presently no absolute indications for hydroxyurea use in this age group. These decisions will always be made with the best interests of the participant in mind and the final determination of therapy will be made by the participant's physician in all cases.

If the participant has two or more episodes of hematological toxicity despite reduction of the study medication to $\leq$ 0.125 ml/kg/day (12.5 mg/kg/day of hydroxyurea or equivalent volume of placebo) or Grade 4 toxicity or recurrent grade 3 toxicity, probably or definitely related to study medication, the study drug will be discontinued.

5.0 EVALUATIONS and FOLLOW UP

5.1 Overview of study procedures

After screening procedures, which include a TCD, MRI, neurological evaluation and neuropsychological testing, children without SCI, stroke, TIA or elevated CBFV (> 170 cm/sec) and meeting the other criteria will be eligible for randomization. Randomized participants will have study visits every 4 weeks with a history and exam, study medication refill, adherence measures, and a CBC with differential and reticulocyte count. A CBC with differential and reticulocyte count will also be obtained 2 weeks after each dose increase and as needed to monitor toxicity. With local institutional review board approval, these visits may occur at satellite sites. Additional labs (Hb F and S quantification, LDH, and comprehensive panel will be obtained 4 times a year and proteomics every 4 weeks). The TCD, sedated MRI/MRA of the brain, neurology and cognitive evaluations will be repeated yearly. Participants with conditional TCDs (CBFV 170-199 cm/sec) or abnormal TCD (CBFV >200 cm/sec) by non-imaging TCD or comparable CBFV by imaging TCD) will have the TCD confirmed by a repeat study for the endpoint. Exit studies are described, along with a summary of procedures in the table below. Tables of evaluations by year are included as appendices 4-6.

Table 1: Schedule of Study Procedures

Procedure	Screening	Pre randomization	Q4 weeks	Yearly	Exit
History and Exam	XX		XX		XX
Transcranial Doppler	XX+			XX	XX
MRI of brain	XX^			XX	XX
Study Medication refill			XX		
Adherence measures		Education Session	XX		
Neurology Evaluation	XX			XX	XX
Cognitive evaluation	XX			XX	XX
CBC, diff., reticulocytes	XX		Q2-4 weeks		
Comprehensive panel, LDH	XX		4 per year		XX

Biorepository samples	XX*		XX		XX
Hb F and S, quantification	XX		4 per year		XX

+The TCD should not be done within 2 months of transfusion
^ To minimize risk, the MRI must be the last scheduled screening procedure, unless performed as a clinical care study
*at time of MRI of the brain

5.2 Screening Visits

Visit 1

Screening visits for participants meeting entry requirements of this protocol will be scheduled by the study coordinator at each site. The first screening visit will consist of a comprehensive history (medical and educational), a screening TCD, a physical examination, including neurologic evaluation by the hematologist, and collection of blood for a CBC with differential, reticulocyte count, LDH, hemoglobin electrophoresis and quantification of HbF, HbS, HbA, and other hemoglobins, F-cells and F-reticulocytes. The TCD should not be done within 2 months of simple or exchange transfusions. The blood draw should prioritize the CBC with differential, reticulocyte count and quantification of hemoglobins, then the LDH, F-cells, and F-reticulocytes. This visit will also include education about neurological complications of sickle cell disease and the rationale for the study.

Visit 2

If the participant meets inclusion criteria, a second visit will be scheduled, including the exam by a pediatric neurologist and neurocognitive assessment, as well as the second hour of education about hydroxyurea and the study. Blood for DNA and to make transformed lymphocytes for the biologic repository will be drawn at this time (see appendix 14). Visit 1 and Visit 2 may be combined or split into additional visits.

Visit 3

This visit will include the sedated MRI of the brain, if the participant still meets all eligibility requirements for the study, and a blood draw for a proteomic sample (see appendix 15). Visit 3 (and the sedated MRI for research) should not occur until after all other study procedures (from Visits 1 and 2) have been completed and the participant has been confirmed to still be eligible for the study.

The site investigator can use an MRI (within 2 weeks of informed consent) or TCD (within 6 weeks of consent), if done for clinical reasons ***and*** if the MRI and TCD meets the qualifying criteria, as judged by the neuroradiology committee (for MRI) and TCD reading center (for the TCD). Note that the clinical TCD must be by the non-imaging technique to qualify.

The participant should be randomized within 2 weeks of the MRI, if at all possible, to avoid the occurrence of endpoint events before treatment is initiated.

5.3 Randomization Evaluation

All participants will have a CBC with differential, reticulocyte count, comprehensive panel (if not obtained in the last 2 weeks for the CBC with differential and reticulocyte count or last 8 weeks for comprehensive panel) at the time of randomization and start the study treatment the same day. If blood for the DNA or proteomic specimens could not be obtained on previous visits, it may be obtained at this time.

5.4 Post-Randomization Evaluations

The following evaluations will be required at each clinical center for all participants **2 weeks** after a dose increase of the study medication:

CBC with differential, reticulocyte count

The following evaluations will be required **every 4 weeks** at each clinical center for all participants:

History and physical exam, including weight, length, blood pressure, respiratory rate, heart rate, temperature, and pulse oximetry

Modified Morisky, review of missed, spilled, or otherwise lost doses, and weighing of the returned study medication
CBC with differential, reticulocyte count, proteomics specimen.
A 3 working day window on either side is allowable for the every 4 week visits (25-31 days after the last visit)

The following evaluations will be required **every 3 months** at each clinical center for all participants:
Comprehensive panel, LDH, hemoglobin electrophoresis and quantification of HbF, HbS, HbA and other hemoglobins, and F-cells

The following studies will be performed **annually on all participants:**
Neurological examination by pediatric neurologist
Cognitive evaluation by a neuropsychologist
MRI of the brain
TCD
F-reticulocytes
A 4 week window on either side is allowable for annual evaluations (using the randomization date as time 0)

If a participant receives a clinical MRI of the brain (not study mandated) during the course of the study, it should be submitted to CRL for review by the Neuroradiology Committee.

5.5 End of study procedures:
After 3 years of therapy, or in the event of an endpoint, the annual visit procedures will be repeated, with a final phlebotomy of 10 ml of venous blood for the biorepository.

5.6 Evaluations for parents/caregivers
Education
Parents/caregivers will participate in a sickle cell disease education program in the pre-randomization phase of this study. This program will increase their knowledge of sickle cell disease (SCD) and its complications, review school related problems in children with SCD, teach identification and management of overt stroke, abnormal TCD, discuss silent cerebral infarct and the explanation of its impact on the educational and vocational attainment for children with sickle cell disease, and describe the risks and benefits of treatment with hydroxyurea in children with SCD. These sessions will occur during two regularly scheduled study visits.

Behavioral Scales
Parents/Caregivers will complete behavior scales about their children, including the ABAS-II (all ages, BRIEF-P (2- 5.99 years old), and BRIEF ($\geq$ 6 years old), depending on the age of the child.

Adherence
Parents/Caregivers will complete the Modified Morisky Scale to evaluate medication adherence, as well as providing information on missed, lost, or spilled doses of the study medication.

5.7 Study exit evaluations for all participants
- MRI of brain
- TCD
- Cognitive and behavioral assessment
- History and physical
- Neurological Assessment
- CBC with differential, reticulocytes, comprehensive panel, LDH, quantification of HbF, HbS, HbA, and other hemoglobins, F-cells, and F-reticulocytes
- Demographic/phenotypic exit form
- Obtain blood for the biologic repository for proteomic studies

5.8 Procedure for missed appointments

In the case of a participant missing an appointment, the study nurse should immediately attempt to contact the participant's caregiver by telephone, to determine why the appointment was missed and reschedule it. All contact attempts, even unsuccessful, should be recorded in the clinic record. When contact is established, the reason why the appointment was missed should be recorded in the clinic record. A make-up appointment should be scheduled as promptly as possible. In cases where non-compliance is becoming an issue that will impact the participant's well-being and data, we invite the investigator or coordinator to contact the Study Chair or Vice Chair, to assist the site in creative ways to improve compliance. We define non-compliance as missing two appointments in a row, or two appointments in a four-month period.

5.9 Addressing New MRI Technology:

If during the course of the trial, upgrades to a 3.0 Tesla MRI scanner from a 1.5 Tesla scanner are planned at a site and a 1.5 Tesla scanner is no longer accessible, an additional two MRI scans will be required: one MRI scan on the 1.5 Tesla scanner before it is discontinued, followed within 3 months by an additional MRI scan on the 3.0 Tesla scanner. These extra MRI scans will only be obtained if sedation is not required so as to minimize risk to the participant. The neuroradiology committee will use these interim 1.5 & 3.0 Tesla scans to compare to the study exit MRI (also completed on a 3.0 Tesla scanner), to determine whether progression has occurred from the baseline scan. This will not be necessary for sites that use a 3 Tesla scanner for all imaging, the preferred field strength for neuroimaging of this study.

6.0 SEDATION OF CHILDREN WITH SCD

MRI of the brain is the only presently available method to detect SCI. For children with the greatest incidence of SCI (less than 5 years old), MRI usually requires sedation. No standard approach for sedation of this population exists, limiting accessibility for screening for SCI in the group with the highest risk. At some medical centers, hematologists and anesthesiologists elect to transfuse red blood cells to all patients with SCD prior to any sedation, in an effort to decrease the rate of complications; however, many centers do not administer transfusions for all sedation procedures. In a recent survey of 21 of 25 SIT Trial Center Investigators, the majority indicated that transfusion was not given before all or some MRIs requiring sedation. At Johns Hopkins and Washington Universities, we evaluate such patients on a case by case basis. Based on data from our co-investigator (J. Kwiatowksi at CHOP) supporting the safety of a structured protocol for patient selection and sedation without antecedent transfusion in over 60 children, we believe that it is reasonable to implement a standardized protocol for sedation at other centers. Demonstrating that MRIs with sedation can be safely obtained without transfusion will greatly increase the feasibility of screening for SCI in the age group with the highest rate of SCI. Early detection of SCI is critical, as additional educational services, even in pre-school, can be mandated by federal law. A successful demonstration that sedation without transfusion using our protocol and procedures is safe would provide necessary information to conduct a definitive phase III trial. It would also provide information that could be applied to other procedures and reduce the use of transfusions and their associated risks.

We are acutely aware of the increased risk of sedation in patients with SCD and that a death occurred during sedation/anesthesia for a research MRI in the BABY HUG study. Our protocol for sedation has been designed to minimize these risks; specifically, children with recent respiratory illnesses and other risk factors will not undergo sedation. This would have excluded the infant who died in the BABY HUG study from our trial. There will be several additional differences from the BABY HUG study: 1) Our sedation team of anesthesiologists have defined a rigorous set of criteria and protocols to minimize risks associated with sedation; 2) The sites will be expected to adhere to the guidelines for sedation that are included in Appendices 12 and 13. Any site team wishing to use a local protocol derived from these guidelines will be required to have their sedation protocol reviewed centrally and approved prior to starting enrollment; and 3) The Sedation Committee will continue to review the sedation protocols and records and be available to address any AE or SAE associated with sedation.

The administration of anesthetics and sedatives to patients with SCD is controversial and there is not consistent agreement amongst anesthesiologists or hematologists as to the best approach. The following

aspires to best practice principles which center on clear and consistent communication between the various providers from hematology, anesthesiology and the sedation team and, most importantly, with the family. These guidelines were reviewed and approved by the Sedation Committee for the HU Prevent Trial and are intended to provide a minimal standard of safety. The actual management of participants should be individualized as necessary, with the safety of the participant being the highest priority at all times; however, the use of propofol as the primary agents for sedation/anesthesia, the exclusion criteria and minimum requirements, such as duration of NPO and baseline oxygen saturation should be adhered to strictly. These requirements may also be exceeded (e.g., longer durations of NPO) based on local judgments.

The children enrolled for imaging will be pre-screened before they receive sedation for an MRI, as the goal of this protocol is to provide the safest environment for these children, minimal disruption for the parents and consistent care with rare cancellations. Children with SCD may have had recurrent hospitalizations and be wary of care providers; they may also have had many previous intravenous (IV) catheters and poor vascular access. The children will be cared for in a warm and supportive manner and the MRI, its indication and the person requesting it should be confirmed with the parent or guardian.

Obtaining an MRI of the brain is considered a research procedure at some sites; however, the sedation procedures planned for this study are standard of care, based on the experience of many centers and the overwhelming majority of investigators in the SIT Trial. The detailed collection of information on the MRIs and sedation in this protocol is for safety, but should help set a standard for sedation of patients with SCD in routine practice.

For the detailed description of the HU Prevent sedation process for MRIs in children, please see Appendix 12.

7.0 IMAGING CENTER AND NEURORADIOLOGY GUIDELINES

Participants with hemoglobin SS or hemoglobin Sβ° 12 to 54 months of age without evidence of overt stroke will be screened for the presence of a qualifying infarct-like lesion using MRI.

The Imaging Center will provide sites with a procedure manual with standardized acquisition guidelines and procedures for handling of imaging data. The Imaging Center will collect images in direct digital form. Each site will obtain de-identified images from their radiology department or use study-provided software to de-identify the image sets. Participant demographic information will be removed and replaced with the study assigned participant identifier and site-assigned 3 letter participant code. These de-identified images will be transmitted to the Imaging Center using a secure HIPPA compliant IRB acceptable method, encrypted VPN connection (for those centers already with this capacity from the SIT Trial, a secured file sharing service, or be sent by express mail on digital media). Sites will complete an image tracking worksheet that matches each DICOM series produced by the MR device to one of the pulse sequences identified in the imaging protocol. The Imaging Center will review and confirm the quality of all images upon receipt are acceptable and will communicate receipt of all incoming data to the clinical center, the Principal Investigator, Vice Chair, Data Coordinating Center, and the Site Investigator. Participant identifier, participant code, study date and study time on the images will be compared with the accompanying forms and imaging worksheet for consistency. The Principal Investigator and Imaging Center will ensure that any confidential information has been masked.

When images are received at the CRL they will undergo secure processing immediately and transfer images onto image review workstation and confirm image quality and data completeness on a diagnostic workstation. The Imaging Center will manage the centralized digital image archive, study database, and back-up storage. The Imaging Center personnel will determine whether images are evaluable for purposes of the study. In the event that a problem with an imaging study is identified, the site will be notified immediately about the nature of any problems and the steps required for corrective action.
The Imaging Center will provide digital images and electronic Case Report Forms (eCRF) to the three-study neuroradiologists (neuroradiology panel members). The neuroradiology panel will perform the evaluation of the images for eligibility screening and analysis of enrolled participants. The presence or absence of cerebral infarcts will be graded according to a 3-point confidence scale. The neuroradiology panel will be blinded to

treatment, participant demographics and clinical history (other than history of sickle cell anemia). They will enter their final evaluations into eCRFs.

The Imaging Center will forward the final imaging evaluation data to the Principal Investigator, Deputy Director, and the Data Coordinating Center weekly. Data concerning participant eligibility and need for referral will be transmitted to the Principal Investigator immediately.

7.1 Imaging Center

A key component of the Imaging Center is the Clinical Research Laboratory (CRL) located at Mallinckrodt Institute of Radiology, Washington University, in St. Louis, MO. The CRL will provide secure office space for the study and has access to all facilities and resources necessary to successfully manage HU Prevent imaging activities. The CRL is configured with new quantitative imaging analysis techniques along with imaging IT systems necessary to support quantitative imaging.. The CRLwill use a commercially available image-processing suite of software programs for secure image activities.

The Imaging Center will customize a procedure manual for its core-imaging laboratory to support the study. The procedure manual will provide the imaging center staff with guidelines for performing quality assurance checks on the received cases and on handling image data. All site investigators will receive written documentation for managing the image-processing technology.

For detailed discussion of Imaging Center process for MRI and TCD protocol, please see Appendices 10 and 11. We will use modifications of the imaging protocols for MRI as the Multicenter Silent Infarct Transfusion Trial (SITT) funded by NINDS; for TCD, we will use the same protocol as the STOP II study funded by NHLBI grant 2UO1 HL52193-061A.

7.2 Definition of an Infarct-Like Lesion

An infarct-like lesion is an MRI signal abnormality visible on two views on the T2 weighted images. The signal abnormality must measure at least 3 mm in one dimension. The lesion must be independently determined to be neurologically silent by the neurology committee. The definition of a silent infarct includes the presence of a silent infarct- like lesion based, on the review of two of the three study neuroradiologists and a normal neurologic examination or an abnormality on neurologic exam that cannot be explained by the location of the MRI lesion. This definition of silent cerebral infarct is operationally the same as that used in the St. Louis Children's Hospital/Washington University School of Medicine Stroke Study Group,[46] the SIT Trial, as well as the definition used in multiple Cooperative Study of Sickle Cell Disease (CSSCD) studies.

7.3 Interpretation of MRI Studies

A panel consisting of three neuroradiologists (Robert C. McKinstry, III, MD, PhD, Washington University School of Medicine, Michael Kraut, MD, PhD, Johns Hopkins University School of Medicine, and Dennis Shaw, MD, Seattle Children's Hospital) will perform the evaluation of images for both eligibility screening and analysis of imaging from enrolled participants, including those with a suspected or confirmed event.

The neuroradiologist in collaboration with the Imaging Center will develop a reference set showing examples of the various diagnostic criteria used in interpretation prior to the start of the study. The neuroradiologists will be responsible for both a qualitative and quantitative assessment of the images. The qualitative assessment will be based on the presence or absence of new cerebral infarcts and will be on a 3-point confidence scale (1=definitely not present, 2=indeterminate, 3=definitely present). This is the same definition that was used by the CSSCD demonstrating a 24% increase in the incidence of silent cerebral infarcts in children with silent cerebral infarcts over 4.8 years. [47] Each neuroradiologist will independently assess and record the presence of a silent cerebral infarct like lesion(s). When there is complete agreement, there is no need for a discussion. When there is discordance a consensus call will be held and a majority vote will decide the status of the participant as to the presence or absence of a silent infarct like lesion. The final decision will be based on a consensus. Quarterly (calendar year) monitoring comparing consensus decision with the independent review will be completed by Data Coordinating Center.

All MRI examinations will be assessed for presence of an abnormality, including presence of infarction, infarct locations, number of infarcts, size in millimeters and significant coincidental findings. The quantitative assessment of the screening MRI by the neuroradiologists will consist of independent measurement of the greatest linear dimension of each infarct-like lesion. At the time of the consensus call, the neuroradiologists will select by consensus the best measurement of the three for posting final data to the database.

Baseline MRI images will be compared side-to-side to all subsequent MRI images, to establish if there has been new cerebral infarction. All time-points for enrolled participants will be read by all three reviewers.

7.4 Quality Assurance for MRI Examination

Prior to commencement of screening MRIs, each neuroradiologist will participate in a screening training and testing program developed by the Imaging Center. The image analysis quality assurance program includes two components: 1) initial training; 2) inter-observer variability testing. Each reader will then independently evaluate a second set of 10 MRI scans in consensus fashion. Inter-observer variability will be assessed and the degree of variability must not exceed a predefined threshold. The Principal Investigator and Chair of the Neuroradiology Committee will agree upon the exact parameters to be assessed and the threshold to be set. If the readers exceed the threshold, then re-training will be required.

7.5 Image Archiving

The Imaging Center will maintain a centralized image archive that will contain every image received from the clinical investigators for the clinical trial. The Imaging Center will provide images that may be used for scientific presentations or publications. Measurements are also stored so that this data may be audited, if necessary. All study data, as well as supporting documentation and administrative records, will be retained for a period of time mutually agreed upon.

7.6 Security of Data

All imaging data will be maintained in a secure environment. Incremental back-ups of the study database will be performed on a nightly basis. Complete back-ups are performed on a weekly basis. Duplicate system back-up tapes will be stored in a secure, off-site facility. Access to the system will be controlled by password protection.

7.7 Masking Procedures

After each film is read according to the prescribed protocol outlined above, each radiologist will record the findings on a CRF. Each radiologist will be unaware of the results of clinical history (other than history of sickle cell anemia), neurological examination and other imaging studies (such as CT or TCD). A side-by-side comparison of the pre-randomization MRI will be made to the exit MRI examination.

7.8 Reinsertion Studies

To monitor assessment of the MRI readings, 10% of previously read studies will be reinserted randomly, to assess the intra-observer and inter-observer reliability and insure fidelity of the SCI endpoint.

8.0 Transcranial Doppler

TCDs may be interpreted locally by radiology or another appropriately credentialed clinical service, in addition to a study interpretation by the staff of the central reading facility. TCDs will be sent by express mail on digital media to the Medical University of South Carolina (MUSC) for quality control and central interpretation by the MUSC Neurology Core. Dr. Robert Adams, Professor of Neurology at the Medical University of South Carolina, is a long-standing consultant to our group and directs the Neurology Core. Dr. Adams will provide training using standardized protocols to assure the validity and reliability of measurements obtained by both imaging and non-imaging TCD at participating sites. Imaging personnel at each site will participate in a screening training and testing program developed by the TCD Imaging Center for non-imaging TCD assessment (described in detail in Appendix 11). The image analysis quality assurance program includes two components: 1) initial training on–site or via videoconference/Webex; and 2) intra-observer variability

testing. Each research study reader will independently evaluate a set of three readings in the same individual within a 120 minute interval. Intra-observer variability will be assessed and the degree of variability must not exceed a predefined threshold. The Principal Investigator and Imaging Center Director (Dr. Adams) will agree upon the exact parameters to be assessed and the threshold to be set. If the readers exceed the threshold, then re-training will be required.

Dr. Adams and his team will evaluate images from both eligibility screening and from enrolled participants, including those with a suspected or confirmed primary endpoint, that is, TCD measurement above a pre-defined threshold (170 cm/sec for non-imaging TCD). He will use a reference set showing examples of the various diagnostic criteria developed prior to the start of the study. The MUSC Neurology Core will be responsible for both a qualitative and quantitative assessment of the images. The qualitative assessment will be based on the presence or absence of TCD measurement reaching the endpoint of the trial (1=definitely not, 2=indeterminate, 3=definitely present). Dr. Adams will independently assess and record whether a TCD endpoint has been reached.

8.1 Quality Assurance and Reproducibility

Each center will meet quality assurance in one of two ways. Centers that participated in STOP or have certified-STOP, STOPII, SWiTCH or TWiTCH TCD technicians will automatically meet quality assurance, after one adequate exam is submitted and reviewed. All other sites must send in at least three TCD evaluations for quality assessment by Dr. Robert Adams. The practice evaluation will be assessed for quality and the technique of acquiring a time-averaged mean maximum velocity.

8.2 Timing of TCD

Usually the TCD will be obtained at the first study visit. Participants with a conditional or abnormal TCD (CBFV$\geq$ 170 cm/sec by non-imaging TCD) will need to have a second TCD with an average velocity <170 cm/sec to be eligible to continue in the study. Performing TCD and all other screening procedures before the MRI assures that participants who do not qualify on the basis of other measures will not be exposed to sedation unnecessarily.

9.0 PSYCHOLOGICAL EVALUATION

9.1 Cognitive Assessment Tools

Bayley Scales of Infant and Toddler Development (Bayley-III)
The Bayley-III provides a comprehensive assessment of early cognitive development in children too young for the measurement of IQ using the Wechsler Preschool and Primary Scale of Intelligence (WPPSI-III). It includes a motor, mental, and behavior rating scale. We will use the Bayley-III [except for the Adaptive Behavior Subtest, as this will be replaced by the Adaptive Behavior Assessment System (ABAS-II)] for children 9 through 29 months of age.

The Wechsler Preschool and Primary Scale of Intelligence-III (WPPSI-III)
The Wechsler Scales are the most widely recognized and administered tools for the assessment of IQ. For children who are 2 years and 6 months up to 3 years 11 months of age, we will administer the Receptive Vocabulary, Block Design, Information, and Object Assembly core subtests of the WPPSI-III. If the participant is less than 3 years and 6 months old and raw scores of 0 are obtained for the first two subtests, the examiner should stop administering the WPPSI-III and administer the Bayley-III. For children 4 to 7 years and 4 months of age, we will administer the Block Design, Information, Matrix Reasoning, Vocabulary, Picture Concepts, Word Reasoning, and Coding core subtests. For children that initially received the Bayley-III, we will administer the WPPSI-III, if the child is 3 years and 6 months of age or older. These core subtests take 30 to 45 minutes in children 2 years and 6 months up to 4 years of age and 45 to 60 minutes in children 4 to 7 years and 3 months of age.

9.2 Behavior Ratings

Adaptive Behavior Assessment System (ABAS-II)

The ABAS-II will be used assess behavior in all children at baseline and, for those randomized, at the annual and exit visit regardless of age. Children up to age 5 years and 11 months will be administered the Infant and Preschool ABAS-II including the motor scale; children 6 years or older will be administered the School ABAS. This system elicits parents' rating of behavior through a questionnaire.

Behavior Rating Inventory of Executive Function Preschool Version (BRIEF-P)
The BRIEF-P (for children 2 to 5.99 years of age) and BRIEF (for children 6 years of older) are sensitive to cognitive changes in children with neurologic decline.[48] For example, the BRIEF-P has recently been demonstrated to be sensitive and specific for reduced inhibition and working memory in children. The BRIEF assesses impairment of executive function in children age 5 through 18 years.[49] For these reasons, the BRIEF-P or BRIEF will be administered at baseline (in children at least 2 years old) and every year and at exit for randomized participants in the study.

Table 2: Cognitive and Behavioral Testing

9 mo - 29 mo	2 yr 6 mo - 3 yr 11 mo
Bayley-III ABAS-II Infant and Preschool BRIEF-P (if ≥ 24 mo)	WPPSI-III (younger) Receptive Vocabulary Block Design Information Object Assembly ABAS-II Infant and Preschool BRIEF-P
4 yr - 5 yr 11 mo	**≥ 6 yr**
WPPSI-III (older) Block Design Information Matrix Reasoning Vocabulary Picture Concepts Word Reasoning Coding ABAS-II Infant and Preschool BRIEF-P	WPPSI-III (older) Block Design Information Matrix Reasoning Vocabulary Picture Concepts Word Reasoning Coding ABAS-II School BRIEF

10.0 NEUROLOGIC EVALUATION

A standardized neurological examination based on the Pediatric Stroke Outcome Measure (PSOM) will be performed by a pediatric neurologist during visit one on all participants who have a TCD below the transfusion threshold, as well as on an annual basis and upon exiting the study. The site may choose to complete the neurological assessment before a TCD. The standardized examination is also completed at months 12, 24, and at study exit. The standardized examination and NIH Stroke Scale will also be carried out after any episode in which neurological symptoms occur. The neurological examination has been standardized, in order to minimize variability that could be created by having multiple examiners at various sites. For participants experiencing an event suspicious for overt stroke or any episode in which neurological symptoms occur, results of their neurologic examination at the time of the acute event will be scored using the NIH Stroke Scale.

The neurological examination, including level of consciousness, cranial nerve testing, tone, coordination, and sensation (light touch, pin prick, vibration, and proprioception) is scored as being normal or abnormal. Strength, proximal and distal in each extremity, is assessed on the Medical Research Council scale, and tendon reflexes are expressed on a 0-4 grading system. Age appropriate cognitive skills are evaluated using naming, comprehension, repetition, and orientation. Children able to read and write will be asked to read and write standardized words and/or sentences; each of these two subtests contains items with an increasing level of difficulty. Finally, several age-specific tasks assessing overall function will be used, including copying of geometric designs, walking on tiptoes, and various aspects of gait.

The evaluation of an event with neurological symptoms will determine the onset and duration of the event, as well as pertinent information about the symptoms. Specifically, the neurological event form contains questions to determine whether there was an altered level of consciousness, headache, hemiparesis/weakness, change in vision, alteration in speech, clumsiness, sensory disturbance, or possible seizure. If the answer to any of these questions is affirmative, details must be provided. At the conclusion of the history and neurological examination in participants with neurological symptoms, the examining pediatric neurologist must make a determination as to the probability that the event was a stroke. The response of the pediatric neurologist is indicated by a 5-point scale ranging from ‘definitely yes’ to ‘definitely no’. The final decision to categorize a

neurological event as a stroke will be made by three members of the adverseadverse committee. The neurology committee is comprised of Lori Jordan, MD, PhD – Vanderbilt University, Rebecca N. Ichord, MD - Children's Hospital of Philadelphia and Michael Noetzel, MD, Washington University School of Medicine.

For participants with an overt stroke as judged by the treating physicians and the site neurologist, the study neurologist will perform the same neurologic examination as the annual monitoring examination. In the course of performing this examination, the participant's deficits will be graded using the PSOM and NIH Stroke Scale.[50] Daily neurologic examination using the standardized protocol will be performed through the first 3 days, or longer as needed, until the severity of the deficit is maximal, at which time the NIH Stroke Scale will be repeated. Follow-up neurologic examination will be performed using the standard protocol and grading on the NIH Stroke Scale 6 months and 12 months after the event, and upon exit from the trial.

10.1 Definition of Transient Ischemic Attacks and Stroke

Table 2 provides the study definition of TIA and stroke for participants presenting with new neurologic findings.

Table 3 Definition of transient ischemic attack and stroke

< 24 hours Neurological Deficit*	< 24 hours Neurological Deficit*	> 24 hours Neurological Deficit*	> 24 hours Neurological Deficit*
Negative MRI	Positive MRI (new vascular lesion which could explain deficit)	Positive MRI (new vascular lesion which could explain deficit)	Negative MRI or Positive MRI and vascular lesion does not explain a deficit
Diagnosis = Transient ischemic attack (TIA)	Diagnosis = Clinical Stroke	Diagnosis = Clinical Stroke	Diagnosis = Clinical Stroke

*The influence of blood transfusion on acute symptoms of stroke has not been well defined. Transfusion therapy may decrease the persistence of neurological findings. Neurologic deficit in this setting means an abnormality consistent with a stroke.

10.2 Determination of Stroke

The HU Prevent protocol has been designed to identify all intercurrent neurological events, either acute, or subacute, that could be consistent with an overt stroke. Information about the signs and symptoms of stroke will be given to all parents/participants who are screened and reinforced with those who are randomized. In addition, family and participants will be instructed to report any significant neurological symptoms. This should minimize any delay in reporting. We anticipate that the majority of participants with neurologic events will be evaluated in close proximity to the event by the local neurological consultant at the individual study site. Participants with acute neurologic events suspicious for a stroke must receive an immediate MRI, preferably following the HU Prevent imaging protocol and including diffusion weighted sequences (within 24 hours of the neurologic event) as part of standard care, as participants may be candidates for erythrocytapheresis or manual exchange transfusion. In addition, the participant should be examined by the study neurologist in close proximity to the MRI, and preferably prior to the decision to proceed with erythrocytapheresis or manual exchange. If the study neurologist is unavailable, every effort should be made by the site to have the neurologist providing clinical care document the neurologic exam and NIH Stroke Scale before performing the transfusion. Historical information and the neurological examination documented in the neurological event form will then be reviewed by the neurology committee consisting of three pediatric neurologists, each of whom has experience with strokes in participants with sickle cell anemia. In addition to the neurological event form, the neurology committee will review all current and prior neurological consultation forms, a copy of any interim progress forms, the intake history form, and all current and prior HU Prevent consensus radiology reports. Any information alluding to the control or treatment status of the participant under consideration will be deleted from data sent to the neurological committee members by the Data Coordinating Center.

Three neurologists will individually review all of the materials and make a determination, based on the data, as to whether the neurological event in question was an overt stroke. When there is complete agreement, there is no need for a discussion. When there is discordance, a consensus call will be held and a majority vote will

decide the status of the participant as to the presence or absence of a stroke. It is anticipated that a final determination of whether a neurological event was stroke will be completed within 4 weeks of the time of the initial report of the neurological event. In addition, the neuroradiology committee will review the event MRI to ascertain whether or not there are new infarct-like lesions following the neurological event. The neuroradiologists will compare all subsequent MRI images to the initial baseline image and will record their findings on case report forms. A neuroradiology review will be forwarded to all members of the neurology committee and will be included as part of the material, to develop a consensus opinion determining whether the neurological event was a stroke.

10.3 Clinical Events or Conditions that Should Trigger an Event-Related Unscheduled Neurologic Assessment

Following randomization, if the participant experiences any of the following situations, the site neurologist should examine the participant as soon as possible, preferably within 24 hours of the suspected event. The site neurologist or their designee should complete the Standardized Neurology Examination CRF 10 for participants <2 years old or CRF 11 for participants ≥2 years old for a clinical event or condition that triggers an event-related, unscheduled neurologic assessment. The forms should be completed and sent to statistical center within one week.

Suspected events should include, but not be limited to:

- Acute neurologic deficit such as confusion, loss of consciousness, hemiparesis, loss of or slurring of speech, visual loss, sensory loss, ataxic gait that has no obvious explanation.
- Any change in neurological function lasting more than 24 hours, even with another explanation.
- Seizure
- Headache with drowsiness or altered level of consciousness, unless there is a history of migraines with similar manifestations.
- New-onset headaches or increasing severity or frequency (days to weeks) of headaches
- Decline or regression of a major life function (school performance, activities of daily living, social relationships, communication) evolving over a short time interval (days to weeks), without clear explanation.

The above-described guidelines should serve to augment the site neurologist's clinical acumen and not substitute for clinical judgment.

In addition, the site PI and study leadership (Chair or vice-Chair) should be notified as soon as possible, preferably within 24 hours, if:

- If the child is suspected by the parent to have an acute neurologic event or change in neurological or functional status, and is brought to the study site for clinical care.
- The child is suspected by the parent to have an acute neurologic event or change in neurological or functional status, and is brought to a facility other than the study site for clinical care.
    - The family or treating physicians at this outside facility notify the site staff that this event has occurred concurrent with assessment and treatment.
    - The family or treating physicians at this outside facility do not notify the site staff that this event has occurred, or only after significant delay (> 1 week).
- The study coordinator, site investigator or any other study personnel learns about a possible acute neurologic event or change in neurological or functional status during the monthly screen; or the study neurologist learns about a possible neurologic event or change in neurological or functional status during the annual screen.
- The child develops an acute neurologic event or change in neurological or functional status, while being treated for a non-neurologic illness.

Parents/guardians will be given a card that they carry with them at all times providing instructions directed to any health care provider who sees the participant for an acute, unscheduled medical care visit, indicating that the provider should contact the on-call hematologist of the study site.

The parents/guardians will be instructed to call the study coordinator immediately, to notify her/him of any instance that they sought medical attention for an acute (unscheduled) medical problem.

For clinical events considered suspicious for an overt stroke or TIA, as described in the list above, a neurological evaluation and an MRI of the brain following the HU Prevent imaging protocol should be completed within 24 hours after consultation with the pediatric site neurologist and site principal investigator.

Participants with acute neurological events suspicious for a stroke (definitely Yes, Probably Yes or Unclear, in answer to question 32 on HU Prevent CRF10) should then have the NIH Pediatric Stroke Scale Summary completed and they must receive an immediate MRI, preferably following the HU Prevent imaging protocol and including diffusion weighted sequences and MRV (within 24 hours of the neurological event), as part of standard care, as participants may be candidates for erythrocytapheresis or manual exchange transfusion. A follow up MRI, 30-60 days after the event is strongly recommended, including MRA (if not done acutely to assess the evaluation of vasculopathy).

If overt strokes or new or progressive silent cerebral infarcts are identified, then the participant would be classified as meeting the primary outcome measure of the study and a repeat MRI will be done annually as part of standard care. We will continue to track the course of the participant throughout the trial, and ask that they continue to receive the scheduled cognitive and behavioral assessment. In the event of an overt stroke, the participant will be offered blood transfusion therapy.

If no overt stroke diagnosis is made or no silent cerebral infarct is identified, the participant will resume their randomly allocated arm and time point in the study.

10.4 Transient Ischemic Attacks

In some participants with acute neurological events, a diagnosis of a TIA may be made (Question 32A on CRF 10). At present there is limited evidence to direct subsequent treatment decisions for these individuals. The most informative data can be found in:

1) Ohene-Fempong, et. al.'s report on Cooperative Study of Sickle Cell Disease data in *Blood* 1998; 91: 288-94. Prior TIA was one of five risk factors for infarctive stroke, including recent acute chest syndrome and elevated systolic blood pressure; however, silent infarction was not measured, the study included many adult participants and the relative risk of TIA was assessed only in the entire cohort; young participants were not evaluated separately.
2) Miller et. al. and the Cooperative Study of Sickle Cell Disease in *J Pediatr* 2001;1*39:38*5-90. Again, using overlapping data to #1, prior TIA strongly was associated with subsequent stroke; however, all stoke participants with TIA also had a silent infarct. In a multivariate analysis, silent stroke and not TIA was associated with an increase rate of overt stroke.
3) AHA/ASA Guidelines (*Stroke* 2006;37:577-617) recommend (on page 600) *that "For* adults with SCD and ischemic stroke or ***TIA*** (emphasis added) …Additional therapies that may be considered include regular blood transfusion to reduce hemoglobin S…" (Class IIb, level of evidence C); however, these guidelines should be considered expert opinion, because TIA is mentioned only in the recommendation and not in the data analysis.

Given that the majority of individuals with SCD presenting with a focal neurological deficit have a MRI of the brain, the decision to perform an exchange transfusion is often based on the results of an acute diffusion weighted image (DWI) MRI and the focal neurological examination; however, in the setting of focal neurological deficit, explicit care must be taken to ensure that there is not over reliance on a negative MRI (absence of an

abnormality on diffuse weighted imaging) to forgo exchange transfusion. Several studies have documented that patients with focal neurological deficits may have a negative MRI-DWI scan, specifically negative diffusion weighted images within 24 hours of the onset of symptoms.[51,52] Such patients may be referred to as having a TIA; however, a proportion of patients that present with a focal neurological deficit and negative MRI, may have repeat MRI of the brain 30 days later revealing the lesion corresponding to the focal deficit.[52] After exclusion of stroke mimics associated with a negative MRI of the brain, such as migraine headache or a seizure that was not witnessed, the possibility of a stroke still exists. Ultimately the diagnosis of an acute overt stroke and the decision to perform a timely exchange transfusion is a multi-disciplinary bedside decision, requiring input from the neurologist, neuroradiologist and hematologist. Figure 3 describes a schematic of the neuroimaging studies and the differential diagnosis of focal neurological deficits.

Figure 3: Evaluation of a Focal Neurological Deficit
Note: MRI or CT may be the first study done, depending the ease and speed with which each study may be done at each institution. MRI is required for complete evaluation when available. A CT of the brain is not required, depending on the availability and results of the MRI.

DVST indicates dural venous sinus thrombosis; PRES, posterior reversible encephalopathy syndrome

The threshold to mandate an initial exchange transfusion in the setting of an acute focal neurological deficit should be low, taking into account the data noted above; however, the threshold to continue exchange transfusion beyond the initial transfusion therapy should be high, if the follow up 30 day MRI is negative, does not reveal any infarct and the patient remains asymptomatic. There may be circumstances where the site PI and neurologist are compelled to treat a patient on study treatment with chronic blood transfusion therapy. We recommend that a study participant with multiple discrete TIAs involving the same vascular should be considered for chronic blood transfusion therapy. Such patients by definition will not have any evidence of a cerebral infarct on MRI, since a new lesion is an endpoint to the HU Prevent Trial. We also would recommend that a repeat MRI be obtained after the start of chronic blood transfusion, approximately 30 to 60 days after the precipitating event. This MRI should be considered standard care to assess the presence of the new lesion associated with the TIA. Finally, if the decision is made to place a child on **chronic** transfusion, then it is imperative to discuss this decision with the study leadership. The use of hydroxyurea has not been shown to be effective in preventing TIAs; the decision to begin this therapy for neurologic symptoms should always be discussed beforehand with study leadership.

If the participant receives a diagnosis of an overt stroke during the course of the trial, we request blood samples for the biologic repository be obtained. Timing of these blood draws will vary, based on participant circumstances. In some cases, it may be feasible to draw daily samples for the first several days; for hospitalized participants, we would then draw samples every 2-3 days, and then weekly for up to 6 weeks, if feasible. A typical schedule might be samples at time 0, 3, 7, and 14 days, but more frequent sampling would be done, if possible.

If the participant receives a diagnosis of an overt stroke during the course of the trial, evaluation per the institution's standard of care is recommended

11.0 SAMPLE SIZE AND STATISTICAL ANALYSIS

11.1 Primary outcome variable

The primary endpoint for the pilot and definitive phase III trial will be prevention of abnormal CBFV, SCI, TIA and stroke.

11.2 Secondary outcome variables

Objective 1:

The secondary outcome variables will be the proportion of screened participants accepting randomization and the proportion of randomized participants with >80% adherence.

Objective 2:

Secondary outcomes will be the proportion of children with: 1) serious adverse events 2) serious adverse events attributed to the study intervention or study procedures.

Objective 3:

The secondary outcomes will be the levels of GFAP, thrombospondin, L-selectin, and apolipoprotein A1 in children with and without SCI. We will also collect plasma specimens to evaluate putative biomarkers of CNS injury as a surrogate outcome of hydroxyurea efficacy in an ongoing, proteomics discovery project.

Objective 4:

Complete the necessary preparations for a definitive phase III trial. During the course of the current study, we will also prepare a manual of operations and case report forms for the proposed trial, develop sample IRB submission templates and organize all committees, collaborators and study procedures necessary for initiation of a successful, definitive, multicenter trial.

11.3 Randomization scheme.

Randomization will be performed using an adaptive randomization scheme, with stratification for age (< 30 months vs. ≥ 30 months) and CBFV <155 vs. 155-169 cm/sec by non-imaging TCD. The randomization will be performed based on information entered by the coordinator using a web-based application, with the randomization code provided to the research pharmacist. Siblings (adoptive or biological) will be randomized to the same treatment to avoid having the participants and their parents compare study treatments or have anxiety related to possible differential treatment. Although this will decrease the independence of the observations, the statistical effect on the study is anticipated to be small and this could enhance recruitment efficiency.

11.4 Statistical Plan

Primary outcome variable: Data analysis will include point estimates and 95% confidence intervals using exact methods of the proportion achieving the primary endpoint for the planned definitive phase III trial (a composite of SCI, CBFV ≥170 cm/sec, TIA or overt stroke, based on MRI, non-imaging TCD, and neurology exam) in each group.

11.5 Secondary outcomes

Objective 1

The proportion of participants accepting randomization. We expect that ~30% will agree to be screened, approximately 80% of screened participants will be eligible and at least 63% will agree to be randomly allocated. If ≤ 40 of the anticipated eligible 80 SCI negative participants screened at all centers are unwilling to undergo random assignment, then our previous estimates of participation in clinical trials may need to be adjusted for the large multicenter setting. For cognitive measures, repeated measures analysis of variance will be used to assess change from baseline between the two treatment groups. In addition to using statistical techniques to evaluate declines in cognitive measures, clinical significance will be evaluated using scores based on normative data. These results will be used to refine the sample size of the definitive Phase III trial.

Objective 2

To determine the safety of study procedures. We will calculate proportions and 95% confidence intervals of participants having severe adverse events using exact methods, as well as the rate of adverse events. We expect the proportion and rate of serious adverse events to be similar in the two groups.

Objective 3

Evaluation of Biomarkers of CNS injury. We will compare the levels of GFAP, thrombospondin, and L-selectin in children with and without SCI by student's t-test or after transformation or with the Wilcoxon rank-sum test, if not normally distributed. These results will provide the necessary preliminary data on the mean and variation of these values in toddlers and preschool children with SCA, to estimate the necessary sample size of biomarker sub-studies of the planned Phase III study.

11.6 Interim Data Analysis and Early Stopping Rules

We will also continuously monitor the safety of study procedures, with reporting to the IRB after every 10 sedated MRIs and planned review by the DSMB after 10, 20, 40, 80, and 120 MRIs (this includes screening and followup MRIs); If the number of SAEs within 72 hours of sedation exceeds the number in Table 3, then the true proportion of SAEs is likely ≥4%; at this point, the MRIs with sedation would be halted temporarily, and we would reconsider the sedation protocol, with DSMB review. The thresholds were determined by finding the 95% confidence interval (one-sided) for the rate of SAEs that did not contain 4%, adjusting for multiple comparisons to give a 5% error rate. We will use univariable and multivariable logistic regression to identify potential risk factors for SCI as exploratory analyses, as the next phase of study will consider adaptive randomization to balance these factors (age, Hb concentration, and/or HbF) between treatment arms. A DSMB will be formed prior to the trial and will include a hematologist with expertise in SCD, a pediatric anesthesiologist, and a biostatistician.

Table 3: Number of Severe Adverse Events to Exclude a Proportion <4% with 95% Confidence

Severe Adverse Events	3	4	6	9	11
MRIs	10	20	40	80	120

In addition, data on the incidence of SAEs in the hydroxyurea and placebo arm will be reported to the DSMB at interim blinded analyses every 6 months after the first 10 participant-years of follow-up has been completed, or at the discretion of the DSMB. If there is a significantly greater proportion of SAEs in one arm (using $p < 0.01$ to account for the multiple comparisons, the decision to halt the trial will be at the discretion of the DSMB.

The DSMB will also review the efficacy outcomes every 6 months or at their discretion, after the first 10, but we do not expect this small study to identify a significant difference between groups. Given the small size for the internal pilot, we will not assess efficacy: therefore, we will not be able to determine futility for the primary endpoint. We do not plan scheduled interim analyses for the primary endpoint other than the DSMB's review.

12.0 REGISTRATION, STUDY MONITORING AND STUDY ORGANIZATION

12.1 Consent Form

The parent/legal guardian of each potential participant will be given consent forms to read and sign prior to the participant's entry into the screening and randomization and treatment phases, if eligible. These forms will contain a description of the objectives of the trial, a description of the examinations and tests which will be given to the participant as each part of the trial, as well as expectations of the participant as a trial participant. This consent forms will be given to the participant's parent or legal guardian by a member of the clinical center staff, who will act as a witness and sign the form below the signature of the parent or legal guardian. All local consent forms will need to be approved by the clinical coordinating center for essential content.

12.2 Participant Registration

Participants will be entered into this clinical trial during the 12 months of active screening at each site. Sites with low accrual after 6 months will have a conference call or site visit by a member of the Executive Committee to aid in the identification of barriers to recruitment and to develop solutions to overcome these barriers. If enrollment remains low, the site may be removed from the study, based on a decision by the study leadership. Enrollment may be extended for 6 months or longer for institutions demonstrating low, but improving, accrual. All participants will be entered and data captured via a web-based data system operated and maintained by the Data Coordinating Center. The studies are to be performed at the participating institutions and centrally reviewed by the respective subcommittees (Neurology, Neuroradiology, and Psychology) or at the Imaging Center.

12.3 Study Organization

The HU Prevent Study will have nine organizational components: (1) 2 initial participating Clinical Sites, pediatric hematology facilities in the United States (with additional sites to be added) (2) Clinical Coordinating Center, (3) Data Coordinating Center, (4) Operations Committee, (5) Executive Committee (6) Neurology Committee, (7) Psychology Committee, (8) Neuroradiology Committee, (9) Data and Safety Monitoring Board.

The Chairs of the Clinical Coordinating Center, Data Coordinating Center, and the Biologic Repository will be located at Johns Hopkins University School of Medicine, Baltimore, MD.

The Chairs of the Neuroradiology, Sedation and Psychology Committees will be located at Washington University School of Medicine in St. Louis, MO.

The Chair of the Neurology Committee will be located at Vanderbilt University, Nashville, TN.

Clinical sites were selected on the basis of their clinical expertise, their past performance in clinical studies involving sickle cell disease, their management and treatment of stroke in patients with sickle cell disease, the numbers of pediatric patients with sickle cell disease treated at their institutions annually, and the presence of pre-existing relationships of working with Drs. Casella and other members of study leadership based on collaborative projects addressing questions regarding the epidemiology and treatment of strokes in sickle cell disease.

12.4 DATA MONITORING AND REVIEW COMMITTEE

Independent local study monitors will be responsible for reviewing lab results and informing the Data Coordinating Center and research team at their center about holds for toxicity or dose escalation. To maintain blinding, simulated dose escalation and holds for toxicity by the central study monitor will occur for the placebo arm, as in previous hydroxyurea trials.[53] More detailed discussion of the study monitors responsibilities and interactions are described in Appendix 20. Adverse events will be recorded at study visits every 4 weeks, using a structured form for common side effects of hydroxyurea and complications of SCD and classified based on seriousness, expectedness, and relatedness by physicians not otherwise involved in this study.[54] Unexpected severe adverse events that are related to study medication or procedures will be reported to the DSMB, FDA, and IRB within 7 calendar days, if fatal or life-threatening, and within 15 calendar days, if not, of the investigators' knowledge of them. All SAE (expected and unexpected) will be provided to the DSMB by the DCC quarterly. All AE (serious and non-serious) will be reported to the DSMB every six months, or at the discretion of the DSMB.

Members of the independent Data and Safety Monitoring Board (DSMB) will be selected by the PIs of the study, subject to NHLBI approval. The DSMB will monitor accruing data in order to confirm that the participants in the trial are being cared for safely. The DSMB will be responsible for:

1. Reviewing and approving the study protocol
2. Reviewing and analyzing the progress of the study;
3. Approving amendments to the trial protocol, if warranted;
4. Monitoring the safety of the study procedures and treatments;
5. Reviewing data quality;
6. Reviewing interim analyses and recommending early stopping or continuation of the trial;
7. Reviewing recruitment, crossover rates, and event rates

The Clinical Coordinating Center and Statistical and Data Coordinating Center will provide information to this committee as requested and every 6 months for all AEs. The DSMB will review study data reports, including primary end point analysis, every six months, either in a meeting or on a conference call. They will review by conference call within 2 weeks of obtaining the required number of studies the severe adverse events related to sedation for MRIs, following the schedule in Section 11.6 .

13.0 Human Subjects Considerations

13.1 General issues of design

Blinding, and justification.
This study will be blinded with a placebo control to minimize cross-overs and bias in the classification of outcomes and adverse effects secondary to hydroxyurea.

Justification for inclusion of a placebo or non-treatment group.
A placebo or non-treatment group is essential to have an appropriate comparison group, as there may be unknown confounders that could not be adjusted for during analysis. Randomization allocation to treatment with hydroxyurea or placebo will minimize the probability of bias.

Procedures regarding treatment for participants when the study ends or if a participant's participation in the study ends prematurely.
Participants will be able to continue or discontinue hydroxyurea after their participation in the study ends at the discretion of their primary hematologist and parents or guardians, as this medication is in clinical use for children with SCD. The study leadership will facilitate their access to hydroxyurea.

Sources of Materials:
Information concerning the child's medical, family, and developmental history will be obtained by interview of the parent or guardian and review of the medical record. Behavioral questionnaires and cognitive testing will be completed for research purposes. Vital signs and other elements of the physical exam, laboratory and imaging studies (MRI of the brain and TCD ultrasound) will be collected and used for research purposes. Some of these results will be released to the participants or their parents and shared with their doctors or schools (but only with the consent of the parent or guardian). Some of this information (history and physical exams from study visits, clinical interpretations of TCDs and MRIs and routine laboratory tests) will be included in the medical record of the participant and therefore directly linked by name and medical record number. Information collected specifically for this research project (cognitive testing, behavioral questionnaires, biorepository specimens) will be directly linked to the study ID. Only the necessary research personnel will have access to the list linking the study ID to personal identifiers of the participant.

13.2 Potential Risks

TCD: There are no known significant risks to TCD testing in children. Children may be uncomfortable having to hold still or have anxiety about the results.

Phlebotomy: At various visits from 2 to 10 ml of blood will be drawn through a peripheral vein for tests and storage in a biological repository. Phlebotomy is often associated with mild pain and occasionally with a small bruise at the site. Rarely, participants feel lightheaded or have syncope. There is also a very small risk of infection at the site of the needle puncture. The maximum amount removed is 1.5% of the blood volume for an 8.6 kg child with SCD (5^{th} percentile of weight for a 1 year old), and has no additional risks, even in an anemic child.

History and physical exam: Some children will become uncomfortable or anxious during the questions or examination.

Neuropsychological tests: Children may occasionally experience some mild anxiety during testing. The results of tests may affect school placement, but the results are only released by parental request. A relaxed environment with frequent breaks will minimize anxiety.

MRI/MRA of the brain: Potential risks due to MR imaging include (1) claustrophobia, (2) movement of paramagnetic materials, (3) identification of an incidental finding. Claustrophobia in the MRI scanner is uncommon in children. Movement of paramagnetic material (either within the participant or in the scanner

room) is rare secondary to the precautions decribed below. All intracranial incidental findings (identified in approximately 5% of children with SCD) will be discussed with the parent of guardian and additional testing may be recommended. Some children are scared by the noise or being alone in the scanner.

Sedation: Potential complications of sedation include respiratory depression leading to hypoxemia and requiring additional support, including oral airway, nasopharygeal trumpet, laryngeal mask or endotracheal intubation to maintain the airway, positive pressure ventilation by mask, or a ventilator. Rarely these complications can include respiratory or cardiac arrest and even death. Sedation may also lead to delayed complications of SCD in the next 48 hours, including acute chest syndrome, pain, or very rarely stroke. Concern has been raised over a possible increased risk of developmental delay after anesthesia, based on animal studies with prolonged high dose exposure and case-control studies of young children undergoing anesthesia. The significance of these findings is controversial and under active investigation in controlled trials. Sedation will require placement of a peripheral intravenous catheter (PIV). This is often associated with mild pain and occasionally with a small bruise at the site or phlebitis. Rarely, participants feel lightheaded or have syncope. There is also a very small risk of infection. Adverse effects related to the medications used for sedation are listed below.

Midazolam: This agent is given prior to sedation to reduce anxiety and to cause amnesia. Common adverse effects include hypoventilation, short periods of apnea, and somnolence. Uncommon adverse effects are nausea, vomiting, hiccoughs, involuntary movements, and headache. Rare adverse effects include respiratory arrest and hypotension.

Propofol: This is a frequently used agent for deep sedation for procedures Common adverse effects include hypotension, short periods of apnea, and pain at the infusion site. Uncommon adverse effects are myoclonus and priapism, and rare adverse effects include dystonia.

Lidocaine: This medication is often administered before the propofol to reduce pain. Adverse effects include nausea (common), drowsiness, labile mood, tinnitus, dizziness, vision changes, tremors, numbness, headache, backache (uncommon), and fever, tachycardia or bradycardia, respiratory distress, seizures, or chest pain (rare).

Sevoflurane: This medication will be used to induce sedation in children that cannot have a PIV placed easily while awake. Common adverse effects are nausea, crying, confusion, hiccups, and hypersalivation. Uncommon adverse effects are emesis, dry mouth, pruritus, rash, fever, and temporary disconjugate gaze. Rare adverse effects include arrhythmias, agitation, difficulty breathing, hypotension, fatigue, or shivering.

Nitrous oxide: This inhalation agent is commonly used for short painful procedures. Common adverse effects include headache, dizziness, confusion, and hypotension and uncommon adverse effects are nausea, emesis, increased middle ear pressure, and apnea. Rare adverse events include elevated transaminases and increased intracranial pressure.

Hydroxyurea: Hydroxyurea has been in clinical use for over 30 years and has several well-recognized potential risks, including reversible bone marrow suppression and macrocytosis that may mask folic acid or B12 deficiency. It is known to cause birth defects in animals. Some patients will have mild nausea or vomiting and rarely darkening of the skin and nails, ulceration of the skin of the legs, or hepatic toxicity (elevated transaminases). Skin necrosis has recently been reported as a rare side effect in patients receiving hydroxyurea for myeloproliferative disorders and men with SCD taking hydroxyurea may have decreased sperm counts and sperm quality.

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.

Alternative treatments: The alternative to participation in this trial is to have usual care, with an MRI of the brain if recommended by your physician and TCD annually beginning at age 2. If a child develops an

abnormal TCD or stroke, transfusion approximately every 4 weeks is usually recommended to decrease the risk of first or recurrent stroke. Monthly transfusion has well-documented risks (transfusion reactions, iron overload, transmission of infectious agents, and volume overload).

Recruitment and Consent: Participants will be recruited through the seven clinical centers. Additonal centers may be added, if recruitment requires this. The parent or guardian of the prospective participant will receive a mailing about the study and also be informed about the study during clinic appointments and admissions. The study will be explained to interested parents. If the parent agrees to participate, informed consent from the parent or legal guardian will be obtained and documented on consent forms approved by the Institutional Review Board. Consent will be obtained by the local investigators, usually in the outpatient clinic.

13.3 Adequacy of protection against risks

Phlebotomy: Venous blood will be collected by trained personnel with experience in pediatric phlebotomy. They will follow standard hospital procedures to minimize risk.

History and physical exam: The exam will be obtained by experienced pediatric providers skilled at minimizing discomfort or embarrassment for the participants and their parents.

Neuropsychological tests: An experienced pediatric neuropsychologist or psychometrician will provide a relaxed environment with frequent breaks to minimize anxiety.

MRI/MRA of the brain: Children will be screened for ferromagnetic materials and implants that may be unsafe during MRI and requiring exclusion.

Sedation: This will be performed using a standardized protocol developed by anesthesiologists and pediatric sedation experts. Sedation will be performed by experienced pediatric providers (anesthesiologists, intensivists, or hospitalists) clinically credentialed to perform sedation at their respective institutions, with direct supervision by an attending anesthesiologist. Participants will be screened by a questionnaire and sedation postponed or cancelled if the participant is at increased risk of complications. All participants will be examined before the sedation procedure by a pediatric hematologist and immediately before the sedation by the sedation provider to identify factors that may increase risk and require that the sedation for the MRI be postponed or not undertaken.

We will also minimize the risk of performing sedated MRIs in children that are not eligible for randomization by the following:

Confirming that potential participants meet all other eligibility requirements for randomization (e.g., no prior stroke or other major neurological disorder, no significant cytopenias or organ dysfunction, cerebral blood flow velocity by transcranial Doppler ultrasound below the threshold for treatment with transfusions) before performing the sedated MRI of the brain. By performing the MRI as the last procedure, as this is the only study procedure associated with significant risk, participants who would otherwise not qualify on other grounds will not undergo this procedure unnecessarily.

In addition, after the first ten participants are screened, we will evaluate all clinical data, to see if any significant predictors of Silent Cerebral Infarction (SCI) can be identified that would tell us these participants should not be screened. We will then continue to do this per the same schedule as monitoring for adverse events associated with the sedated MRI.

Hydroxyurea: The risk of bone marrow suppression will be minimized by frequent monitoring of the participants with a CBC with differential and reticulocyte count every two to four weeks. The participants will be carefully followed to identify other adverse effects (comprehensive panel every three months to evaluate hepatic and renal function) from the medication and to intervene early to minimize the risk of serious adverse

events. We will ask the parents of the participants to use precautions while administering the study medication to avoid direct contact with it.

Participant confidentiality: To protect of the confidentiality of the participants, names will be removed from each participant's file and replaced with an identification code. All testing protocols will use these codes to identify subjects. A list that contains the match between the code and name will be kept in a secure location only accessible to the investigators and study personnel. All data will be retrieved using the identification code. Data will be maintained on computer database only accessible to the investigators and study personnel.

13.4 Potential benefits of the proposed research to the subjects and others

Probable benefits for the participant and for society.

This study will help to evaluate the efficacy of hydroxyurea for the primary prevention of abnormal TCD, silent cerebral infarct, cognitive impairment and stroke in children with HbSS. If hydroxyurea is efficacious in preventing the CNS complications of SCD, the participants taking the drug will benefit. If hydroxyurea is efficacious, it could also prevent the need to initiate transfusion in many children with HbSS. Many patients who do not meet the current criteria for transfusion also suffer these complications, and hydroxyurea may decrease the risk of stroke in this group. Ultimately, many more CNS complications could be prevented than with chronic transfusions, given the lower cost and relative ease of administration of hydroxyurea compared to transfusions. Also, the side effects of hydroxyurea and burden to patients may be perceived to be lower and less severe by many patients than chronic transfusions. The patients who receive placebo will be followed more closely (visits every 4 weeks) and this will provide additional opportunity for education about SCD, prompt recognition of complications of their disease and disease prevention. The potential direct benefits to the participants and for other patients with SCD appear to outweigh the risks of the study.

13.5 Importance of knowledge to be gained

The efficacy of hydroxyurea in treating neurologic complications of SCD is not well established, nor are the indications for hydroxyurea in childhood. This study will evaluate therapy to prevent progression to abnormal TCD, silent cerebral infarct, cognitive impairment and stroke. This study will also provide the necessary feasibility data to potentially evaluate hydroxyurea for the primary prevention of CNS complications of HbSS in a definitive Phase III trial. Further studies will be necessary to determine whether patients perceive the burden of hydroxyurea to be less than that of transfusions, but the present study may help shed some light on this as well. It is expected that a larger group of patients at risk could be treated with hydroxyurea than transfusions. This would be a tremendous benefit in the treatment of SCD and prevention of CNS complications. It may also clarify whether all children with SCD should be treated with hydroxyurea, barring contraindications.

Collection of specimens for a biological repository with detailed information on MRI, cerebral blood flow velocity and other phenotype information will improve our understanding of SCD and potentially provide compelling evidence that hydroxyurea can prevent chronic neurological complications of SCD. The studies using this repository may identify new prognostic factors, biomarkers and targets for therapy.

Risk/Benefit: This study will hopefully provide the necessary feasibility data to proceed with a Phase III trial of hydroxyurea to prevent CNS complications in children with HbSS and HBSβ^0. Treatment with hydroxyurea and sedation is associated with moderate risk, but this risk can be minimized with standardized protocols and monitoring. The degree of risk is reasonable, given the morbidity of neurological injury in children with SCD, the morbidity of current treatments for stroke and impending stroke (transfusions and chelation), the potential for direct benefit for the children and the possible importance of the results.

13.6 Data and safety monitoring plan

Safety assessments

Participants will be evaluated every two weeks after a change in dose and otherwise every four weeks. An interval history of medical events and potential adverse events will be obtained at these visits. This information will be collected for 4 weeks after the participant has stopped the study medication. Conditional and abnormal TCDs will be repeated within defined intervals to detect progression and the need for transfusions.

Safety information will be sent to the coordinating center led by Daniel Hanley, MD and supported by the Johns Hopkins ICTR. This center will be responsible for data management, data quality assurance, random stop orders and dose escalation orders for hydroxyurea, and providing information on results and analyses to the DSMB.

Adverse events: Data will be collected on adverse events (AE), any abnormal finding (history, physical exam, laboratory, or imaging) of a participant in the study.

Unexpected AEs are adverse reactions that are not consistent (nature or severity) with the available information on hydroxyurea or SCD.

Serious adverse event (SAE)

- Results in death
- Life-threatening (participant at risk of death at the time of the event)
- Requires or prolongs hospitalization.
- Causes persistent or significant disability.
- Results in a congenital anomaly.
- Other medical events (in the opinion of the investigator) that may put the participant at risk or require intervention to prevent a serious AE.

AEs will be scored using the Common Toxicity Criteria for Adverse Events (CTCAE) 3.0 of the National Cancer Institute and the standard nomenclature for defining the causal relation between the AE and study drug (unrelated, probably not related/remote, possibly related, probably related, definitely related)

AEs will be followed until they resolve or stabilize. AEs will be classified as ongoing, resolved without sequelae, resolved with sequelae, or death. The response to the AE will be recorded.

Reporting of events
Serious safety issues will be reported to the DSMB, which will make recommendations to the sponsor (NHLBI) about the study.

Unexpected SAE will be reported to the DSMB and FDA within 7 calendar days, if fatal or life-threatening, and within 15 calendar days if not.

All SAE (expected and unexpected) will be provided to the DSMB by the DCC quarterly.

All AE (serious and non-serious) will be reported to the DSMB every six months or at their discretion.

Safety monitoring
The DSMB will include a pediatric hematologist, pediatric anesthesiologist, and a biostatistician with expertise in clinical trials. The DSMB will review the safety data, as well as information on enrollment at their meeting approximately every six months after the first participant is enrolled or at the discretion of the DSMB, based on patient accrual. Upon the review of the safety data as well as interim data analysis of enrolled subjects, they will make a recommendation to continue or stop the trial.

13.7 Payment and Remuneration

The participating families will receive $20 at each visit and an additional $20 for each visit that requires extra

time (sedated MRI, TCD, and cognitive testing) to assist with the expense of meals, parking, and babysitting. We estimate that the average randomized participant will have 16 visits in the first year and 15 visits in the second and third years. If the participant completes all of the study visits, they will receive $1100 ($920 for the regular visits and $180 for sedated MRI, TCD, and cognitive testing) over 3 years to help defray any study associated costs.

Costs

The following will be paid for by the study: History and Physical, Neurology evaluation, Neuropsychological evaluation, Sedation (except when clinically indicated), Study medicine (hydroxyurea or placebo), LDH, quantification of Hb S and F, biorepository specimen collection, processing, and storage, TCD (except when clinically indicated), and MRI/MRA of the brain (except when clinically indicated).

13.8 Inclusion of Women and Minorities

The study should include a similar number of males and females, based on the sex of participants with HbSS seen at the Pediatric Hematology clinics. We will invite any eligible participants (male or female) to participate. We expect that most of our participants will be of African descent, given the ethnic distribution of SCD in the United States. We will invite participants of other ethnic backgrounds to participate, if they meet the eligibility requirements for the study.

13.9 Inclusion of Children

We plan to enroll only children with SCD ages ≥ 12 and < 48 months of age, given the high incidence of progression to abnormal TCD, first silent cerebral infarct, and stroke in this age group. Older children have a lower incidence of these complications. We do not include adults, because the overall incidence of these events is much lower or unknown and many adults have established vasculopathy that may not respond to treatment with hydroxyurea or transfusions.

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Appendix 1 Parent and Participant Sickle Cell Disease Education Program

Parents participating in this program will increase their knowledge of sickle cell disease (SCD) and it's complications, learn the school-related problems in children with SCD, obtain help for a child with education problems in school, learn about the identification and management of overt stroke, discuss silent cerebral infarct and the explanation of its impact on the educational and vocational attainment for children with sickle cell disease and become familiar with the risks and benefits of blood transfusion and hydroxyurea therapy in children with SCD.

Visit I-1 hour
Sickle Cell Disease Overview - Study Coordinator, Social Worker, & Principal Investigator
Objectives:

- Provide definition of silent and overt strokes
- Explain the importance of TCD screening in stroke prevention and the role of blood transfusion for treatment in stroke prevention
- Explain silent cerebral infarct and ramifications for education
- Discuss barriers to the educational process for children with sickle cell disease, particularly those with silent cerebral infarcts
- Provide recommendations to improve educational achievement such as developmental assessment and early intervention programs for at risk toddlers and preschool children, intermittent home bound education, and an evaluation for an individual educational plan (IEP)

Visit II - 1 hour
Discussion of hydroxyurea and sedation risks & benefits - Study Coordinator & Principal Investigator
Objectives:

- Discuss implications of hydroxyurea therapy
- Discuss the potential benefit of hydroxyurea therapy, including the decrease in the frequency of painful episodes, acute chest syndromes, and possibly decreasing the progression of neurologic injury
- Discuss risks associated with sedated MRI and precautions taken to decrease these adverse events
- Discuss possible financial implications of study participation to the family and encourage discussion with their insurance providers
- Review the HU Prevent Trial and respond to questions from parents and potential participants

Appendix 2 Imaging Center

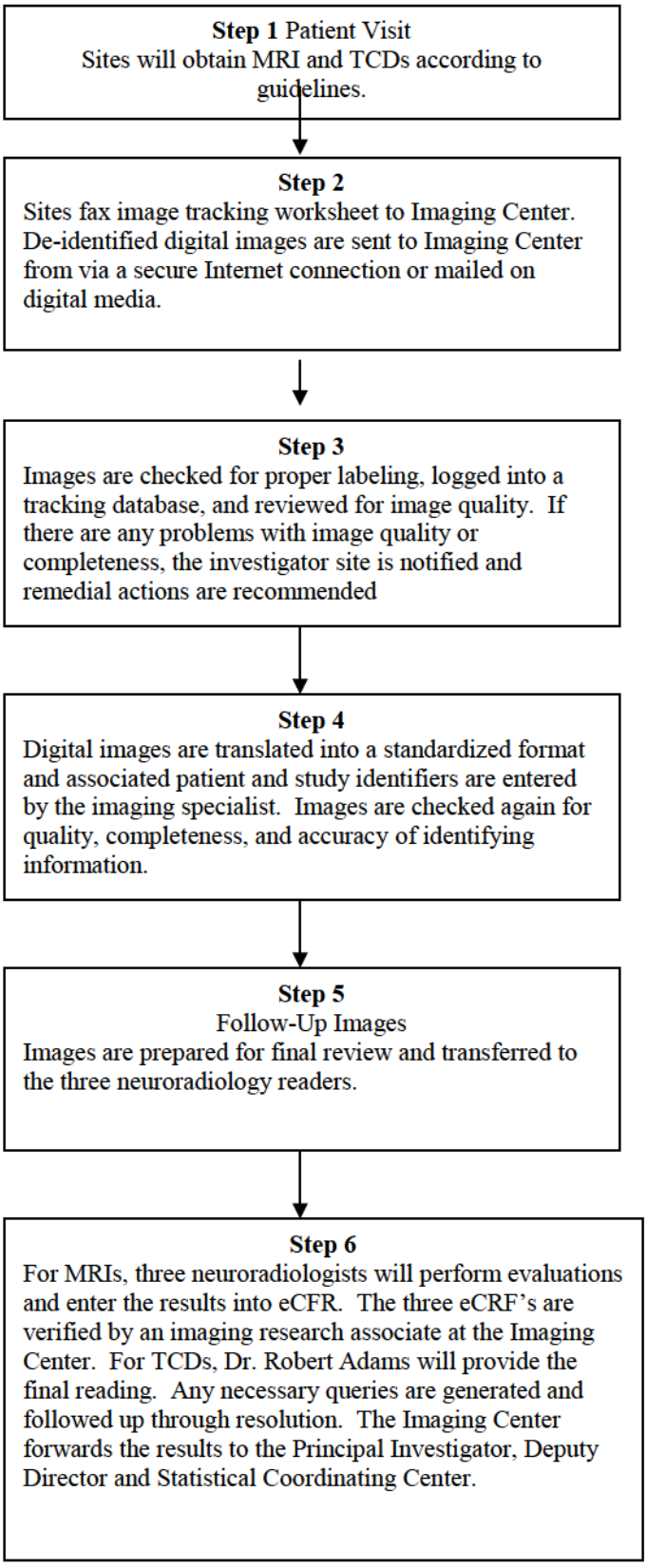
The Imaging Center will provide sites with standardized acquisition guidelines and procedures for handling of imaging data. The Imaging Center will collect images in direct digital form. Each site will deidentify images and then send images using a secure VPN, a secured file sharing service or on digital media to the Imaging Center. A coordinator at each site will use software provided by the Imaging Center or institutional software to de-identify the image sets or otherwise de-identify the images. Participant's demographic information will be removed and replaced with the study- assigned participant identifier and site-assigned 3 letter participant code. The laptop system will transmit the de-identified images to the Imaging Center using a secure, encrypted VPN connection or it will be sent by express mail on digital media. Sites will complete an image tracking worksheet that matches each DICOM series produced by the MR device to one o f the pulse sequences identified in the imaging protocol. The Imaging Center will check the quality of all images upon receipt and will communicate receipt of all incoming data to the clinical center, the Principal Investigator, Vice Chair, Data Coordinating Center, and the site investigator. Participant identifier, participant code, study date and study time on the images will be is compared with the accompanying forms and imaging worksheet for consistency. The Principal Investigator and Imaging Center will ensure that any confidential information has been masked.

The Imaging Center will then transfer images onto image review workstation and confirms image quality and data completeness on diagnostic workstation. The Imaging Center will manage centralized digital image archive, study database, and off-site back-up storage.

The Imaging Center neuroradiology reviewers will determine whether images are evaluable for purposes of the study. In the event that a problem with an imaging study is identified, the site is immediately notified concerning the nature of any problems and the steps required for corrective action.

The Imaging Center will provide digital MRI images and electronic Case Report Forms (eCRF) to the three-study neuroradiologist (neuroradiology panel members) and the clinical coordinating center Principal Investigator. The neuroradiology panel will perform the evaluation of the images for eligibility screening and analysis of enrolled patients. The presence or absence of cerebral infarcts will be graded according to a 3-point confidence scale. The neuroradiology panel will be blinded to treatment, patient demographics and clinical history (other than history of sickle cell anemia). They will enter their final evaluations into electronic Case Report Forms (eCRF).

TCDs images will be sent to the Neurology Core at the University of South Carolina for review.
The imaging center will forward the final imaging evaluation data to the Principal Investigator, Deputy Director, and the Statistical Coordinating Center weekly. Data concerning patient eligibility and need for referral will be transmitted to the Principal Investigator immediately.



Appendix 3. Evaluations for screening / pre-randomization

All participants	Screening Visit 1 +	Screening Visit 2+	Screening Visit 3 MRI
Informed consent	X		
Demographic/phenotypic form	X		
Repository Studies (DNA Specimens)	X^		
CBC, Diff, Retic, LDH, Comprehensive Panel, Hemoglobin analysis	X		
Medical history and physical exam by hematologist	X		
TCD	X		
† Neurology Exam with neurological event form after TCD (if TCD <170cm/sec)		X	
Cognitive Testing		X	
Sedation evaluation	X	X	
Sedation checklist		X	
MRI			X
*Parent & Participant Education	X	X	X
Repository Studies (Plasma Specimens for Proteomics)			X

† Site neurologist blinded to location of lesion

* Parents and participants will be encouraged to participate in these formal sessions prior to enrollment on this study; however, sickle cell disease education, management and emphasis on keeping the participant on track for preschool development and in school as an older child should continue throughout the duration of the study as standard of care for patients with sickle disease.

+ The procedures in the first and second visits can be combined or split into additional visits. Laboratory values not obtained successfully at the first visit may be done on the second or an additional visit or visit, as long as no more than 10cc of blood is drawn on a given day.

^ This specimen can be obtained at any of the screening visits, including the MRI

Appendix 4 Schedule of Study Procedures After Treatment Initiation- Year 1

	Visit 0 Randomization	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	Visit 10	Visit 11	Visit 12	Visit 13
H & P by Hematologist	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Study Medication Refill	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Adherence Measures		x	x	x	x	X	x	x	x	x	x	x	x	x
* CBC, diff, retic	x Repeat in 2 weeks	x	x	x	x	X	x	x	x	x	x	x	x	x
Education	x	x	x	x	x	X	x	x	x	x	x	x	x	x
Comprehensive panel, LDH				x			x			x				x
Hb F and S, and Other Hemoglobin Quantification				x			x			x				x
Samples for Proteomics	x +	x	x	x	x	X	x	x	x	x	x	x	x	x
Transcranial Doppler														x
Sedation Evaluation														x
MRI of Brain														x
Cognitive Evaluation														x
Neurology Evaluation														x
Randomization	X													

*CBC Diff, Retic may need to be done every 2 weeks

+ Proteomic sample not necessary, if done with the MRI and randomization is ≤ 2 weeks after MRI

Appendix 5 Schedule of Study Procedures Year - 2

	Visit 14	Visit 15	Visit 16	Visit 17	Visit 18	Visit 19	Visit 20	Visit 21	Visit 22	Visit 23	Visit 24	Visit 25	Visit 26
H & P by Hematologist	x	x	x	x	x	x	x	x	x	x	x	x	x
Study Medication Refill	x	x	x	x	x	x	x	x	x	x	x	x	x
Adherence Measures	x	x	x	x	x	x	x	x	x	x	x	x	x
* CBC, diff, retic	x	x	x	x	x	x	x	x	x	x	x	x	x
Education	x	x	x	x	x	x	x	x	x	x	x	x	x
Comprehensive panel, LDH			x			x			x				x
Hb F and S, and Other Hemoglobin Quantification			x			x			x				x
Samples for Proteomics	x	x	x	x	x	x	x	x	x	x	x	x	x
Transcranial Doppler													x
Sedation Evaluation													x
MRI of Brain													x
Cognitive Evaluation													x
Neurology Evaluation													x

* CBC Diff, Retic may need to be done every 2 weeks

Appendix 6 Schedule of Study Procedures Year – 3

	Visit 27	Visit 28	Visit 29	Visit 30	Visit 31	Visit 32	Visit 33	Visit 34	Visit 35	Visit 36	Visit 37	Visit 38	Visit 39
H & P by Hematologist	x	x	x	x	x	x	x	x	x	x	x	x	x
Study Medication Refill	x	x	x	x	x	x	x	x	x	x	x	x	x
Adherence Measures	x	x	x	x	x	x	x	x	x	x	x	x	x
* CBC, diff, retic	x	x	x	x	x	x	x	x	x	x	x	x	x
Education	x	x	x	x	x	x	x	x	x	x	x	x	x
Comprehensive Panel, LDH			x			x			x				x
Hb F and S Quantification			x			x			x				x
Samples for Proteomics	x	x	x	x	x	x	x	x	x	x	x	x	x
Transcranial Doppler													x
Sedation Evaluation													x
MRI of Brain													x
Cognitive Evaluation													x
Neurology Evaluation													x

* CBC Diff, Retic may need to be done every 2 weeks

Appendix 7 Schedule of Toxicity Evaluations After Treatment Initiation - Year 1

	Visit 0	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	Visit 10	Visit 11	Visit 12	Visit 13
H & P by Hematologist, PA or NP	x	x	x	x	x	x	x	x	x	x	x	x	x	x
* CBC, diff, retic	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Comprehensive panel,				x			x			x				x
Sedation Evaluation														x
Sedation Follow-up calls														x

* CBC Diff, Retic may need to be done every 2 weeks

Appendix 8 Schedule of Toxicity Evaluations - Year 2

	Visit 14	Visit 15	Visit 16	Visit 17	Visit 18	Visit 19	Visit 20	Visit 21	Visit 22	Visit 23	Visit 24	Visit 25	Visit 26
H & P by Hematologist, PA or NP	x	x	x	x	x	x	x	x	x	x	x	x	x
* CBC, diff, retic	x	x	x	x	x	x	x	x	x	x	x	x	x
Comprehensive panel,			x			x			x				x
Sedation Evaluation													x
Sedation Follow-up calls													x

* CBC Diff, Retic may need to be done every 2 weeks

Appendix 9 Schedule of Toxicity Evaluations - Year 3

	Visit 27	Visit 28	Visit 29	Visit 30	Visit 31	Visit 32	Visit 33	Visit 34	Visit 35	Visit 36	Visit 37	Visit 38	Visit 39
H & P by Hematologist	x	x	x	x	x	x	x	x	x	x	x	x	x
* CBC, diff, retic	x	x	x	x	x	x	x	x	x	x	x	x	x
Comprehensive panel,			x			x			x				x
Sedation Evaluation													x
Sedation Follow-up calls													x

* CBC Diff, Retic may need to be done every 2 weeks

Appendix 10 Cranial Magnetic Resonance Imaging Protocol

Magnetic Resonance Imaging of the brain performed at 1.5 or 3 Tesla

I. **Required Sequences**
   a. Scout locator-3 planes
   b. Axial T2W FLAIR
      i. 3 mm contiguous slices for whole brain coverage
      ii. Concatenated acquisitions for even/odd slices
   c. Coronal T2W FLAIR
      i. 3 mm contiguous slices for whole brain coverage
      ii. Concatenated acquisitions for even/odd slices
   d. T2W axial-FSE or local equivalent
      i. 3 mm contiguous slices for whole brain coverage
      ii. 21 cm FOV
   e. Axial and coronal T1W [can be replaced by T1W 3D sequence (IIb under supplemental)]
   f. Diffusion weighted imaging with ADC Maps [(can be replaced by DTI (IIc under supplemental)]

II. **Supplemental sequences (in order of priority)**
   a. 3D Time-of-Flight (TOF) MRA-This sequence will provide information about cerebral vasculopathy, a common finding and the major mechanism of ischemic stroke in children with SCD
      i. 4 overlapping slabs for coverage from C2 through the level of the centrum semiovale
      ii. 320 x 100 matrix with submillimeter resolution (ideally voxel of 0.3 x 0.3 x 0.6 mm)
      iii. Slice thickness 0.6 mm
      iv. 20 cm FOV
      v. Somersault (tumbling) and Exorcist (spinning) MIP reconstruction
   b. Sagittal T1W MPRAGE T1W 3D isotropic (or local equivalent-IR-FSPGR on GE scanners. This sequence will provide images for volumetric analysis of brain structures that may correlate with cognitive deficits.
      i. 1x1x1mm resolution (can replace axial and coronal T1W sequences)
   c. Diffusion tensor imaging (DTI) (can replace diffusion weighted imaging with ADC Maps). The DTI sequences will permit analysis of fiber tracking and white matter integrity, two potential contributors to the common deficits in attention and other aspects of executive function in children with SCD.
      i. 2x2x2mm resolution at 3T, 2.5x2.5x2.5mm resolution at 1.5T
      ii. 12 or more diffusion encoding directions
      iii. Automatic computation of ADC and FA
   d. Susceptibility Weighted Imaging (SWI). These sequences are very sensitive for the detection of acute or chronic hemorrhage.
      i. Source images
      ii. Phase maps
      iii. Source images masked by phase maps
      iv. Minimum Intensity projection reconstruction
   e. Arterial Spin Labeling (ASL). The various ASL techniques (pulsed, continuous, pseudocontinuous) are used to measure cerebral blood flow, a potential predictor of the risk of silent and overt stroke that may correlate with performance on cognitive testing.

f. Functional Connectivity Mapping with MRI (fcMRI, a 4 to 8 minute BOLD run without stimuli). This sequence is used to study the resting state correlations of the BOLD signal among various brain regions.
g. MR venography (MRV)-This sequence is used to evaluate for cerebral sinus thrombosis and should be obtained in children with a clinical presentation concerning for stroke, cerebral sinus thrombosis or intracerebral hemorrhage.

Appendix 11 Transcranial Doppler (TCD) Protocol

During the TCD recording IDENTIFY the vessel and OPTIMIZE the waveforms. The following describes the Transtemporal Identification Labels & Flow Direction:

	Label	Vessel	Flow Direction
1.	M -1	Shallowest depth of the MCA	Toward the probe
2.	MCA	Middle Cerebral Artery	Toward the probe
3.	BIF	ICA bifurcation	Bi-directional signal
4.	ACA	Anterior Cerebral Artery	Away from the probe
5.	dICA	Distal Interior Carotid Artery	Toward the probe
6.	PCA	Posterior Cerebral Artery	Toward the probe
7.	TOB	Top of the Basilar	Bi-directional signal

Recording the TCD Exam Step-by-Step

The M-1 and the Middle Cerebral Artery (MCA):
Begin the TCD exam by detecting a signal through the temporal window with a depth setting of ~50mm. Once this signal is detected, increase or decrease the depth until a bidirectional "bifurcation" (BIF) signal is identified. Once the BIF has been identified verify that the artery being insonated is the MCA by tracking the vessel to its shallowest depth. The shallowest portion of the MCA is labeled M-1. Record ONE scan of the M-1.

After recording the M-1, increase the depth of insonation in 2 mm steps and record one scan of each waveform using the MCA label until the Bifurcation is reached. If HIGH VELOCITY is found then take as many scans as necessary to record the HIGH VELOCITY.

The Bifurcation (BIF)
The intracranial internal carotid artery (ICA) terminates by dividing into the MCA and the ACA. This bifurcation, known as the "BIF", is identified as a bi-directional signal. The flow above the zero line and flowing towards the probe represents the MCA while the flow below the zero line and away from the probe represents the ACA. Once the BIF is identified, attempt to obtain a signal with equal "intensity" above and below the line. Record TWO scans of the BIF at the same depth.

The Anterior Cerebral Artery (ACA)
After identifying the ACA vessel as the flow away from the probe below the Zero line at the Bifurcation, increase the depth by 4 mm and record the ACA. To better isolate the ACA, angle the probe anteriorly/superiorly. Record TWO ACA waveforms: one scan should be recorded at 4 mm past the bifurcation, then a second scan at 6mm past the bifurcation UNLESS the ACA at 6mm past the bifurcation is not a good signal. In this case, record two scans of the ACA at 4 mm past the bifurcation.

The Distal Internal Cerebral Artery (dICA)
Return to the BIF landmark and angle slightly inferiorly to locate the dICA. Increase the depth 4 mm to isolate and optimize the signal. Record TWO dICA waveforms: one scan should be recorded at 4 mm past the bifurcation, then a second scan at 6mm past the bifurcation UNLESS the dICA at 6mm past the bifurcation is not a good signal. In this case, record two scans of the dICA at 4 mm past the bifurcation. Due to the unfavorable angle of insonation, the dICA signal is often "damped" or diminished as compared to the MCA signal and may have a "gruff" audio quality.

The Posterior Cerebral Artery (PCA)

Return to the BIF landmark. Increase the depth 4mm and angle the transducer posteriorly/ inferiorly to record the PCA. Track and record the PCA in 2mm steps until reaching the Top of the Basilar. The mean velocity of the PCA is normally about 1/2 to 2/3 of the mean velocity of the MCA.

The Top of the Basilar (TOB)
The top of the basilar is identified by tracking the PCA in 2mm increments until bidirectional flow is identified at the midline. Record TWO scans of the TOB at either one or two depths.

The following describes the Transforaminal Examination:

Label	Vessel	Flow Direction
PR-BAS	Proximal Basilar	Away from probe
DIS-BAS	Distal Basilar	Away from probe

Record one scan of the Proximal Basilar (PR-BAS) and one scan of the Distal Basilar (DIS-BAS)

Follow the same instructions for each temporal side of the head. EXAMPLE of TCD Scanning & Labeling when the Head Diameter is 120mm (Midline=60mm; Bifurcation will be seen around 50mm)

Right Temporal Side :		Left Temporal Side :	
R-M1	40	L-M1	40
R-MCA	42	L-MCA	42
R-MCA	44	L-MCA	44
R-MCA	46	L-MCA	46
R-MCA	48	L-MCA	48
R-BIF	50	L-BIF	50
R-BIF	50	L-BIF	50
R-ACA	54	L-ACA	54
R-ACA	56	L-ACA	56
R-dICA	54	L-dICA	54
R-dICA	56	L-dICA	56
R-PCA	54	L-PCA	54
R-PCA	56	L-PCA	56
R-PCA	58	L-PCA	58
R-TOB	60	L-TOB	60
R-TOB	60 or 62	L-TOB	60 or 62
PROX-BAS	72		
DIST-BAS	74		

Appendix 12 Sedation Protocol Using Propofol or Pentobarbital as the Primary Agent

The administration of anesthetics and sedatives to patients with SCD is controversial and there is not consistent agreement amongst anesthesiologists or hematologists as to the best approach. The following aspires to best practice principles which center on clear and consistent communication between the various providers from hematology, anesthesiology and the sedation team and, most importantly, with the family. These guidelines were reviewed and approved by the Sedation Committee for the HU Prevent Trial and are intended to provide a minimal standard of safety. The actual management of participants should be individualized as necessary, with the safety of the participant being the highest priority at all times; however, the use of propofol or as the primary agents for sedation/anesthesia, the exclusion criteria and minimum requirements, such as duration of NPO and baseline oxygen saturation should be adhered to strictly. In addition, the administration of propofol should be performed by or occur under the direct supervision of an anesthesiologist. These requirements may also be exceeded (e.g., longer durations of NPO) based on local judgments.

The children enrolled for imaging will be pre-screened before they receive sedation for an MRI, as the goal of this protocol is to provide the safest environment for these children, minimal disruption for the parents and consistent care with rare cancellations. Children with SCD may have had recurrent hospitalizations and be wary of care providers; they may also have had many previous intravenous (IV) catheters and poor vascular access. The children will be cared for in a warm and supportive manner and the MRI, its indication and the person requesting it should be confirmed with the parent or guardian.

1) Sedation Exclusion Criteria:

Above and beyond adhering to all prevailing local standards and procedures for MRI screening and safety, the following exclusion criteria will be followed:

a. Children who do not pass the MRI screening checklist.
b. Children with obstructive sleep apnea [OSA] who are receiving therapy [e.g. continuous positive airway pressure (CPAP)], or who are being evaluated or followed by a specialist for management of severe OSA.
c. Children who present with room air oxygen saturations 5% below their baseline will not be anesthetized on that day and will be referred to their hematologist.
d. Children who present with room air oxygen saturation less than 90% will not anesthetized on that day and will be referred to their hematologist.
e. Children with an allergic reaction such as urticaria or bronchospasm, or previous adverse reaction to propofol, eggs or soy products.
f. Children with known major chromosomal abnormalities.
g. Children with known airway abnormalities that would increase the risk of sedation/anesthesia.
h. The case will be cancelled in the event that the participant has a temperature of > 38˚ C, symptoms of an upper or lower respiratory infection in the previous 4 weeks, active bronchospasm, acute chest syndrome, sequestration or other acute complications of SCD in the last 4 weeks, or pain crisis within two weeks requiring treatment with opiates.
i. Children whose parents refuse to participate.

2) NPO Times and Pre-Sedation Hydration:

a. The NPO time will be kept to a minimal duration and the child should be encouraged to take oral hydration so as to remain hydrated prior to propofol administration.
b. NPO for 2 hours for clear liquids and at least 6 hours for solids.
c. It is imperative that the child remains well hydrated prior to the NPO period. To achieve this goal, the family will be requested to administer clear liquids up until the time of absolute NPO.
d. If the child is deemed inadequately hydrated upon arrival, one of the following will occur:
    1) An IV will be placed and the child will be hydrated with an isotonic intravenous fluid.
    2) The child will be orally hydrated and the sedation time will be delayed.
    3) If the above cannot be performed, the case will be cancelled and rescheduled.

3) Pre-Sedation:

a. The child must have a history and physical documented with the last 30 days.
b. Due to the increased risk of perioperative complications in children with SCD, the child must be otherwise well at the time of this elective MRI.
c. Prior to the day of the MRI, the child will be screened by a checklist for acute illnesses, coexisting diseases, or OSA. This will usually be done by telephone.
d. The case will be cancelled in the event of an upper or lower respiratory infection in the previous 4 weeks, active bronchospasm, or acute chest syndrome in the last 4 weeks, or pain crisis requiring treatment with opiates within two weeks, sequestration or other acute complications of sickle cell disease.
e. A thorough preoperative history and physical, including baseline vital signs [weight, heart rate, respiratory rate, temperature, blood pressure, room air pulse oximetry and pain score], shall be performed and documented in the child's medical record.
f. A hemoglobin will be obtained within the previous 30 days; if the hemoglobin is <6.5 g/dL the child will not be sedated and instead will be referred to their hematologist.
g. It will be confirmed that a hospital bed is available, in the event of complications.
h. Informed consent for administration of sedation will be obtained from the child's parent or guardian according to local practice. This will include the potential for the development of abnormal vital signs, the possibility of hospitalization and a risk of sickle cell crisis. There is also a low potential risk for cardiopulmonary arrest, stroke or death. The case will be cancelled if the family does not consent to the MRI or to the administration of the sedatives to be used as part of this protocol.
i. If the parent indicates that the child typically tolerates placement of an IV catheter, a topical local anesthetic patch [e.g. Synera, ELA-Max] or local anesthetic infiltration will be placed at the potential site for venous access. After the skin is anesthetized, the IV will be placed.
j. Midazolam may be used as an oral premedication for IV placement at a dose of 0.5 mg/kg [to a maximal dose of 10 mg].

4) Sedation Regimen using Propofol

a. Preadmission vital signs will be reviewed and recorded as part of the pre-sedation evaluation.
b. The participant will have continuous monitoring of vital signs during the induction, maintenance, and recovery period in the recovery area.
c. Standard American Society of Anesthesiology [ASA] monitoring will be utilized including EKG, pulse oximetry, respiratory rate, blood pressure, temperature and end tidal carbon dioxide. Vital signs will be documented at least every 5 minutes using the institution's form.
d. Vital signs will be maintained within an age appropriate range as determined by the attending anesthesiologist or propofol-trained hospitalist, or other appropriate personnel under the direct supervision of an anesthesiologist
e. Oxygen will be administered by nasal cannula or mask to maintain oxygen saturation above 95%.
f. Following intravenous placement, intravenous lidocaine, 1 mg/kg up to 20 mg, will be given intravenously followed by a bolus of propofol, 2 mg/kg. Additional propofol at 1 mg/kg increments may be administered at the anesthesiologist's/propofol-trained hospitalist's discretion until an adequate level of anesthesia is obtained.
g. If the parent indicates that the child does not tolerate IV catheter placement, a mask inhalation induction will be performed by *an anesthesiologist* and an IV catheter placed when the child is sufficiently anesthetized.
h. For children who require a mask induction for IV catheter placement, all monitors will be placed, following by an inhalation induction until an adequate depth of anesthesia is achieved. After the IV is inserted, the child will be given 100% oxygen and the bolus of propofol administered.
i. Propofol will be titrated between 0 and 250 micrograms/kg/minute as needed to maintain normal vital signs and adequate sedation.
j. Additional propofol at 0.5 mg/kg increments will be administered at the anesthesiologist's or propofol-trained hospitalist's or other appropriately trained personnel's discretion, under the direct supervision of an anesthesiologist until an adequate level of anesthesia is obtained.

k. Following completion of the non-contrast MRI of the brain, propofol will be discontinued and the child will promptly be brought to the recovery area.
l. Following standard recovery, with the child achieving pre-procedure vital signs, having no nausea or vomiting, and tolerating clear liquids, the child will be discharged to home.
m. The family will be given the phone number of the anesthesiologist or propofol-trained hospitalist and the hematologist to call for any questions or concerns.
n. The family will be contacted the following day by the anesthesiologist or propofol-trained hospitalist or their representative to ascertain any problems or concerns of the child's parent.

Appendix 13 Checklist for MRI with Sedation

Excludes participation until resolved

To be completed by phone screener on the day prior to the sedation.

- ☐ Parent refusal to participate
- ☐ Obstructive sleep apnea (OSA) and receiving therapy (e.g. CPAP), or being evaluated or followed by a specialist for management of severe OSA.
- ☐ Asthma exacerbation in previous 4 weeks
- ☐ Upper respiratory infection in previous 4 weeks
- ☐ Acute chest syndrome, sequestration or other acute complications of sickle cell disease in the previous 4 weeks or pain crisis within the last 2 weeks requiring opiate treatment
- ☐ Hospitalization in the previous 4 weeks
- ☐ Unrepaired complex congenital heart disease
- ☐ Single ventricle physiology
- ☐ Pulmonary hypertension
- ☐ Chromosomal abnormalities
- ☐ Known airway abnormalities that would increase the risk of sedation/anesthesia
- ☐ Current fever or other signs of acute illness
- ☐ Morbid obesity
- ☐ ASA PS ≥ 3
- ☐ Hemoglobin ≥ 6.5 g/dl not documented within 30 days of procedure

Appendix 14 DNA Sample Protocol

Genetic repository specimen –

Ten to fifteen milliliters (ml) of blood is to be drawn and added to ACD (6 ml yellow top, fill completely) and K_3EDTA vacutainer tubes (5 ml purple (lavender) top, fill to 4 ml). Immediately following blood collection, ACD and EDTA tubes should be gently inverted 8-10 times, to mix blood with preservative or anti-coagulant. If less than 10 ml of blood is obtained, preferentially fill the ACD tube. If 13-15 ml of blood is obtained, fill a second ACD tube with 3-5 mls, after putting 4 ml in the K_3EDTA tube. See table below.

Blood draw	6 ml ACD vacutainer (yellow top)	5 ml K_3EDTA vacutainer (purple top)	Extra 6 ml ACD vacutainer (yellow top)
10 ml	6 ml	4 ml	
<10 ml	6 ml	Rest of blood available	
13-15 ml	6 ml	4 ml	3-5 ml

The ACD and EDTA tubes should be packed and shipped the same day as collection using the provided materials and following Department of Transportation (domestic) or IATA (international) guidelines for the shipment of human blood. Blood should be shipped at room temperature (22-24°C). An ambient shipping box will be used. It can contain up to 8 tubes. Since each study participant that is screened will provide 2 to 3 tubes, each shipping box can contain tubes for up to 4 study participants.

Shipping / tracking CRFs (CRF 03 and 04) need to be completed for each study participant (e.g., if there is only one study participant having tubes shipped, then only one set of CRFs is required; if two study participants are having tubes shipped, there needs to be two sets of CRFs enclosed in the box).

On each shipping / tracking CRF, a duplicate tube label needs to be affixed for each set of tubes being shipped).

The clinical site will also fax or scan and e-mail the shipping / tracking CRF to Dr. Barron-Casella's lab at 410-966-8208. Please also email Emily Barron-Casella at ebarron1@jhmi.edu and Kim Jones at kjones62@jhmi.edu, that a shipment is to be expected. This approach provides advanced notice to Dr. Barron-Casella's lab that a particular study participant will be having a certain number of tubes shipped to the lab soon.

Place each participant's tube in a biohazards bag with paper towels inside the bag and arranged so that they will fit in slots in the multitube Styrofoam container of the shipper. Close and seal the Styrofoam container with several wraps of tape. The Styrofoam box, together with the filled CRFs, should be slipped into the outer cardboard box of the shipper. The cardboard box should be closed and the ends sealed with tape.

Affix the Biological Substance Category B UN3373 label on the outer cardboard box and put the shipper in the UN3373 bag, seal, attach the filled air bill and schedule the FedEx pick-up.

Please ship all specimens Monday through Thursday to:

Attn: Kim Jones/Emily Barron-Casella, PhD
Department of Pediatric Hematology
Johns Hopkins University School of Medicine
Ross 1125
720 Rutland Avenue
Baltimore, MD 21205
Phone 410-955-6132

If a Friday shipment is needed, please contact Kim Jones or Emily Barron-Casella before shipping, if at all possible (410-614-0708 or 410-955-6132).

All specimens must arrive by 2 PM on Friday, unless alternative arrangements are made. International specimens should only be collected on Monday, Tuesday, or Wednesday, so that they will arrive before this deadline.

Appendix 15 Plasma Samples For Proteomic Analysis

The purpose of this protocol is to get the freshest and best preserved plasma samples as possible from this important group of participants. Please do not rush the procedure; however, the faster the blood samples are processed and the plasma is frozen, the less proteolysis will occur. These very high quality samples will be used by laboratories to discover potential protein markers for silent cerebral infarcts.

Required materials on site:

- Low speed centrifuge (must be able to spin 5 ml Vacutainer tubes 1800-2000 x g)
- -80ºC Freezer
- Dry ice

Required materials from JHU: To be obtained from Emily Barron-Casella. Please submit request via email ebarron1@jhmi.edu or fax 410 955 8208.

- Purple (Lavender) top, 5 ml glass Vacutainer tubes (2 tubes; one is spare).
- 2.0 ml screw cap freezer tubes (12 tubes; two are spare)
- 15 ml polypropylene tube (12 X 75 mm) (2 tubes; one is spare)
- Disposable graduated transfer pipets (2 pipets)
- Pre-printed white labels for freezer tubes
- Freezer box labeled HU Prevent Proteomics Samples
- Containment biohazard bag with adsorbent pad
- Styrofoam carrier outer cardboard box
- Dry ice label
- Biological Substance Category B UN3373 label
- FedEx airbill with Hopkins ship-to information filled out

1. Before starting
   a. Participant's identity should be confirmed verbally.
   b. Please mark participant's ID number on 1 x 15 ml polypropylene tube
   c. Please complete labels for 10 screw top 2.0 ml freezer tubes. They should contain:
      - Participant's ID and barcode (given)
      - Please fill in date
      - Please enter which visit (ie. 0, 3, 6, 9, 12, 18, 24, 30 or 36 months) or for acute stroke participants the days post-event (ie. 0, 3, 7, 14, …days)
   d. Labels should be affixed to 10 screw top 2.0 ml freezer tubes (Note that the barcode on the label should go down, not around, the tube). Make sure you can clearly see the graduation marks on the small freezer tubes.
   e. We are supplying and prefer that all blood be collected in **glass** Vacutainers, but if plastic tubes are used, please make a notation on the case report form.

Until the samples are aliquotted, all samples should be kept at room temperature (21 °C).
Do NOT put sample on ice at any time during protocol. Freeze at -80°C after aliquoting.
Plasma samples should be completely processed <u>within 4 hours.</u>

2. Phlebotomy
   a. Identify vein, and cleanse surrounding area thoroughly with ethanol
   b. Venipuncture should be performed using a 23 gauge (or larger) needle, and sufficient blood should be obtained to fill one purple top Vacutainer tube (5 mls).
   c. Gently invert tube several times to mix.

3. Centrifugation

a. Centrifuge the Vacutainer tube for 8 minutes at 3000 RPM (1900 x g) at room temperature (21ºC). (Remember to balance tubes in the centrifuge.
b. Transfer as much plasma (i.e. top clear layer) as possible (about 3 mls) into the labeled 15 ml polypropylene tube (12 X 75 mm).
c. If a clean transfer of plasma is obtained, proceed to aliquotting the plasma. If contaminated with some cells, this tube can be spun at same speed for 8 min and plasma carefully transferred to remove cells.

4. Plasma aliquotting
   a. Gently mix the plasma in the polypropylene tube so that it is homogeneous in color. Aliquot plasma with disposable transfer pipet into 2.0 ml screw top freezer tubes as follows:
      - Five tubes with 250 ul each
      - Remainder of plasma should be distributed in 500 ul aliquots
      - If there is additional plasma, please add this remainder to the final 500 ul tube and put a plus (+) sign on the cap to indicate more than 500 ul
   b. Freezer tubes should be placed HU Prevent Proteomics Samples freezer box and immediately frozen by placing in a -80ºC freezer. Please note the location of the box (preferably in a rack). Boxes are easily lost in big, multi-user freezers. Samples must be frozen solid prior to shipping.

5. Additional notes
   a. From the time the plasma is withdrawn from Vacutainers, plasma samples should be completely processed (i.e. aliquoted and placed in -80ºC for storage) **within 4 hours.**
   b. Samples that remain at room temperature for a longer than 4 hours can be processed, but the actual time required to process the samples completely should be recorded on CRF.
   c. Samples should be stored at -80ºC until it is a good day to ship. Good days are Mondays, Tuesdays, and Wednesdays (domestic) or Mondays and Tuesdays (international). **Samples must be taken from -80°C freezer and directly put on dry ice for shipping. Do not let thaw or put on wet ice.**
   d. Samples should be shipped to Johns Hopkins University School of Medicine in batches every three months.
   e. Vacutainers should be filled completely to ensure adequate plasma sample.
   f. When filling Vacutainer, use the vacuum to allow the tube to fill with blood. Do not force blood into the tube with a syringe, which may cause hemolysis.
   g. If a clot forms in the needle and prevents plasma transfer, change needles and proceed.

6. Shipping

Domestic shipments

1. Please contact us ahead of time about shipping. Please email Emily Barron-Casella at ebarron1@jhmi.edu, Kim Jones at kjones62@jhmi.edu, and Diane Weiss at dweiss14@jhmi.edu that a shipment is to be expected.
2. Please store samples at -80ºC until it is a good day to ship. Good days are Mondays, Tuesdays, and Wednesdays (domestic).
3. Shipping must follow the guidelines of DOT/IATA for "Biological Substance Category B UN3373." There must be 3 levels of containment.
4. Place HU Prevent Samples freezer box containing samples in small containment bag with pad. The pad is to absorb any biohazardous material if compromised. Put bagged box in the bottom of the Styrofoam carrier/cardboard box.
5. Add approximately **5 lbs (=2.5 kg)** of dry ice on top of bagged box. Put Styrofoam top on carrier, but do NOT tape (CO_2 from dry ice must be able to escape).
6. Between Styrofoam and cardboard box, tuck in the completed Case Report Form. Close cardboard top and tape.
7. Complete and apply dry ice label and "Biological Substance Category B UN3373" labels to outside of cardboard box.
8. Complete and apply USA FedEx Airbill and schedule a pick-up.

International shipments

1. Please contact us ahead of time about shipping. Please email Emily Barron-Casella at ebarron1@jhmi.edu, Kim Jones at kjones62@jhmi.edu, and Paul Kamate at pkamate1@jhmi.edu that a shipment is to be expected.
2. Please store samples at -80°C until it is a good day to ship. Good days are Mondays and Tuesdays.
3. Shipping must follow the guidelines of DOT/IATA for "Biological Substance Category B UN3373" shipments. There must be 3 levels of containment.
4. Place HU Prevent Samples freezer box containing samples in small containment bag with pad. The pad is to absorb any biohazardous material if compromised. Put bagged box in the bottom of the Styrofoam carrier/cardboard box.
5. Add approximately **15 lbs (=7.5 kg) of dry ice** on top of bagged box for shipping. Put Styrofoam top on carrier, but do NOT tape (CO_2 from dry ice must be able to escape).
6. Between Styrofoam and cardboard box, tuck in the completed CRF. Close cardboard top and tape.
7. Complete and apply dry ice label and "Biological Substance Category B UN3373" labels to outside of cardboard box.
8. Complete and apply International FedEx Airbill, the commercial invoice and any country-specific required forms and schedule a pick-up.

Appendix 16: Committees

Executive Committee: This is the day-to-day decision-making body and is responsible for the successful completion of the study, including sustaining target enrollment and protocol adherence. Members include the Study Chair, who will head the committee, the Principal Investigator (PI) from the DCC, the study Vice-Chair, John Strouse, Co-Investigator, the Chairs of the Neurology, Neuroradiology, Neuropsychology, Imaging and Sedation Committees, as well as a representative from the TCD Reading Center, 3 rotating study center Principal Investigators and the NHLBI Project Officer. On issues requiring a vote, one vote per member will be allowed. During early phases of the Trial, this Committee will meet by conference call no less than every other week during the study, to review trial enrollment, conduct of the protocol, feasibility (participant burden, site burden and cost), scientific merit, and issues raised by the Clinical Sites, and no less than monthly thereafter.

The Executive Committee will meet in person at least every twelve months, in Baltimore. Frequent conference calls will ensure smooth day-to-day operations of the trial and help to identify issues that need to be brought before the Operations Committee, which will meet biweekly. The Executive Committee will also make recommendations on trial-related issues and publications.

Publication Committee: A Publications Committee consisting of the Executive Committee and the Site Investigators will review and report results for data analyses from the DCC and will review all proposals for and final versions of research abstracts, presentations, and manuscripts to be submitted to journals and national meetings. A Site Investigator Committee will discuss and find solutions to problems that may arise at each Clinical Site.

Committee Meeting Schedule For HU Prevent Trial

Committee	Participants	Schedule
Executive	Principal Investigator, Vice-Chair, Statistical Coordinating Center PI, Co-Investigator, Discipline Chairs, Imaging Core and TCD Reading Center Representatives, Consultants, Administrative Office Staff (if needed)	Phone conference every month, meeting in Baltimore every 12 months for one day meeting
Operations	Principal Investigator, Vice Chair, John Strouse, Co-investigator, Representative from Statistical Coordinating Center, Lead Study Coordinator.	
Site Hematologists / Neurologists	Site Investigators Meeting	Annual Investigators Meeting in Baltimore or via Webinar
Neurology	Site Neurologists & Neurology Committee	Annual Investigators Meeting in Baltimore
Neuropsychology	Neuropsychology Committee	Annual Investigators Meeting in Baltimore
Neuroradiology	Neuroradiology Committee Members, and Imaging Core Investigators	Annual Investigators Meeting in Baltimore

Sedation	Sedation committee	Annual Investigators Meeting in Baltimore

Appendix 17: Hydroxyurea and Placebo Formulation

HYDROXYUREA ORAL SOLUTION (100 MG/ML)

Ingredients
Hydroxyurea capsules 500 mg #10
Distilled water qs ad 25 mL
Simple Syrup 50 mL

Directions
1.Empty capsules into 25 mL water to form a slurry in a 30 mL Wheaton vial.

2.Shake well for 1-2 minutes continuously every 10-15 minutes for 1 hour until all granules are in solution.

3.Filter solution using a 5 micron filter (the active ingredient is soluble and will be in the filtrate).

4.Add 25 mL simple syrup (measured with syringe) and shake well.

Labeling
Keep refrigerated. Special handling required.

Expiration
3 months

Reference
Heeney, Matthew, "Chemical and Functional Analysis of Hydroxyurea Oral Solutions," J Pediatr Hematol Oncol 2004, Vol 26 (3):179-184.

PLACEBO ORAL SOLUTION

ingredients
Distilled water 50 ml
Simple Syrup 50 ml

Mix all ingredient to make 100 ml of placebo solution

Labeling
Keep refrigerated. Special handling required.

Expiration
3 months

Appendix 18: Anesthesia/Sedation Data Collection Instrument

Age ☐☐ years ☐☐ months Weight ☐☐.☐ kg

Gender ○ male ○ female

Diagnosis ○ HbSS ○ HbSβ^0

Coexisting Illnesses ○ Asthma ○ Other _____

ASA Status ☐ (1-5)

Home medications ○ Penicillin ○ Folic acid ○ Ibuprofen ○ Albuterol

○ Other _____ ○ Other _____ ○ Other _____ ○ Other _____ ○ Other _____ ○ Other _____

NPO duration ☐☐ hours for clear liquids ☐☐ hours for solids

Case Cancellation ○no ○ yes

Cancellation Reason ○ NPO violation ○ Acute Illness ○ Other_____

Premedication ○ None ○ Lorazepam ○ Other _____

IV placed ○Before anesthesia ○After inhalational agent ○Unknown

Start of procedure ☐☐:☐☐ (24 hour time)

Intravenous agent	Dose for induction	Total Dose for the Procedure
○ Lidocaine	☐☐☐ mg	☐☐☐ mg
○ Propofol	☐☐☐ mg	☐☐☐ mg
○ Pentobarbital	☐☐☐ mg	☐☐☐ mg

Inhalational agent

○ Sevoflurane Concentration ☐☐% Duration ☐☐☐minutes

○ Nitrous Oxide Concentration ☐☐% Duration ☐☐☐minutes

Additional agents used

○ Other _____ Details _____

○ Other _____ Details _____

Airway device ○ None ○ LMA ○Endotracheal Tube ○ Tracheostomy Tube

Change in airway device ○no ○ yes

Type of airway change ○ None to LMA ○ None to ETT ○LMA to ETT ○ Trach to ETT

Reason for change ○ Coughing ○ Secretions ○ Laryngospasm
○ Upper airway obstruction ○ other _____

Duration of MRI ☐☐☐minutes

Duration of anesthetic/sedation ☐☐☐minutes

Duration of recovery ☐☐☐minutes

Complications

Desaturation <90% for > 60s ○ no ○ yes ○ unknown/missing

Desaturation >10% from baseline > 60s ○ no ○ yes ○ unknown/missing

Lowest desaturation ☐☐☐%

Duration of lowest desaturation ☐☐seconds ☐☐minutes

Respiratory ○ Apnea ○ Aspiration ○ Bronchospasm
○ Stridor ○ Laryngospasm
○ Other _____

Cardiovascular ○ Bradycardia ○ Tachycardia ○ Hypotension ○ Hypertension
○ Other _____

Core Temperature ○ <36.0° ○ 36.0°-38.1° ○ >38.1° ○unknown/missing

Emergence Delirium ○no ○ yes ○ unknown/missing

Emesis ○ no ○ once ○ ≥2 times ○ unknown/missing

Other ○no ○ yes

If yes please describe_____

Acute Serious Adverse Events

Hospitalization	○ no	○ yes	○ unknown/missing
Pain	○ no	○ yes	○ unknown/missing
Acute chest	○ no	○ yes	○ unknown/missing
Stroke	○ no	○ yes	○ unknown/missing
Fever	○ no	○ yes	○ unknown/missing
Aspiration	○ no	○ yes	○ unknown/missing
Hypoxemia	○ no	○ yes	○ unknown/missing
Other	○ no	○ yes	○ unknown/missing

If yes, describe _____

CPR	○ no	○ yes	○ unknown/missing
Death	○ no	○ yes	○ unknown/missing
Permanent neurologic injury			○ no ○ yes ○ unknown/missing
Permanent end-organ injury			○ no ○ yes ○ unknown/missing
Unanticipated endotracheal intubation			○ no ○ yes ○ unknown/missing
Other			○ no ○ yes ○ unknown/missing

If yes, describe _____

Elective Hospitalization for Observation is not considered in itself an adverse event

Appendix 19: Sedation Follow-up Questionnaire

The following script is to be used for telephone follow-up 24 hours after sedation is administered for a study MRI. The same questions should be administered at 14 days either at a follow-up visit or by telephone.

Hello, this is **name of caller** from **name of PI, name of institution**. May I please speak ***to name of participant*** mother or father (or other caregiver). I am calling to see how **name of participant** is doing after their MRI on *date of MRI.* Is now an OK time to ask you a few questions?

1) Has **name of participant** had any pain?-Yes, No, Unknown, Missing
    a. If yes, where was the pain? (choose multiple from list-head, arms, hands, legs, chest, back, abdomen/belly, feet, other)
    b. If yes, when did it start? Day/month/year military time
    c. If yes, when did it stop? Day/month/year military time
    d. If yes, how severe (0 no pain, 10 worst pain ever) (drop down menu 0-10, 99 for missing
    e. If yes, were they given any pain medicine? Yes/No/Unknown/Missing
    f. If yes, what type of medicine-choose all from list
        i. Tylenol/acetaminophen
        ii. Motrin/Advil/Ibuprofen
        iii. Aleve/Naproxen
        iv. Acetaminophen with codeine/Tylenol with codeine, Tylenol #3
        v. Oxycodone
        vi. Morphine
        vii. Dilaudid/hydromorphone
        viii. Other_____
2) Has **name of participant** had any breathing problems-Yes, No, Unknown, Missing
    a. If yes, when did it start? Day/month/year military time
    b. If yes, when did it stop? Day/month/year military time
    c. If yes, type-mark as many
        i. Cough
        ii. Shortness of breath
        iii. Wheezing
        iv. Breathing hard
        v. Other _____
    d. If yes, did your child receive any treatment for it
        i. None
        ii. Albuterol/Proventil
        iii. Antibiotics
        iv. Over the counter cough medicine
        v. Prescription cough medicine
        vi. Corticosteroids by mouth (prednisone, prednisolone, Prelone, Pediapred)
        vii. Other_____
3) Has **name of participant** had any other health problems since the MRI? Yes/No/Unknown/Missing
    a. If yes, please describe_____
4) Has **name of participant** had any health care visits since the MRI?
    a. None
    b. Regular visit-If Yes
        i. Date (day/month/year)

ii. Reason-follow-up of problem, health care maintenance, other_____
iii. Description-hematatogy, primary care, other_____

c. Sick visit to doctor's office, if yes
   i. Date (day/month/year)
   ii. Reason-follow-up of problem, health care maintenance, other_____
   iii. Description-hematatogy, primary care, other_____
d. Sick visit to urgent care, if yes –date, reason, description_____
   i. Date (day/month/year)
   ii. Reason-follow-up of problem, health care maintenance, other_____
   iii. Description-hematatogy, primary care, other_____
e. Sick visit to emergency department, if yes –date, reason, description_____
   i. Date (day/month/year)
   ii. Reason-follow-up of problem, health care maintenance, other_____
   iii. Description-hematatogy, primary care, other_____
f. Hospitalization, if yes –start and end date, reason, description_____
   i. Date (day/month/year)
   ii. Reason-follow-up of problem, health care maintenance, other_____
   iii. Description-hematatogy, primary care, other_____

Appendix 20: Medical Monitor Responsibilities

1. Laboratory Monitoring
   - Patients will have lab testing done locally.
   - Each site will have a local medical monitor (LMM) who will review all blinded laboratory testing. The LMM will still be blinded to treatment assignment.
   - The LMM will issue stop orders for patients (on HU or on placebo) who meet toxicity criteria.
   - The LMM will notify Donna Whyte-Stewart, MD, the central medical monitor (CMM) about all toxicities that result in stop orders. Donna Whyte-Stewart's cell phone number is 315-378-2455.
   - This will allow:
     a. Central review of criteria for stopping study drug.
     b. Pairing of placebo patients for random stops.
   - The LMM will also notify the CMM when restart occurs.
   - The CMM will review all labs in REDCap weekly, to be sure that no toxicities are missed.

2. Random Stop Orders
   - When a placebo patient is enrolled, the CMM will assign a HU patient at another site to be a "mirror" subject.
   - If the HU patient in the pair is issued a stop order, the CMM (who will be notified of the stop as above) will call the local monitor for the paired placebo patient and have the LMM issue a stop order for that patient after the next set of labs, regardless of the lab results.
   - Restart will be similarly paired.
   - If a placebo patient has a toxicity (viral suppression for example) and receives a stop order, the paired HU patient will NOT be issued a stop order.

3. Dose Escalation
   - When an HU patient has a dose escalation, the LMM will notify the CMM. The CMM will keep track of the timing of this escalation (weeks from study initiation) and will notify the LMM about timing of dose escalations on the placebo patient paired to that HU patient.

4. Summary Points/Flow of Communication
   - LMMs will call CMM (315-378-2455) about:
     a. Toxicities requiring stop orders
     b. Restarts
     c. Dose escalations
   - The CMM will call LMMs regarding:
     a. Random stops on placebo patients
     b. Re-starts on placebo patients
     c. Dose escalations on placebo patients
     d. Missed toxicities after weekly review
   - The CMM will be notified of all newly randomized patients to allow mirror subject pairings.