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Version 4.1 (02/14/2017)

TREATMENT OF ADULT PATIENTS WITH HEMOGLOBIN SC DISEASE

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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 HbS β^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 Adult QL™ 3.0 Sickle Cell Disease Module by comparing scores at entry and after 6 months of hydroxyurea therapy at maximum tolerated dose.

Secondary objectives for this trial are the following:

- Effect of hydroxyurea on the following laboratory values:
 - Whole blood viscosity
 - Red cell density
 - 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)
- Patients with an AdultQL™ 3.0 Sickle Cell Disease Module demonstrating less than a 10 point improvement after 6 months of hydroxyurea therapy at MTD will be offered therapeutic phlebotomy. Clinical improvement on this second line therapy will be assessed with the AdultQL™ 3.0 Sickle Cell Disease Module as well.
- 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.

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This study will enroll patients with HbSC disease from 18 through 69 years of age, who have evidence of disease-related complications or an AdultQL™ 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 AdultQL™ 3.0 Sickle Cell Disease Module scores after 6 months of hydroxyurea therapy at MTD compared to score at entry.

1.2 Secondary objectives of this study:

1. Assess the role of phlebotomy for patients who fail to show a 10 point improvement on the AdultQL™ 3.0 Sickle Cell Disease Module scores after 6 months at MTD of hydroxyurea.
2. To investigate the effect of hydroxyurea on HbSC rheology, including viscosity, %dense red blood cells (%DRBC), red cell deformability, and time of transit through an artificial microvascular network.
3. To investigate changes in the following laboratory values of HbSC patients using serial measurements every two months 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. (Optional) Genomic analysis of variants associated with HbF response and disease related complications. Expression level changes from RNA obtained at baseline and at stable MTD.

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

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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 Role of whole blood viscosity in the pathophysiology of 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.^{1,8} Pain events and ACS, while less frequent than in HbSS, still commonly 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 had recommended phlebotomy for all symptomatic adult HbSC patients with Hb >10.5 g/dL. In this phlebotomy therapy applied to adult HbSC patients, the Hb set point reduction is achieved by inducing iron deficiency. Later publications noted the lack of linear relationship between hemoglobin levels and whole blood viscosity. Current recommendations are to base phlebotomy decisions on individual's whole blood viscosity measurements.⁹

2.3 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.¹⁰ 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.¹¹ 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.¹²

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

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(HUG-KIDS) demonstrated similar safety and hematological toxicities and efficacy at MTD as seen in adults.¹³ Hydroxyurea was well tolerated and had no negative effects on growth or development.¹⁴ 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%.¹⁵ 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.¹⁶

A report by Dr. 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

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.¹⁷ Of note, review of the medical charts of HbSC patients treated with hydroxyurea in our institution found 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;¹⁸ therefore, it is likely that a HbSC patient will reach MTD more rapidly, typically after 1-2 dose increases.

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. Unfortunately, clinical conclusions from this trial were limited because of its incomplete enrollment and early termination.¹⁹

2.4 Effect of Hydroxyurea on whole blood viscosity

Elevated hemoglobin has been implicated in several clinical complications of sickle cell disease. The Cooperative Study of Sickle Cell Disease (CSSCD) identified increased hemoglobin as a major risk factor for development of acute chest syndrome (ACS) in adults. Higher hemoglobin values appear to correlate with increased frequency of pain crises.^{20,21} Since hemoglobin concentration is the largest contributor to viscosity, it can be inferred that increased hemoglobin concentration resulted in higher viscosity. A study of packed red blood cell rheology of HbSS patients showed a strong correlation between increased viscosity and end organ damage.²² Patients with SCD are often transfused with non-sickled RBC in order to prevent or reverse severe complications such as stroke or acute chest syndrome. These transfusions reduce blood viscosity and increase oxygen transport effectiveness.²³ However, if patients are transfused above a hematocrit of 30%, hyperviscosity could worsen the condition the transfusion was initiated to treat or prevent.²⁴ This consideration must also guide any pharmacological therapy that may increase hemoglobin, and therefore whole blood viscosity. It is of even higher

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importance in patients with HbSC, who already have higher baseline hemoglobin values than most HbSS individuals.

2.5 Hydroxyurea Response Variability in Sickle Cell Disease

The response to hydroxyurea therapy in SCD is highly variable.²⁵ Patients are typically escalated to the MTD based on laboratory myelosuppression, aiming for an absolute neutrophil count of $1.0 - 3.0 \times 10^9/L$. With proper compliance, virtually every patient 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 patient 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 is of major importance in the pathophysiology of HbSC disease. A small trial of hydroxyurea in HbSC patients showed a statistically significant rise in hemoglobin levels, which may increase whole blood viscosity.²⁶ In our study, patients who do not show clinical improvement on hydroxyurea will be placed on therapeutic phlebotomy if their whole blood viscosity is at or above baseline at either shear rate. Patients will not be taken off of hydroxyurea when phlebotomy is initiated. They will not be on study therapy, but will continue to be monitored clinically, and rheological testing performed. This allows collection of preliminary data to assess an alternative therapy widely used in adult HbSC patients in Europe.²⁷

2.7 AdultQL™ 3.0 Sickle Cell Disease Module

The AdultQL™ 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. It contains questionnaires to be administered to the patient and the parent if present. 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.

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 18 to 69 years of age.
- 3.1.3 Score of 80 or lower on the AdultQL™ 3.0 Sickle Cell Disease Module, or any disease related complication, including, but not limited to, one or more pain events per year, proliferative sickle retinopathy, avascular

necrosis, cholelithiasis, or any thrombotic event. If the subject has a score >80, they may still enroll on the trial, and be analyzed for secondary endpoints. They will be excluded from analysis of the primary endpoint.

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.
- 3.2.6 Current phlebotomy therapy

3.3 Research Participant Recruitment and Screening

Study participants will be patients with HbSC disease who receive medical care from the Texas Children's Cancer and Hematology Centers (TCC/HC)/Baylor College of Medicine (BCM) and the University of Texas Houston Hematology Center. Recruitment of external participants will consist of those physicians and patients who contact the Texas Children's Hospital principal investigator and/or the University of Texas Houston Hematology Center 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 the Texas Children's Cancer and Hematology Centers (TCC/HC)/Baylor College of Medicine (BCM) and the University of Texas Houston Hematology Center, all patients with HbSC disease will be screened with AdultQLTM 3.0 Sickle Cell Disease Module. Those with a score of 80 or lower, or any disease related clinical complication, 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 her designee.

3.4 Enrollment on Study

Subjects will be recruited from the patient population followed at the Texas Children's Cancer and Hematology Centers (TCC/HC)/Baylor College of Medicine (BCM) and the University of Texas Houston Hematology Center. Eligibility to the study will be confirmed by the Principal Investigator or her designee based on the inclusion/exclusion criteria defined in Section 3.0 of the protocol. All patients who meet eligibility criteria will be approached for consent at their scheduled physician/clinic visit. The investigational nature and objectives of the study, the procedures involved and their attendant risks and discomforts, and potential alternative options will be carefully explained to the patients. If they would like to proceed, written consent will be obtained from eligible subjects, in conformity to the BCM and UT human research policies and to the federal regulations.

4.0 STUDY DESIGN AND METHODS

4.1 Study Design

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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 AdultQL™ 3.0 Sickle Cell Disease Module after 6 months in the study, compared to entrance scores.

Also examined will be the effects of hydroxyurea on viscosity, red cell density, Hb, HbF, MCV, MCHC, ANC and ARC. LDH and unconjugated 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.²⁸

Patients who do not show improvement after treatment with hydroxyurea for 6 months at MTD will be offered phlebotomy, and observed clinically and rheologically on study. Phlebotomy may be required monthly, but only visits two months apart will include questionnaire administration. A sample for rheological measurements will be obtained with each phlebotomy, but no less than every two months. Since initially phlebotomy will be performed monthly, rheological analysis may occur more often than every two months. Hydroxyurea will not be continued, and iron deficiency will not be prevented. Standard phlebotomy guidelines will be followed. Induction of iron deficiency has been shown to be beneficial in reducing viscosity-related complications in sickle cell disease, and will reduce the erythropoietic drive, prolonging the effects of phlebotomy.²⁹⁻³¹

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.³² 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°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°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 and University of Texas Houston Hematology Center clinical laboratories.

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Research lab assays will be performed in Dr Sheehan’s laboratory. ADVIA and measurements will be used for research purposes only, to determine red cell density.

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

Table 1: Study Roadmap

	Entry	At MTD	Every two months	Exit
AdultQL™ 3.0 Sickle Cell Disease Module	x		x	x
X-rays of bilateral² shoulders and hips	x			x
Retinal exam¹	x			x
Sensorineural hearing test²	x			x
Genomic DNA extraction from peripheral blood	x			
RNA from peripheral blood	X	x		
CBC with differential ARC Hemoglobin profile CMP LDH	x		x	x
Microalbumin³	x			x
Rheological measurements⁴	x		x	x
Pregnancy testing⁵	X		X	X

1. May be obtained up to 2 months after enrollment
2. Optional exam (voluntary assessment)
3. If abnormal at study entry, microalbumin will be checked at every study visit
4. May be performed with each phlebotomy, no less than every two months, but potentially monthly

5. Only for female subjects who are sexually active and do not report a highly effective method for contraception

Hydroxyurea Dosing Parameters:

- **Initiation:** Initiate hydroxyurea 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.
- **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 (see table below).
- **Response to 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.

Table 2: 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

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.

Determination of MTD and Dose Adjustments:

Subjects will be followed for a minimum of eight weeks after initiation and each dose adjustment to determine if they meet criteria for MTD at this dose. **The definition of MTD for study purposes will be the dose at which the subject maintains an ANC, ARC, or platelet count in the target range (defined in Table II) over one CBC check performed at eight weeks after the last dose adjustment.**

If criteria for toxicity are met, treatment will be suspended for one week and will then be resumed at the same dose after count recovery is documented. If toxicity persists past one week or if it occurs more than once at the same dose, therapy will be suspended until count recovery and will then be restarted at a dose 2.5 mg/kg less than the prior dose.

Any deviations from this dose adjustment regimen, including suspension of therapy, dose reduction, or dose escalation, that do not meet the criteria detailed above must be reviewed with the study P.I. before being put into effect. Certain circumstances will be considered as grounds for such deviations without being considered a violation of study procedures:

(1) In consultation with the study P.I., the subject's primary clinician may choose not to escalate the hydroxyurea dose despite 8-10 weeks of therapy at the current dose and CBC parameters meeting criteria for escalation if the trajectory of changes in the subject's CBC parameters (a rapid rise in MCV; a rapid drop in ANC, ARC, or platelet count) suggests that the subject would not tolerate further dose escalation. In this situation, the primary clinician, after consultation with the study PI, may choose to observe the subject at the current dose for an additional four weeks before escalating therapy.

(2) In consultation with the study P.I., the subject's primary clinician may choose not to escalate the hydroxyurea dose despite 8-10 weeks of therapy at the current dose and CBC parameters meeting criteria for escalation if there is evidence of non-adherence to therapy over this period. In this circumstance, the primary clinician must discuss the importance of adherence with the subject and re-evaluate the suitability of dose escalation after an additional four weeks of therapy at the current dose.

(3) In the event of significant illnesses such as severe systemic infection, acute renal insufficiency or hepatic dysfunction, acute gastroenteritis, or other significant illnesses, the primary clinician may decide, in consultation with the study P.I., to suspend therapy or delay a scheduled dose escalation until the acute illness has resolved.

The reasons for and duration of any deviations from the scheduled dose escalation protocol should be clearly documented. In all cases, a lack of dose escalation over a 12-14 week period should lead to a review of the subject's status and suitability for continued participation in the study.

Indications for Initiation of Phlebotomy:

AdultQL™ 3.0 Sickle Cell Disease Module score after 6 months of hydroxyurea therapy at MTD unchanged or reduced compared to baseline.

Phlebotomy Schedule: The first phlebotomy volume will be 7 ml/kg, but may be increased to 10 ml/kg to obtain the target Hb of 9-10 g/dL. Initially, phlebotomy is to be performed monthly. If Hb is

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≤9.0 g/dL on a phlebotomy visit, phlebotomy will not be performed, and phlebotomy visits will be spaced 2 months (8 weeks) apart.

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. Red cell deformability will be measured with a RheoScan ektacytometer. Microfluidics will be analyzed with an artificial microfluidics network, proprietary design of Dr. Shevkopyas.³³⁻³⁵ All necessary equipment is present in the PI, Dr. Sheehan's laboratory, located on the 10th floor of the Feigin Center, or in the Shevkopyas laboratory in the Biomedical Engineering department at University of Houston.

5.1.1 Laboratory Evaluations: All laboratory testing, except whole blood viscosity and red cell density, 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.

The PI, Dr. Sheehan, will receive DNA samples and laboratory data from study participants with HbSC disease enrolled on the IRB approved study at the University of Texas Houston Hematology Center.

DNA samples will be coded by researchers at the University of Texas Houston Hematology Center to preserve anonymity prior to distribution to BCM. The samples will be transferred, evaluated and stored in Dr. Sheehan's laboratory. Once the coded samples have been obtained from the University of Texas Houston Hematology Center the BCM investigator will perform experimental rheology lab tests of viscosity and percent dense cell measurements, and all the data analysis. The Shevkopyas lab will perform ektacytometry and artificial microvascular network analyses.

5.2 Medical Record Review

Study personnel at Texas Children's Hospital/Baylor College of Medicine and the University of Texas Houston will review the subject's medical record to abstract results of the above tests that have been done as routine care prior to participating in this study. Results will be considered to be baseline (pre-therapy) data for the purpose of this protocol if they were obtained within 12 months of hydroxyurea treatment initiation. Texas Children's Hospital/Baylor College of Medicine and University of Texas Houston Hematology Center healthcare providers and study personnel will have access to subject's past medical history.

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

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participant names will be recorded on documents used in data analysis, or used on any documents submitted for publication of study results.

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.

Additionally, we would like to monitor significant clinical events on an ongoing basis. 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. These clinical complications are part of the sickle cell disease process, and have not been linked to hydroxyurea use. Development of these complications will not prompt removal from the hydroxyurea arm of the study. Since there is a correlation between stroke and more severe anemia in HbSS, development of a stroke or TIA will prompt removal from the study if the patient is on the phlebotomy arm.

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

5.3 **Off-Study Evaluations**

No off-study evaluations are planned.

6.0 OUTCOME MEASURES

6.1 AdultQL™ Sickle Cell Module scores

6.2 Whole blood viscosity

6.3 Red cell density

6.4 Red cell deformability

6.5 Transit time through and artificial microfluidics network

6.6 Expression level changes due to hydroxyurea therapy

6.7 Laboratory analysis

6.8 Observed disease related complications during study period, compared to same duration of time immediately preceding study entry.

6.9 Variability in Hydroxyurea Response: The genetic basis for hematological variability of HbF induction for patients 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

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- 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) 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.
- 6) Death

8.0 SAFETY AND ADVERSE EVENT REPORTING 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 a minimum of 12 months. Patients requiring more than 6 months to reach MTD will have the duration of study increased to permit 6 months observation at MTD. HbSC patients often reach MTD at significantly lower doses of hydroxyurea than HbSS, hastening achievement of MTD, often at 15-20 mg/kg/day. Placement on phlebotomy will extend the study by an additional 6 months to permit 6 months observation on this therapy.

During study visits, patients will be closely monitored for any clinical or laboratory evidence of toxicity associated with hydroxyurea or phlebotomy. 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 \geq 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. Adverse events related to loss of confidentiality will be reported.

All safety data collected on study participants will be reviewed by the Texas Children's Non-Cancer Data Review Committee (TXCH DRC).

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

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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 co-investigators 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.

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 appropriately monitored. The most common side effects associated with hydroxyurea therapy are a drop in infection fighting cells, or white blood cells, platelets, which help the blood clot, stomach discomfort, skin discoloration or roughness, and mild hair loss. Most patients taking hydroxyurea do not experience these symptoms when dosed appropriately.

Potential risks include the risks associated with phlebotomy, 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 6 mL per sampling point) being procured for this study is well within our local institutional guidelines for maximal volume allowed.

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 AdultQL™ 3.0 Sickle Cell Disease Module 3.0 Sickle Cell Disease Module score after 6 months of hydroxyurea therapy at MTD compared with baseline. Previous research suggests that the standard deviation (SD) of scores

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is expected to be about 20 points.³⁶ 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 after 6-months of hydroxyurea therapy at MTD 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 AdultQL™ 3.0 Sickle Cell Disease Module 3.0 Sickle Cell Disease Module score at 6-months of hydroxyurea therapy at 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, q2 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 viscosity, %DRBC, red cell deformability, transit time through an artificial microfluidics network, Hb, HbF, MCV, MCHC, ANC, ARC, LDH and unconjugated bilirubin will be similarly assessed. The proportion of patients requiring phlebotomy will be estimated with 95% exact, binomial confidence intervals. Summary statistics for baseline characteristics will also be stratified by phlebotomy group. Univariable analysis will compare baseline characteristics between groups using independent, two-sample t-tests, Wilcoxon rank sum test, or Fisher's exact test as appropriate. A multiple logistic regression model will be used to estimate odds ratios with 95% confidence intervals comparing patients requiring phlebotomy versus those who do not require the procedure.

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".

Phlebotomy will be offered to patients who do not show improvement in QoL scores after 6 months of hydroxyurea therapy at MTD. All primary and secondary outcome measures will be assessed as described above. It is not known how many patients will meet criteria for phlebotomy. Patients who meet criteria for phlebotomy may refuse phlebotomy, and elect to exit the study or remain in the study on hydroxyurea therapy. Statistical analysis and calculation of power needed to see effect therefore cannot be calculated. Inclusion of phlebotomy will allow us to obtain preliminary data needed to design a clinical trial to assess phlebotomy therapy in HbSC individuals who do not experience clinical improvement on hydroxyurea.

Whole exome sequencing will be performed on genomic DNA. Samples will be included in a larger study of genetic variants involving pediatric and adult SCD genomes associated with HbF

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levels at baseline and HbF levels at hydroxyurea MTD already underway (H31356). Linear regression analysis and T1 gene based testing will be performed for each variable, with genotype and age as an independent variables. Whole genome analysis may be performed in the future.

RNA will be obtained from CD71+ cells at baseline and at MTD. RNASeq will be performed on study samples, as well as HbSS samples collected on protocol H35374. Fractional counts will be quantile-normalized followed by t-test for change in expression between paired samples pre-hydroxyurea and at MTD.

We will identify individual transcripts, splice isoforms, allele-specific effects and gene networks that are altered by hydroxyurea, and the association between the degree or type of alteration and HbF response to hydroxyurea. We will also compare expression levels at baseline between individuals, to determine if expression levels differ between individuals with higher or lower endogenous HbF levels.

11.0 OBTAINING INFORMED CONSENT

The process of obtaining informed consent will follow UT institutional guidelines. Informed consent will be obtained by the attending physician or his/her designee. After the diagnosis of HbSC is confirmed and the research participant is deemed eligible, consent will be obtained from the patient. Informed consent will be obtained prior to administration of the AdultQL™ 3.0 Sickle Cell Disease Module questionnaire. If the subject has a score >80, they may still enroll on the trial, and be analyzed for secondary endpoints. They will be excluded from analysis of the primary endpoint.

12.0 STUDY DURATION

This protocol describes a planned 12 months of therapy. Patients requiring more than 6 months to reach MTD will have the duration of study increased to permit 6 months observation at MTD. Placement on phlebotomy will extend the study by an additional 6 months.

At the end of this period, study participants on hydroxyurea, on phlebotomy, or on no disease specific therapy will be asked if they would like to participate in a 2 year observation period. In this observation period, the laboratory tests and AdultQL™ 3.0 Sickle Cell Disease Module scores that were obtained every two months during the planned 12 months of the study will be administered at each clinic visit, scheduled according to clinic guidelines and individual need.

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