Treatment of Hemoglobin SC Disease

Principal Investigator

Vivien Sheehan MD, PhD

Co-Investigators

Donald Mahoney MD
Amber Yates MD
Alex George MD, PhD
Ionela Iacobas MD

Statistician
Charles Minard, PhD

Texas Children's Hospital
6621 Fannin St.
Houston, TX 77030-2399

Abstract

Decades of observational data, including landmark natural history studies from the Cooperative Study of Sickle Cell Disease (CSSCD), have documented that sickle cell disease (SCD) is a severe, debilitating hematological disorder. Hydroxyurea has emerged as an excellent therapeutic agent for the pharmacological induction of HbF in patients with SCD, due to its ease of oral administration, modest toxicity profile, and clinical efficacy for preventing acute vaso-occlusive events. However, all completed clinical trials have excluded patients with hemoglobin SC (HbSC), restricting the inclusion criteria to patients with HbSS or HbSβ^0 disease. HbSC differs significantly in pathophysiology from HbSS, as HbC does not sickle, but instead causes cellular dehydration which potentiates sickling of HbS. HbSC patients demonstrate a wide variability of clinical courses and a rate of life threatening complications much higher than the general population.[1] Many severely affected HbSC patients have been placed on hydroxyurea on a case by case basis,[2,3] but there is no large scale prospective data on safety or efficacy of hydroxyurea in this subset of SCD patients.

The primary objective of this Phase II clinical trial is to treat symptomatic HbSC patients prospectively with hydroxyurea to maximum tolerated dose (MTD), and monitor for improvement using the PedsQL™ 3.0 Sickle Cell Disease Module by comparing scores at entry and at 6 months.

Secondary objectives for this trial are the following:

- Effect of hydroxyurea on the following laboratory values:
    - Whole blood viscosity
    - %DRBC
    - Fetal hemoglobin (HbF) levels
    - Mean corpuscular volume (MCV)
    - Mean corpuscular hemoglobin concentration (MCHC)
    - Hemoglobin (Hb) levels
    - Absolute reticulocyte count (ARC)
    - Absolute neutrophil count (ANC)
    - Liver function tests (LFT)
    - Creatinine
    - Lactate dehydrogenase (LDH)
    - Unconjugated bilirubin (u.bili) levels
    - Microalbuminuria as measured by routine urinalysis (UA)

- Genomic analysis of variants associated with HbF response and disease related complications. Sequencing will be performed by whole exome sequencing (WES) and traditional sequencing as indicated.

- Assessment of effect on retinopathy, sensorineural hearing loss or avascular necrosis (AVN) if found to be present at entrance screening.

This study will enroll patients with HbSC disease from 5 through 21 years of age, who have a PedsQL™ 3.0 Sickle Cell Disease Module score of 80 or lower.

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1.0 OBJECTIVES

1.1 **Primary objective of this Phase II study**: To determine if hydroxyurea, dosed to MTD, improves PedsQL™ 3.0 Sickle Cell Disease Module scores after 6 months on hydroxyurea at MTD compared to score at entry.

1.2 **Secondary objectives of this study**:

1. To investigate the effect of hydroxyurea on HVR in HbSC patients treated with hydroxyurea.

2. To investigate the effect of hydroxyurea use on %DRBC in HbSC patients.

3. To investigate changes in the following laboratory values of HbSC patients using serial measurements monthly for the duration of the study:

- Fetal hemoglobin (HbF) levels as determined by HPLC in the hemoglobin profile
- Mean corpuscular volume (MCV)
- Mean corpuscular hemoglobin concentration (MCHC)
- Hemoglobin (Hb) levels
- Absolute reticulocyte count (ARC)
- Absolute neutrophil count (ANC)
- Complete metabolic panel (CMP)
- Lactate dehydrogenase (LDH)
- Unconjugated bilirubin (u.bili) levels
- Microalbuminuria as measured by routine urinalysis (UA)

4. Genomic analysis of variants associated with HbF response and disease related complications in HbSC.

5. Assessment of effect of hydroxyurea therapy on retinopathy, sensorineural hearing loss or avascular necrosis (AVN) if found to be present at entrance screening.

2.0 BACKGROUND AND RATIONALE

2.1 **Hemoglobin SC disease**

Patients with hemoglobin SC disease are compound heterozygotes. One beta globin gene contains the sickle mutation, glutamic acid to valine change at the 6th codon, and the other beta globin gene contains the C mutation, a change from glutamic acid to lysine, also at the 6th codon. Each erythrocyte contains HbS and HbC in an approximate 50:50 ratio.

Hemoglobin SC disease is one of the most common inherited diseases in the United States, affecting approximately 1 in 833 African-American live births.[4] HbS undergoes intracellular polymerization in the deoxygenated state, leading to deformation of the red cell membrane and alteration of cellular physiology. While HbC does not polymerize, it increases the mean corpuscular hemoglobin concentration (MCHC) of the cell via volume regulated K+ efflux, thereby potentiating the polymerization of HbS.[5] In vitro studies of HbSC red blood cells found that reduction of MCHC from 37 to 33 g/dL would restore normal red blood cell morphology.[6]

Clinical manifestations of HbSS disease result primarily from chronic severe hemolytic anemia and the effects of repeated intravascular sickling of erythrocytes within the capillaries and small venules. Hemolysis leads to chronic anemia, gallstone formation, and intimal damage/hyperplasia within the arterial vasculature. Red blood cell sickling leads to acute vaso-occlusive events with varied presentations, including painful events, priapism, splenic sequestration, acute chest syndrome, or stroke. These processes occur in HbSC disease as well, but at 25-50% the frequency. Hemolysis in HbSC is reduced due to longer RBC survival, approximately 25% of a normal individual, or 28.9 ($\pm$4) days, compared to less than 21 days in HbSS disease.[7]

2.2 Hydroxyurea Therapy for HbSC disease: HbF response

Low levels of HbF in patients with SCD are associated with a variety of vaso-occlusive complications and an increased risk for early death. In patients with HbSS, hydroxyurea provides an important therapeutic option for patients with SCD, since it increases the amount of HbF within circulating erythrocytes, can be administered orally with once-daily dosing, has minimal short-term adverse effects, and is clinically effective. In a randomized, double-blinded, placebo-controlled Phase III trial involving severely affected adults with homozygous HbSS, hydroxyurea therapy was associated with significantly fewer painful vaso-occlusive events, episodes of acute chest syndrome, erythrocyte transfusions, and hospitalizations compared to observation alone.[8] The Multicenter Study of Hydroxyurea in Sickle Cell Anemia trial reported that adult patients treated with hydroxyurea had a 40% reduction in overall mortality after 9 years of follow-up.[9] In adults with SCD, hydroxyurea is well tolerated and leads to increases in hemoglobin concentration, mean corpuscular volume (MCV), and percentage HbF, as well as decreases in white blood cell and reticulocyte counts.[10]

Similar efficacy for hydroxyurea therapy has been observed in children with HbSS. A Phase I/II trial of hydroxyurea therapy in children 5 to 15 years of age with severe clinical manifestations (HUG-KIDS) demonstrated similar safety and hematological toxicities and efficacy at MTD as seen in adults.[11] Hydroxyurea was well tolerated and had no

negative effects on growth or development.[12] The most common short-term hydroxyurea toxicity was transient and reversible myelosuppression, primarily of the granulocyte series. While there is compelling evidence for the effectiveness of hydroxyurea in HbSS, HbSC patients were not included in these studies.

It has been hypothesized that HbSC patients may not benefit from hydroxyurea because adult patients have baseline HbF<5%, and exhibit little or no HbF induction on hydroxyurea.[13] In a cohort of 10 adult HbSC patients treated with hydroxyurea at doses ranging 500-1000 mg per day, a significant increase in MCV was noted. No significant change in HbF was noted for the group but one individual had a 6.8% rise in HbF.[14]

A report by Dr. Russell Ware of 6 pediatric patients with HbSC disease treated with hydroxyurea at MTD between 1995 and 2002 in the Duke Pediatric Sickle Cell Program supports the clinical and hematological efficacy of hydroxyurea therapy in children with HbSC disease. MCV increased significantly without increase in hemoglobin. Additionally HbF percentage and percentage of F-cells increased.[3] CHAMPS, a Phase II double blinded multicenter trial examined the efficacy of hydroxyurea and magnesium pidolate in children and adults with HbSC. In this trial, participants who received hydroxyurea (± magnesium pidolate) had significant increases in MCV and HbF, without changes in hemoglobin level. Additionally, children treated with hydroxyurea had greater increases in MCV and HbF after 24 weeks than adults. Unfortunately, clinical conclusions from this trial were limited because of its incomplete enrollment and early termination. [15]

Another study of eight pediatric HbSC patients placed on hydroxyurea therapy demonstrated an increase in MCV, but only two patients had an increase in HbF. However, the hydroxyurea dose was not increased to MTD, but remained stable at 15 mg/kg.[16] Of note, review of the medical charts of HbSC patients treated with hydroxyurea in our institutionfound the dose of hydroxyurea needed to reach MTD ranged between 15-25mg/kg, average 20 mg/kg. This is significantly lower than the average dose of 30 mg/kg needed to reach MTD in HbSS patients;[17] therefore, it is likely that a HbSC patient will reach MTD more rapidly, typically after 1-2 dose increases. Two patients had hydroxyurea held for thrombocytopenia, two more for neutropenia. These reports also highlight the importance of establishing safe drug dose initiation and titration in HbSC patients, who may be more sensitive to the myleosuppressive effects of hydroxyurea than HbSS patients.

2.3 **Role of whole blood viscosity and hematocrit to viscosity ratio (HVR) in the pathophysiology of HbSS and HbSC disease:**
Recent reports have shown higher whole blood viscosity to correlate with more frequent pain events, leg ulcers, and ACS in patients with HbSS. Patients with HbSC disease have similar rates of AVN, and higher rates of proliferative sickle retinopathy (PSR), than HbSS patients. Pain events and ACS, while less frequent than in HbSS, still occur in HbSC, especially in adolescence. These observations lead some providers to offer therapeutic phlebotomy to symptomatic HbSC patients. A large series of 179 HbSC patients treated with phlebotomy, reported 71% had significant reduction in pain events. The authors have recommended phlebotomy for all symptomatic adult HbSC patients with Hb greater than 10.5 g/dL. In this phlebotomy therapy applied to adult HbSC patients, the Hb set point reduction is achieved by inducing iron deficiency, an undesirable event in a developing child. It is therefore imperative that alternative therapies like hydroxyurea be thoroughly tested in pediatric HbSC patients prior to investigating phlebotomy as a therapeutic option.

A more specific measure of SCD pathology is the HVR, a measure of oxygen carrying capacity, calculated by the ratio of hematocrit to viscosity at various shear rates. It is abnormally low at both high and low shear in HbSS and HbSC patients. Individuals with sickle cell related leg ulcers have a statistically significant lower HVR compared to sickle cell patients without leg ulcers. Since microcirculatory dysfunction is the root of SCD pathology, contributing to pain events, avascular necrosis and chronic leg ulcers, it is essential that SCD therapies are evaluated for their effect on HVR. Chronic transfusion therapy does not improve HVR, and may decrease HVR at low shear rates that model the venous circulation.

The HVR has been calculated at high and low shear rates for 98 HbSS patients on hydroxyurea, 53 HbSC (untreated) and 43 HbSS (untreated), Table 1. Hydroxyurea treatment improved HVR in HbSS patients, indicating an increase in oxygen carrying capacity. HVR did not correlate with HbF in any of the three groups individually, or as a whole. It is encouraging that HVR, low in both HbSC and HbSS patients, significantly improves on hydroxyurea in a HbF independent manner, especially since few HbSC patients demonstrate a meaningful rise (final %HbF of 20% or more) in HbF on hydroxyurea. A secondary endpoint of this protocol is to evaluate the effect of hydroxyurea on HVR in HbSC patients.

Table 1:

	HbAA n=19	HbSS, untreated n=43	HbSS, on Hydroxyurea n=98	HbSC n=53
Age (years)	15.4 ± 3.8	10.4 ± 5.1	10.7 ± 3.4	10.5 ± 4.3
Hemoglobin (gm/dL)	13.5 ± 1.7	8.5 ± 1.0	9.9 ± 1.4	11.0 ± 1.2
Hematocrit (%)	40.9 ± 5.3	25.5 ± 3.1	28.4 ± 3.7	31.3 ± 3.2
Viscosity (cP) at $45s^{-1}$	5.3 ± 0.9	4.6 ± 1.2	4.3 ± 0.9	5.5 ±0.9
HVR at $45s^{-1}$	7.5 ± 0.9	5.8 ± 1.1	6.75 ± 1.0	5.77 ± 0.7
Viscosity (cP)at $225s^{-1}$	3.8 ± 0.5	3.3 ± 0.5	3.4 ± 0.5	4.1 ± 0.5
HVR at $225s^{-1}$	10.3 ± 0.7	7.7 ± 0.8	8.53 ± 0.8	7.72 ± 0.6

2.4 Role of % dense red blood cells (HVR) in the pathophysiology of HbSS and HbSC disease:

Another contributor to SCD pathology is the abnormal density of the red cells. K+ efflux from red blood cells (RBC) decrease water content and increase hemoglobin concentration. Dense red blood cells, or DRBC's, have a intracellular hemoglobin concentration of 40-50 g/dL, compared to a normal cell, with a hemoglobin concentration of approximately 33 g/dL. DRBCs are highly prone to sickling, have increased rigidity, and decreased stability. %DRBC correlated significantly with skin ulcers, priapism, and renal dysfunction. Hydroxyurea has been shown to decrease %DRBCs by 34% after 6 months of therapy in HbSS patients, independent of degree of HbF induction. Its impact on DRBC's in HbSC patients is unknown; this determination is another secondary endpoint of our protocol. %DRBCs have been measured using Pthalate oil mixtures in the past; our ADVIA hematology system is able to measure % RBC in an automated fashion.

2.5 Hydroxyurea Response Variability in Sickle Cell Disease

The response to hydroxyurea therapy in SCD is highly variable.[18] Patients are typically escalated to the MTD based on laboratory myelosuppression, aiming for an absolute neutrophil count of 1.0 – 3.0 x 10^9/L. With proper compliance, virtually every child with SCD will increase their HbF during hydroxyurea therapy at MTD, but the magnitude of the response varies from 10% to >30% in HbSS. Currently, there is no way to predict which child will have a high or low HbF response to hydroxyurea prior to reaching MTD for HbSS patients, much less the HbSC subtype. We hypothesize that genetic differences beyond the sickle gene mutation influence the HbF response to hydroxyurea therapy, and that these differences may be found in the various SCD subtypes as well as HbSS.

2.6 **Treatment Rationale**

Patients with HbSC generally have a milder course than HbSS patients. However, a select number will have significant clinical complications in childhood, and a majority of patients with HbSC develop chronic organ damage in adulthood. The potential efficacy of hydroxyurea to postpone, prevent, or even reverse chronic organ damage in patients with HbSC disease has not been determined for either children or adults. Viscosity and HVR is of major importance in the pathophysiology of HbSC disease. A small trial of hydroxyurea in HbSC patients showed a statistically rise in hemoglobin levels, which may increase whole viscosity.[19] However, even a rise in hemoglobin may lead to an improvement in HVR, if other rheological benefits, observed in hydroxyurea treated HbSS patients, such as a reduction in dense cells, are also experienced in HbSC patients on hydroxyurea. In our study patients whose HVR drops 20% below baseline levels will be removed from the study. Serial measures of HVR in the same individual on different days in the PI's laboratory exhibit 20% variability. This is an important safety measure for patients who may experience a rise in whole blood viscosity and viscosity related complications on hydroxyurea, a theoretical concern.

2.7 **PedsQLTM 3.0 Sickle Cell Disease Module**

The PedsQL™ 3.0 Sickle Cell Disease Module is based on frequently used generic health related quality of life tools (HRQL). It is a 43 item module, divided onto age ranges 5-7 years, 8-12 years, 13-18 years, and adult. It contains questionnaires to be administered to the patient and the parent. This clinical research tool has been validated, found reliable and feasible in a multi-center trial. Texas Children's Hematology Center participated in this trial, where it was self-administered in the clinic setting where appropriate. The PedsQL™ 3.0 Sickle Cell Disease Module is not a standard clinic procedure, therefore utilization of the module will occur after consent.

3.0 RESEARCH PARTICIPANT ELIGIBILITY CRITERIA AND STUDY ENROLLMENT

3.1 **Inclusion Criteria**

3.1.1 Diagnosis of HbSC disease
3.1.2 Age from 5 to 21 years of age.
3.1.3 Score equal or lower than 80 on the PedsQL™ 3.0 Sickle Cell Disease Module
3.1.4 Have experienced a sickle cell disease related complication

3.2 Exclusion Criteria

3.2.1 Failure to meet inclusion criteria
3.2.2 Hydroxyurea usage in the last 3 months.
3.2.3 Chronic RBC transfusion therapy
3.2.4 Packed red blood cell transfusion in the last 3 months (temporary exclusion).
3.2.5 Pregnancy, or refusal to use medically effective birth control if female and sexually active.

3.3 Research Participant Recruitment and Screening

Study participants will be patients with HbSC disease who receive medical care from the Hematology Center of Texas Children's Hospital. Recruitment of external participants will consist of those physicians and patients who contact the Texas Children's Hospital principal investigator and express an interest in participating. Physicians and patients may learn of this study either through the www.clinicaltrials.gov website or via presentations at scientific meetings. Of patients followed at Texas Children's Hospital, all patients with HbSC disease will be consented prior to screening for eligibility with the PedsQLTM 3.0 Sickle Cell Disease Module. Those with a score equal to or lower than 80, will be offered hydroxyurea therapy and invited to participate in this study. The protocol will be explained to them and consent obtained by the Principal Investigator or designee.

3.4 Subject Recruitment and Informed Consent

Subjects will be recruited from the patient population followed at Texas Children's Hospital. All pediatric patients who meet eligibility criteria will be approached for consent at their scheduled physician/clinic visit.

The Principal Investigator, Study Coordinator, or another member of the research team will confirm eligibility based on the criteria listed above, explain the protocol, and obtain informed consent from the subject's legal guardian(s) in accordance with federal regulations and IRB policies at Baylor College of Medicine. Where applicable, assent from the subject will also be obtained. No undue coercion will be placed on subjects or parents to participate in the study

4.0 STUDY DESIGN AND METHODS

4.1 Study Design

The primary objective of this Phase II study is to prospectively and uniformly treat symptomatic HbSC patients to MTD on hydroxyurea, and assess for clinical improvement using the PedsQL™ 3.0 Sickle Cell Disease Module after 6 months at MTD, compared to entrance scores.

Patients will be consented prior to screening for eligibility with the PedsQLTM 3.0 Sickle Cell Disease Module.

Also examined will be the effects of hydroxyurea on HVR, %DRBC, Hb, HbF, MCV, MCHC, ANC and ARC. LDH and u bilirubin will be monitored as indirect measures of hemolysis. Hydroxyurea dose escalation to a stable MTD with evidence of mild myelosuppression will occur according to published guidelines.[20]

This is an unblinded Phase II trial; all patients enrolled will be placed on open-label hydroxyurea. Drug effect will be determined based on change in laboratory values from baseline. All laboratory tests, red cell density and viscosity measures will be performed every two months. Testing will be performed on peripheral blood obtained during hydroxyurea monitoring visits.

4.2 METHODS

4.2.1. Viscosity measurements

Measures of whole blood viscosity will be performed according to current guidelines.[21] Whole blood will be obtained at routine clinic visits and collected in EDTA tubes, and analyzed within 4 hours of venipuncture. All specimens will be warmed to 37^{o}C prior to measurement. A Brookfield cone and plate viscometer in Dr. Sheehan's laboratory will be used. Samples will be run at moderate and high shear stress, 45 s^{-1} and 225 s^{-1} respectively, at 37^{o}C. 0.5 mL of whole blood will be used for each measure. The sample used for viscosity measures will also be analyzed on the ADVIA 120 hematology analyzer in order to measure the red cell density.

4.2.2 Laboratory measurements

Routine clinical laboratory assays will be performed at Texas Children's Hospital clinical laboratories. Research lab assays will be performed in Dr Sheehan's laboratory. ADVIA and measurements will be used for research purposes only, to determine %DRBC.

4.2.3. Studies of phenotypic variability

The HbF response will be evaluated as both a continuous and a categorical variable. Change in HbF at MTD will be analyzed as a continuous variable using linear regression analysis. Peripheral blood mononuclear cells will be isolated from 5mL venous blood, and genomic DNA will be purified using standard laboratory techniques. Small aliquots of DNA will then be tested for genetic variations that could modify the baseline and treatment HbF levels. Analyses will be performed to identify gene polymorphisms associated with HbF response and other laboratory and clinical variables.

5.0 CLINICAL AND LABORATORY EVALUATIONS

Hydroxyurea Dosing Parameters:

- **Initiation:** Initiate hydroxyurea (HU) at 10 mg/kg daily, with the exact dose rounded up or down to the nearest practical dose based on formulation. The actual starting dose should be within 2.5 mg/kg of the calculated 10 mg/kg dose. A liquid formulation of hydroxyurea may be used for children unable to swallow pills, or to improve dosing accuracy.

- **Dose escalation:** Escalate HU dose by 5 mg/kg/day every 8 weeks up to a maximum dose of 35 mg/kg/day if blood counts meet escalation criteria prior to dose increase (Table 3).

- **Response to Toxicity:** Hematologic toxicity: If one or more blood counts fall into the toxic range, stop hydroxyurea and recheck blood counts weekly. Restart hydroxyurea at the same dose if the affected blood counts recover within 1 week. Reduce hydroxyurea dose by 2.5 mg/kg if toxicity persists for more than 1 week or if there is a previous history of toxicity at the current dose.

  Non-hematologic toxicity: Hydroxyurea will be held if liver function tests rise to twice the upper limit of normal, or if creatinine rises to 1.5 the upper limit of normal. Relevant laboratory values will be rechecked weekly, and hydroxyurea restarted at a reduced dose, 2.5 mg/kg lower than the dose at which non-hematologic toxicity was experienced.

Definition of MTD: dose of hydroxyurea at which ANC is between 1000 and 3000/μL, OR ARC is between 70,000 and 100,000/μL, or platelet count is between 70,000 and 100,000/μL on CBC.

In order to evaluate the effectiveness of hydroxyurea therapy in HbSC patients, patients must remain at MTD through the 6 month evaluation period. MTD is defined in this protocol. If a study participant is not at MTD at a given clinic visit after achieving MTD at the current dose, the laboratory values and PedsQLTM 3.0 Sickle Cell Disease Module scores will not be included in the study analysis, and an additional month will be added to the study duration.

Table 2: Schedule of Evaluations

	Entry	Scheduled Clinic Visits *	Exit
PedsQL™ 3.0 Sickle Cell Disease Module	x	x	x
X-ray bilateral shoulders	x		x
X-ray bilateral hips	x		x
Retinal exam	x		x
Sensorineural hearing test	x		x
Genomic DNA extraction from peripheral blood	x		
CBC with differential ARC Hemoglobin profile CMP LDH u. bili	x	x	x
UA/microalbumin *	x		x
Whole blood viscosity	x	x	x
%DRBC	x	x	x

** Microalbumin will be measured at study entry and exit only if within normal limits at entry. If above normal limits, it will be measured at * scheduled clinic visits occuring every two months .Retinal exam, x-rays, and sensorineural hearing tests must be no older than one year, or obtained within 2 months of study entry. Baseline studies may be obtained at or before second study visit.*

5.1 **Special Instructions for Evaluations**

Whole blood viscosity measures will be performed on blood collected in EDTA vacutainer tubes, on a Brookfield cone and plate viscometer. Measures will be taken no more than 4 hours after sample collection, at shear rates of 45 and 225 s^{-1}. Red cell density will be obtained using an ADVIA hematology analyzer.

5.1.1 Laboratory Evaluations: All laboratory testing, excepting microalbumin, whole blood viscosity and red cell density measurements, are currently obtained as a part of routine clinical management every 2 months in patients with SCD who are initiating hydroxyurea therapy.

Vital Signs, weights and measures will be recorded during the subject's participation in the study. Results of laboratory studies for all study participants will be collected in the study database for future analysis.

Table 3: Criteria for Hydroxyurea escalation and cessation

Laboratory value	Toxicity Criteria (Any)	Escalation Criteria (All Must be Met)
ANC (per uL)	< 1000	> 3000
ARC (per uL)	<70,000	> 100,000
Platelets (per µL)	< 70,000	> 100,000

5.2 **Medical Record Review**

Study personnel will review the subject's medical record to abstract all laboratory test results and other testing obtained as described for this study, for the study duration. We will also review the history of disease-related complications from birth. We would like to monitor significant clinical events on an ongoing basis as well, for the duration of the study and the two year post-study observation period if indicated. Key areas to be collected include information such as: 1) pain episodes 2) acute chest syndrome 3) stroke or transient ischemic attacks, 4) acute splenic sequestration, 5) death.

Specific diagnoses, rather than signs or symptoms will be recorded whenever possible.

5.3 **Off-Study Evaluations**

No off study evaluations will be required.

6.0 OUTCOME MEASURES

6.1 Peds QL Sickle Cell Module™ scores
6.2 Whole blood viscosity and HVR
6.3 %DRBC
6.4 Laboratory analysis
6.5 Observed disease related complications during study period, compared to same duration of time immediately preceding study entry.
6.6 Variability in Hydroxyurea Response: The genetic basis for hematological variability of HbF induction for children receiving

hydroxyurea therapy at maximum tolerated dose (MTD) will be assessed, through collection of genomic DNA, which will be submitted for whole exome sequencing under NHGRI U54HG003273. This sequencing information may also be used to investigate associations between variants and disease related complications.

7.0 CRITERIA FOR REMOVAL FROM PROTOCOL AND OFF-STUDY CRITERIA

7.1 Off-Study Criteria

1) Subject decides to no longer participate in the study

2) Pregnancy

3) Investigators may discontinue any subject at their discretion, if in their professional opinion, the subject's health, safety, and/or well-being is threatened by continued participation in the study.

4) Subject decides to stop hydroxyurea treatment permanently

5) Reduction of more than 20% below baseline of HVR

6) Lost to follow-up—a letter will be sent to the last known address of study participants who are lost to follow up informing them that they are being taken off-study.

7) Death

8) If a study patient requires a transfusion for any reason, they will be removed from the study for a duration of 3 months, while continuing on hydroxyurea during that period. At the end of the 3 months, they will resume all normal study activities.

8.0 SAFETY AND ADVERSE EVENT REPORTING REQUIREMENTS

This protocol has a category 2 risk, research involving greater than minimal risk, but presenting the prospect of direct benefit to the individual subjects. Monitoring and reporting of adverse events will comply with the BCM Institutional Review Board requirements.

Subjects on study will have scheduled clinic visits every two months, and laboratory evaluations throughout the period of dose titration to MTD and clinical observation, totaling 12 months. During these visits, they will be closely monitored for any clinical or laboratory evidence of toxicity associated with hydroxyurea or study participation. All toxicities and adverse events will be scored utilizing criteria listed in the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 and toxicities ≥ grade 3 will be included in the visit data collection. Further assessment, testing, and interventions for any adverse events, including hydroxyurea dose reduction or interruption of therapy, will be performed according to section 5.0, Response to Toxicity. Subjects with severe (grade 3 or worse) toxicities or adverse events will be reviewed in detail by the study P.I. in conjunction with the Study Coordinator and primary clinician and will be considered for termination from the study. The Principal Investigator will provide a Continuing Review Report to the Baylor College of Medicine IRB at least annually. In addition, all serious adverse events and UPIRSOs that meet reporting criteria as defined by the BCM IRB will be submitted to the BCM IRB per their policy. Expected adverse events will not be reported, only adverse events related to hydroxyurea and study participation.

All safety data collected on study participants will be reviewed by the Texas Children's Non-Cancer Data Review Committee (TXCH DRC). An interim analysis for safety and efficacy will be performed when half of the enrolled participants have reached the 6 month point.

9.0 DATA COLLECTION, STUDY MONITORING, AND CONFIDENTIALITY

Data collection and data management for this investigation will be conducted through the BCM Department of Pediatrics, Section of Hematology-Oncology. Co-investigators assigned to this protocol will be responsible for assisting the PI in assuring protocol compliance as well as reviewing, transcribing and tracking of all clinical and safety related data. All personnel will receive Human Subjects Protection training with appropriate annual updates as required.

Each subject's medical record will be reviewed and existing data will be collected that is relevant to this study.

Clinical data will be transcribed from source documents directly into an electronic database. This database will be password protected and accessible only to the Principal Investigator and her designated staff.

Copies of the subject's Informed Consent document will be maintained in a locked file in the BCM Department of Pediatrics, Section of Hematology-Oncology

Confidentiality
Protocol Accession Numbers assigned in the Patient and Protocol Management (PPM) will be used in place of an identifier such as a medical record number. No research participant names will be recorded on documents used in data analysis, or used on any documents submitted for publication of study results.

9.1 Potential Risks

All patients in this study will be on hydroxyurea, which has repeatedly been shown to be safe and cause minimal side effects in patients with sickle cell disease when used with appropriate clinical monitoring. The most common side effects associated with hydroxyurea therapy are a drop in infection fighting cells, or white blood cells, a drop in platelet count, stomach discomfort, skin discoloration or roughness, blood may become thicker and less efficient at carrying oxygen and mild hair loss. Most patients taking hydroxyurea do not experience these symptoms.

Potential risks include the risks associated with laboratory blood draws, which will be minimized by the use of careful sterile technique when drawing blood from peripheral veins or venous access lines that are already in place. The blood volume (total 3 mL per sampling point) being procured for this study is well within our local institutional guideline for maximal volume allowed for the smallest potential study subject.

Subjects may risk loss of confidentiality pertaining to protected health information obtained during the study. To minimize this risk, these data will be collected and stored using coded identifiers, locked storage facilities and password-protected databases, each accessible only to the investigators.

There exists a small potential for special risks to privacy relevant to the collection and storage of specimens for DNA, and the planned posting of coded genomic sequences to dbGaP. As the study of these specimens will be restricted solely to identify gene polymorphisms in low versus high Hydroxyurea responders, and stored samples will be labeled only by coded identifiers, these special risks will be minimized.

10.0 STATISTICAL CONSIDERATIONS

The primary outcome measure of interest is the change in PedsQL 3.0 Sickle Cell Disease Module score at 6 months after achieving MTD compared with baseline. Previous research suggests that the standard deviation (SD) of scores is expected to be about 20 points.[22] Assuming SD=20 at each time point and a correlation between repeated measures of 0.50, a sample size of 34 subjects would be required to detect a 10 point change in scores at 6 months versus baseline with 80% power using a two-sided, paired t-test. Therefore, a total of 41 subjects will be recruited for this study to allow for a 20% attrition rate. Baseline patient characteristics will be summarized by means with standard deviations, medians with 25th and 75th percentiles or frequencies with percentages as appropriate.

A two-sided, paired t-test will be used to test the primary null hypothesis that there is no change in PedsQL 3.0 Sickle Cell Disease Module score at 6 months after achieving MTD versus baseline. Statistical significance will be assessed at the 0.05 level. This hypothesis will also be tested using a general linear mixed model. The mixed model will use all available data, include a fixed effect for time (discrete) and will assume an unstructured matrix of correlated error terms. Approximate normality will be qualitatively assessed using quantile-quantile plots, and data transformations will be used if required. The mixed model will also be used to explore associations between the primary outcome measure and baseline characteristics, simultaneously adjusting for time. A similar mixed model will also be used to simultaneously assess differences from baseline at all repeated, every two months time points up to 12 months post-study entry. P-values will be adjusted using Holm's step-down Bonferonni correction, and statistical significance will be assessed at the 0.05 corrected level.

Secondary outcome measures, including HVR, %DRBC, Hb, HbF, MCV, MCHC, ANC, ARC, LDH and unconjugated bilirubin will be similarly assessed. Baseline values will be compared to laboratory values obtained at stable MTD (5 months or longer at MTD) using a two-sided paired t-test. Laboratory values that differ significantly between baseline and MTD will be analyzed by simple linear regression.

Samples collected for genetic testing will be subjected to whole exome sequencing, and analyzed for the association between change in HbF at MTD and exonic variants using linear association and burden analysis. Other phenotypes of interest, such as HVR, %DRBC, or SCD related complications may also be investigated in a similar manner, together with non-study participants

Subjects who stop HU therapy for any reason (e.g., pregnancy, toxicity, SCD complications requiring other intervention, non-adherence to medication dosing, patient or family's request to discontinue therapy) will be considered off-therapy.

Subjects who miss two or more follow-up evaluations will be sent a letter indicating that they are “off-study”.

11.0 STUDY DURATION

This protocol describes a planned 12 months of therapy. At the end of this period, study participants will be asked if they would like to participate in a 2 year observation period. In this observation period, the laboratory tests and PedsQL™ 3.0 Sickle Cell Disease Module scores that were obtained every two months during the 12 months of the study will be administered at each clinic visit, scheduled according to clinic guidelines and individual need.

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