

CLINICAL RESEARCH PROJECT**Protocol: 14-H-0172****IND/IDE: Exempt**

NHLBI Protocol: *Pilot Study to Assess Algorithm-Based Hydroxyurea Dosing in Subjects with Sickle Cell Disease*

Abbreviated Title: Algorithm Hydroxyurea Dosing

Keywords: Hydroxyurea, dosing algorithm, fetal hemoglobin, sickle cell disease

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Subjects of study:

Number

24

Sex

Male/Female

Age range

≥15 years

Project involves ionizing radiation?

No

Off site project?

No

Multi-Institutional project?

No

DSMB involvement?

No

Tech Transfer: CRADA, MTA

No

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1.0 PRECIS

Sickle cell disease (SCD) is associated with significant morbidity and early mortality. Despite the discovery of the disease more than 100 years ago, only one drug, hydroxyurea (HU), has been FDA-approved. Hydroxyurea exerts its beneficial effects largely by inducing fetal hemoglobin (HbF) and thereby inhibiting red blood cell sickling. Hydroxyurea has been shown to decrease the frequency of acute complications such as painful crises and acute chest syndrome. However, previous studies are conflicting regarding whether HU improves survival; 2 long-term studies where HU was titrated to the maximum tolerated dose show that HU improves survival. However, multiple studies performed in the era post-FDA approval of HU show no change in median survival. We and others have found that patients with SCD who die prematurely have more evidence of renal, hepatic, and cardiopulmonary damage. Our work also suggests that HU treatment per se is not sufficient to improve survival and decrease organ damage in patients with homozygous SCD (HbSS). Instead, patients treated with the highest HU doses and who had the highest HbF levels appeared more likely to survive and had less evidence of organ damage over time. Hydroxyurea management can be intimidating; therefore, many adults with HbSS are either not treated with HU or are treated with doses below that which are FDA-approved. A HU dosing algorithm may simplify dosing such that not only are more patients treated with HU, but more may be titrated to the maximal tolerated dose which may be necessary to prevent organ damage and prolong survival. Further, myelosuppression beyond what has traditionally been recommended may further maximize HbF response. This protocol is a prospective pilot study which follows a 2 month run-in period. Hydroxyurea dosing will be based on a written algorithm which will be derived manually, and by a computer program which was developed at the NIH Clinical Center. Clinical, laboratory, and echocardiographic parameters will be monitored at baseline and after treatment to further study the effect of maximum HbF response on acute complications associated with HbSS and organ function.

2.0 OBJECTIVES

- 2.1 Determine whether a hydroxyurea (HU) dosing algorithm leads to a statistically significant change in fetal hemoglobin (HbF) response as compared to baseline HbF in patients with homozygous sickle cell disease (HbSS). The 95% confidence interval of the change will be calculated to see if the change is statistically significant.
- 2.2 Determine whether maximum HbF response improves organ function in patients with HbSS.

3.0 BACKGROUND

3.1 Pathophysiology

Homozygous sickle cell disease was first described when sickled red blood cells (RBC) were found on a peripheral blood smear.¹ Decades later, an abnormal hemoglobin was identified in patients with the disease.² Sickle hemoglobin forms polymers upon deoxygenation^{3,4} and transforms RBC into rigid structures which occlude the microvasculature. Vessel occlusion leads to acute complications such as recurrent painful crises and acute chest syndrome. One study evaluated 232 patients with sickle cell disease (SCD) who completed a daily pain diary for up to 6 months.⁵ While pain was reported in 54.5% of patient-days, only 3.5% of patient-days involved presentation to an emergency room, hospital, or clinic. Therefore, patients frequently experience

pain at home without presenting for evaluation and treatment. Patients may also experience chronic organ injury such as sickle nephropathy, sickle hepatopathy, and cardiopulmonary complications. Further, organ damage has been associated with premature mortality in patients with HbSS.⁶⁻¹⁶ In a retrospective analysis involving our own cohort of patients with SCD with a mean follow-up of 3.6 years, deceased patients were more likely to have laboratory and echocardiographic evidence of organ injury at the time of enrollment and at most recent follow-up as compared to subjects remaining alive (Table 1).¹⁷

Table 1: Multiple Logistic Regression Analysis of Variables Associated with Deceased Subjects

Variable ^a	Chi-Square Score	P-value
First Visit		
↑ Alkaline Phosphatase	25.14	<0.0001
↑ Creatinine	8.57	0.02
Last Visit		
↑ Direct Bilirubin	11.43	0.0007
↓ Ejection Fraction	4.16	0.04
↑ Tricuspid Regurgitant Velocity	31.59	<0.0001

↑: Higher value is associated with deceased status. ↓: Lower value is associated with deceased status.

3.2 Treatment Options

To date, HU is the only FDA-approved drug for the management of patients with HbSS. The drug was approved based on the multi-center study of HU (MSH) where patients with HbSS were randomized to receive HU starting at a dose of 15 mg/kg/day and increased as tolerated to a maximum dose of 35 mg/kg/day or placebo.¹⁸ The trial was stopped early because patients who received HU had significantly decreased hospitalizations for pain crises and acute chest syndrome and had a decreased transfusion requirement. Hydroxyurea was also found to significantly decrease the number of days that patients needed analgesic medications at home as compared to the placebo group.¹⁹

HU largely exerts its effect by increasing HbF and inhibiting RBC sickling.²⁰⁻²³ Long-term HU studies further demonstrate improved survival.^{14,15} Conversely, studies from the post-HU approval era found that patients continue to die by the fifth decade of life.^{9,12,13,16,24} Further, health database studies have shown no significant change in mortality rates between the years before and after HU approval.^{16,25} However, HU dosing was not considered by these studies, and only 7-37% of patients were prescribed HU at the time of death.^{9,12,13,24} When we retrospectively queried data regarding patients with SCD screened at the NIH, we found that HU dosing was independently associated with prolonged survival (p=0.006).^{26,27}

Lastly, our recent retrospective analysis suggests that patients with the highest HbF response were more likely to survive and also had less evidence of organ damage over time. The mean HbF seen throughout the period of follow-up in the highest HbF quartile group was 26 as compared to 1.5% in the lowest HbF quartile group, and the median HU doses were 24.1 versus 4.8 mg/kg/day, respectively^{26,27}. Patients in the highest HbF quartile group had less evidence of renal, hepatic, and cardiopulmonary dysfunction at enrollment and most recent follow-up as compared to the lowest HbF quartile group (Table 2).

Table 2: Comparison of Organ Function Parameters in Patients with HbSS Based on HbF Quartile

Variable	First Visit			Last Visit		
	Low HbF	High HbF	P-value	Low HbF	High HbF	P-value
Ejection Fraction (%)	56.2	59.9	0.004	57.5	60.2	0.04
Alanine Aminotransferase (U/L)	34.6	25.7	0.01	38.9	28.5	0.03
Aspartate Aminotransferase (U/L)	52.0	41.4	0.04	54.7	33.7	<0.0001
Creatinine	1.7	0.7	0.002	2.0	0.9	0.004

A study performed in infants with HbSS which employed a fixed dose of HU at 20 mg/kg/day did not meet the primary endpoints of preventing organ damage.²⁸ However, a small case series reported splenic regeneration in patients with HbSS when HbF increased to 30%. These results were supported by two larger pediatric studies where patients were treated with maximally tolerated doses of hydroxyurea,^{29,30} one confirming that HbF 25% was more likely to be associated with some splenic uptake as compared to HbF 14%.³⁰ It is possible that HbF approaching 30% may be crucial to prevent organ injury caused by sickled RBC-induced vascular damage.^{8,31,32} However, improvement in secondary markers of splenic function seen in the infant study²⁸ suggests that even modest increases in HbF can be beneficial.

Multiple studies have evaluated medication adherence in patients who take HU. The MSH study evaluated adherence by counting capsules that were left in bottles, percentage of clinic visits that were kept, serum HU levels, and percentage of patients who reached toxic myelosuppression.³³ Adherence was defined as administering at least 80% of capsules and keeping at least 80% of clinic visit appointments. They found that patients with HbF levels in the highest quartiles had medication adherence rates ranging from 81-83%. Conversely, adherence rates were 56 to 71% in patients in the lowest HbF quartiles. Other studies have reported adherence rates ranging from 35-58% in 2 studies^{34,35} to 75-88.9% in 3 studies.³⁶⁻³⁸ As expected, adherence rates were higher in the last 3 studies since patients were followed prospectively as compared to the previous 2 studies which were retrospective analyses.

3.3 Clinical and Scientific Justification

The most common indications for HU initiation in patients with HbSS are recurrent pain crises and acute chest syndrome. Hydroxyurea has also been used in patients with severe anemia since it significantly improves hemoglobin levels. The most frequent adverse effect associated with HU is myelosuppression. The MSH study permitted HU titration as long as the following parameters were not met: absolute neutrophil count (ANC) less than 2,000/uL and an absolute reticulocyte count (ARC) and platelet count less than 80,000/uL or a dose of 35 mg/kg/day.¹⁸ These

parameters now define what is considered the maximum tolerated dose (MTD) in standard clinical practice. Only 65% of patients with HbSS screened at the NIH Clinical Center were treated with HU despite the vast majority meeting disease criteria severe enough to warrant initiating HU. Because we are a referral center and most patients are managed by their outside hematologist, we have not been able to control what percentage of patients who are followed at outside institutions start HU. However, as ANC ranged from 5,600 to 4,900/uL, ARC ranged from 267,000 to 222,800/uL, and platelet count ranged from 399,700 to 353,100/uL between patients who had ever administered HU at the time of enrollment and at most recent follow-up, respectively, MTD was not reached in our cohort. Further, the median HU dose in all patients with HbSS was only 19.4 mg/kg/day. In the MSH study, only 33% of patients in the HU group were administering HU at MTD or had received a higher dose that was later decreased within 6 months of initiating HU.¹⁸ By the end of the study, 51% of the patients were on a MTD or close to maximal dose.

Since the definition of MTD based on blood counts was arbitrary, it may be necessary to tolerate lower blood counts in order to maximize HbF induction. In the most recent years, we at the NIH have been tolerating an ANC as low as 1,500/uL when titrating HU dosages in patients with HbSS. The goal has been to start HU at 15 mg/kg/day and increase the dose by 5 mg/kg every 2 months until MTD is reached. However, due to the complexity involved in their care, often the focus has not been to push the HU to MTD. Ideally, a dosing algorithm would make the HU dose titration process easier, more effective, and less intimidating for primary providers who frequently manage adult patients with HbSS. Further, a computer program which is able to calculate a HU dose based on patients' blood counts and the timing of most recent HU dose titration would improve the percentage of patients whose HU is increased to MTD. Proof of principle is evidenced by multiple computer-based warfarin dosing studies which increased confidence regarding warfarin dosing for doctors and nurses, decreased the number of venipunctures, and either produced dosing results that were equal to or superior to manual dosing recommendations.³⁹⁻⁴¹

As described above, HU has been shown to decrease acute complications associated with SCD. Further, HU, particularly when pushed to MTD and thereby maximizing HbF response, may improve survival and prevent organ damage. While HU commonly leads to myelosuppression, other serious side effects are rare. There has been no evidence that HU increases the risk of secondary malignancies above that in the general population of patients with HbSS.⁴² Therefore, treatment of all adult patients with HbSS, and not just those with recurrent acute complications, is justified.

4.0 STUDY DESIGN

This is a one arm, open-label, non-randomized pilot study to evaluate the effect of algorithm-based HU dosing on the HbF response, the ability to titrate each patient to the MTD of HU, acute complications, and organ function in patients with HbSS. The primary outcome will be the change of HbF level from baseline with algorithm-based HU dosing. The detailed definitions about primary endpoints and secondary endpoints are given in Section 12.0.

The study will follow a 2 month run-in period during which time 3 visits will occur (first visit and then another visit 1 and 2 months later). During the run-in period, patients will remain off of HU

or continue their current dose of HU, and baseline data will be gathered. Patients will then be treated with HU for 1 year according to a dosing algorithm.

The IT and computational support is provided by the NHLBI DIR Scientific Information Office, and Information Technology and Applications Center. The program features a Php/HTML built front-end and a MySQL database in the background. Hydroxyurea doses will be derived manually according to the written algorithm and also be determined by the computer program.

A licensed independent practitioner (LIP) or pharmacist will review laboratory data for all patients on this protocol.

Samples will be collected to monitor HbF, red blood cells with HbF (F-cells), and reticulocytes with HbF (F-reticulocytes). Frequency of hospitalizations and transfusion requirements will be monitored as well as pain scales and quality of life. Further, as we found that patients with renal, hepatic, and cardiopulmonary dysfunction were more likely to die prematurely and there was also evidence that HU may prevent or reverse damage involving those organs, renal, hepatic, and cardiopulmonary function will be monitored closely. To assess kidney function, urine albumin and protein, urine osmolality, serum creatinine, glomerular filtration rate (GFR), and cystatin C will be monitored. Regarding the liver, alkaline phosphatase, direct bilirubin, transaminases, and gamma-glutamyl transpeptidase (GGT) will be followed. To evaluate cardiopulmonary function, ejection fraction, tricuspid regurgitant velocity (TRV), pro-brain natriuretic peptide (BNP), 6 minute walk distance, and oxygenation with ambulation will be followed.

5.0 SUBJECT RECRUITMENT AND/ REGISTRATION

5.1 Recruitment efforts

The study will be listed on the clinicaltrials.gov, Clinical Center research studies, and the National Heart, Lung and Blood Institute patient recruitment websites. We will also ask local providers who follow patients with HbSS such as Howard University Hospital and Children's National Medical Center to refer patients. Further, local patients who are followed on our National History Study and our Screening Study will be asked to participate in this study. If at least 6 patients are not enrolled every 6 months, a recruitment plan will be developed by the Clinical Center Office of Patient Recruitment.

6.0 ELIGIBILITY ASSESSMENT

6.1 Inclusion Criteria

- 6.1.1** Age \geq 15 years
- 6.1.2** Homozygous sickle cell disease (HbSS)
- 6.1.3** Patients with recent transfusion must have HbA $<15\%$ prior to enrollment
- 6.1.4** ANC $\geq 2,000/\mu\text{L}$, platelets $\geq 150,000/\mu\text{L}$, Hb $>5.4\text{g/dL}$, and ARC $\geq 100,000/\mu\text{L}$ (unless the Hb is $>8\text{g/dL}$) at baseline
- 6.1.5** Patients on angiotensin-converting enzyme inhibitors and angiotensin receptor blockers should be on a stable dose for 2 weeks prior to initiating or adjusting HU

6.2 Exclusion Criteria

- 6.2.1** Pregnant or lactating women or patients planning to get pregnant during the study period
- 6.2.2** Patients unwilling to use two forms of contraception throughout the period of HU administration
- 6.2.3** Patients receiving chronic transfusion therapy
- 6.2.4** Patients receiving a HU dose of ≥ 20 mg/kg/day
- 6.2.5** Patients with history of allergy or intolerance to HU judged by the investigator to be prohibitive against restarting HU
- 6.2.6** Patients with end stage renal disease defined as GFR < 10 mL/min/1.73m²
- 6.2.7** Patients being treated with antiretroviral agents (such as didanosine and stavudine) because of a higher risk for potentially fatal pancreatitis, hepatic failure, hepatitis, and severe peripheral neuropathy when co-administered with hydroxyurea.
- 6.2.8** Participation on any other chronic investigative treatment studies
- 6.2.9** Unable to understand the investigational nature of the study or give informed consent

7.0 CLINICAL EVALUATION OF THE PATIENT

7.1 Pre-study evaluation will be performed under our screening protocol (08-H-0156) or our natural history study (04-H-0161)

- 7.1.1** History and physical examination
- 7.1.2** CBC with differential, ARC
- 7.1.3** Hemoglobin electrophoresis
- 7.1.4** Acute care panel, hepatic panel
- 7.1.5** HIV antibody
- 7.1.6** Urine or serum pregnancy test in women of childbearing potential
- 7.1.7** Eligible patients will sign informed consent

7.2 On-study baseline evaluation and evaluation during the run-in period:

The following studies will be repeated every month +/- 7 days for a total of 2 months:

- 7.2.1** CBC with differential, ARC
- 7.2.2** Hemoglobin electrophoresis, F-cells, F-reticulocytes
- 7.2.3** Acute care panel, hepatic panel, GGT, BNP, cystatin C, lactate dehydrogenase (LDH), Erythropoietin (EPO)
- 7.2.4** GFR: The following equation will be used to calculate GFR (Chronic Kidney Disease Epidemiology Collaboration, CKD-EPI)⁴³⁻⁴⁵

$$\text{GFR (mL/min/1.73 m}^2\text{)} = 141 \times \min(\text{Scr}/k, 1)^\alpha \times \max(\text{Scr}/k, 1)^{-1.209} \times 0.993^{\text{Age}} \times 1.018 \text{ (if female)} \times 1.159 \text{ (if African-American)}$$
 where Scr is serum creatinine, k is 0.7 for females and 0.9 for males, α is -0.329 for females and -0.411 for males, min indicates the minimum of Scr/k or 1, and max indicates the maximum of Scr/k or 1.

- 7.2.5** Urine protein/creatinine ratio, urine albumin/creatinine ratio, urine osmolality

7.3 Evaluation prior to starting or increasing HU:

The following studies will be performed within one month +/- 7 days of starting or increasing HU unless otherwise indicated:

- 7.3.1** A pain diary which surveys pain intensity on a 0-10 scale and medications required will be completed at home daily for a 2 week period
- 7.3.2** Transthoracic echocardiogram
- 7.3.3** 6-minute walk
- 7.3.4** PROMIS quality of life questionnaire (see Section 9.6)
- 7.3.5** The concentration of HbF within F-cells
- 7.3.6** RBC Deformability
- 7.3.7** Urine or serum pregnancy test will be performed in women of childbearing potential within 48 hours of ordering the first dose of HU in patients not already taking HU

7.4 Outpatient monitoring

- 7.4.1** Patients will have CBC with differential and ARC drawn 2 weeks +/- 7 days after every HU dose adjustment
- 7.4.2** Patients will be seen once monthly +/- 7 days before MTD is reached to check pill count, to continue to encourage compliance, and to evaluate for adverse effects
- 7.4.3** CBC with differential, hemoglobin electrophoresis, ARC, acute care panel, and hepatic panel will be checked once monthly +/- 7 days until patients reach MTD
- 7.4.4** History and physical will be performed every 2 months +/- 7 days
- 7.4.5** CBC with differential, hemoglobin electrophoresis, ARC, acute care panel, and hepatic panel will be checked every 2 months +/- 7 days after patients reach MTD
- 7.4.6** After reaching MTD, patients will be called every month that they are not seen in clinic to encourage compliance with HU administration
- 7.4.7** F-cells, F-reticulocytes, the concentration of HbF within F-cells, RBC Deformability, GGT, BNP, LDH, EPO, cystatin C, GFR, urine protein/creatinine ratio, urine albumin/creatinine ratio, and urine osmolality will be checked every 4 months +/- 7 days
- 7.4.8** Urine or serum pregnancy test will be checked in women of childbearing potential every 4 months +/- 7 days
- 7.4.9** A pain diary will be completed at home daily for a 2 week period every 4 months +/- 7 days
- 7.4.10** PROMIS quality of life questionnaire will be performed every 4 months +/- 7 days (see Section 9.6)
- 7.4.11** Transthoracic echocardiogram will be performed at 12 +/- 1 month
- 7.4.12** 6-minute walk will be performed at 12 +/- 1 month

8.0 TREATMENT PLAN

The dosing algorithm is as follows:

- 1) Starting dose: Normal renal function- 15 mg/kg/day (rounded to the nearest 200, 300, or 500 mg capsule), given in a single daily dose. Abnormal renal function (10 to 60 mL/min/1.73m²) 7.5 mg/kg/day⁴⁶ (rounded to the nearest 200, 300, or 500 mg capsule).

- 2) Patients already receiving/taking HU will initially stay on their current dose unless the dose is less than the starting dose above.
- 3) If tolerated for at least 8 weeks without toxicity, increase HU dose by 5 mg/kg/d in patients with normal renal function and 2.5 mg/kg/d in patients with abnormal renal function and monitor as above (up to a maximum of 35 mg/kg/day). The weight of the patient at the time of the HU adjustment will be used to calculate the next dose.
- 4) Check complete blood count (CBC), differential, and absolute reticulocyte count (ARC) two weeks after every dose adjustment.
 - a) Stop HU if any of the following are detected on CBC:
 - i) ANC <1,500/uL
 - ii) Platelets <80,000/uL
 - iii) Hb <5.4 g/dL
 - iv) ARC <80,000/uL only if Hb < 8 g/dL
 - b) If HU is held, recheck CBC and ARC within 1-2 weeks. If counts are above toxicity parameters, restart at the same dose.
 - c) If the blood counts are still abnormal after 1-2 weeks, recheck again in 1-2 weeks. If counts are above toxicity parameters, restart at the same dose.
 - d) If toxicity recurs or persists, stop as above, and later resume therapy with a dose reduced by 2.5 mg/kg/day.
- 5) Blood counts will be repeated 4-5 weeks after every dose adjustment.
 - a) The same holding parameters will then be followed as described above.
- 6) If we are unable to complete evaluations listed above as scheduled due to subject experiencing a complication such as vaso-occlusive crisis, then the evaluations will be done at the subject's next study visit.
- 7) Subjects will be provided with a reserve supply, with a maximum of 7 doses of HU at each visit to ensure that they will not run out of study drug in between study visits. If they have not used the reserve supply provided from the previous visit, then no additional reserve supply will be provided.
- 8) If total Hb increases to ≥ 12 g/dL, patients are at risk for hyperviscosity. Therefore, serum ferritin will be checked and one unit of blood can be phlebotomized up to every 14 days to keep hemoglobin <12 g/dL. If hemoglobin remains above 12 g/dL after 2 phlebotomy attempts, HU dose will be decreased by 2.5 mg/kg/d.
- 9) If a patient experiences intolerable (defined as grade 2 or higher) gastrointestinal toxicity (vomiting and/or diarrhea) that is not helped by anti-emetics, the prescribed daily dose will be divided and administered twice daily. If the patient continues to have grade 2 or higher gastrointestinal toxicity after the dose is divided, then the hydroxyurea dose will be decreased by 2.5 mg/kg/d.
- 10) Patients must be at least 70% compliant during the 8 week period in order for the HU dose to be increased.

- 11) Toxicity is defined as significant myelosuppression as defined in section 8.0, #4a or total Hb that remains ≥ 12 g/dL despite 2 phlebotomy attempts as stated in section 8.0, #6.
- 12) MTD is defined as 2.5 mg/kg/d lower than the dose that reaches toxicity after 2 different attempts OR 2.5 mg/kg/d lower than the dose that takes more than 2 weeks to recover counts OR 2.5 mg/kg/d lower than the dose that causes grade 2 or higher gastrointestinal toxicity despite splitting the dose OR a dose of 35 mg/kg/d.
- 13) The results of pregnancy tests will be reviewed by either the LIP, research nurse, or pharmacist. The study drug will be ordered prior to obtaining the test results, but will not be dispensed until the results are reviewed.
- 14) Hydroxyurea may be held or the dose may be split into two equal doses for adverse events at the discretion of the PI, until the AE sufficiently resolves. The PI will consider the nature of the adverse event and the likely relation to drug therapy to make a determination of whether to restart at the same dose level or reduce the dose, or recommend discontinuation of HU and removal from the protocol.

Patients will be followed at the NIH for 1 year after HU initiation or dose increase. If a subject develops an acute vaso-occlusive crisis requiring hospitalization during that time, they will be encouraged to return to the NIH Clinical Center for admission, where they will receive standard of care. After patients complete the one year period of the study drug, they may be enrolled on to a different protocol if available or HU management will be transitioned to their primary hematologist.

9.0 RESEARCH STUDIES

9.1 RBC deformability

Decreased deformability is a characteristic of erythrocytes from patients with SCD. Ektacytometry allows for the determination of red cell shape in response to defined shear stresses. Methods have recently been reported that correlate deformability characteristics and number of irreversibly sickled cells or percentage of HbS⁴⁷. The deformability and aggregation characteristics of red blood cells will be monitored at baseline and every 4 months +/- 7 days by ektacytometry. Data will be analyzed in terms of both absolute deformability and deformability as a function of hemoglobin composition.

9.2 F-Cells and F-Reticulocytes

The percentage of red blood cells and reticulocytes that contain HbF will be monitored at baseline and every 4 months +/- 7 days. We will also assess the percentage of HbF within F-Cells since the F-Cells with the highest percentage of HbF are more likely to inhibit sickle hemoglobin polymerization.⁴⁷

9.3 Cystatin C

Cystatin C is a ubiquitous protein that is freely filtered at the glomerulus and not reabsorbed. After filtration, it is catabolized and is not detected in the urine. Serum cystatin C-based GFR may be a more accurate measurement for GFR as compared to creatinine-based GFR in patients with SCD.⁴⁸ Cystatin C will be measured at baseline and every 4 months +/- 1 week.

9.4 Pain Diary

A pain diary will be administered which will evaluate a pain intensity score from 0-10 and pain medications required. These measurements will be collected daily over a 2 week period.¹⁹ A comprehensive pain score will be calculated by adding these pain scores over each two week time period. Pain diaries will be administered at baseline and every 4 months +/- 1 week.

9.5 Quality of Life

PROMIS quality of life questionnaire will be administered at baseline and every 4 months +/- 1 week. The specific forms that will be used are the Pain Interference - Short Form 8a, Fatigue-Short Form 8a, Ability to Participate in Social Roles and Activities- Short Form 8a, and Physical Function- Short Form 8b. Each of the PROMIS short forms will be administered in paper and pencil format, and each takes less than five minutes to complete. Each of the PROMIS short forms has items on a 5-point Likert scale. The total raw score is derived from summing the individual item responses, and raw scores are converted to standardized t-scores, with a mean of 50 and standard deviation of 10.

10.0 SAMPLE COLLECTION, STORAGE AND TRACKING PLAN

10.1 Sample storage

Research samples will have patient identifiers and be stored in the secure laboratory of the principal investigator or Core Lab. The PI will be responsible for overseeing entry of data into an in-house password protected electronic system. Research samples will be stored using BSI in accordance with NHLBI DIR Biospecimen policy.

Data gathered via the computer program will be stored in a MySQL database. Access to the computer program is password protected through the standard NIH portal.

10.2 Intended use

During the course of participating on this study, blood and data will be collected for correlative laboratory research studies. Specimens collected strictly for research purposes will not be read by a pathologist.

10.3 Tracking

Samples will be ordered and tracked through CRIS Research Screens. Should a CRIS screen not be available, the NIH form 2803-1 will be completed and will accompany the specimen and be filed in the medical record.

10.4 End of study procedures

The study will remain open so long as sample or data analysis continues. Samples from consenting subjects will be stored until they are no longer of scientific value or if a subject withdraws consent for their continued use, at which time they will be destroyed.

10.5 Loss or destruction of samples

Should we become aware that a major breech in our plan for tracking and storage of samples has occurred, the IRB will be notified.

10.6 Publication Policy

Given the research mandate of the NIH, patient data including the results of testing and responses to treatment will be entered into an NIH-authorized and controlled research database. Any future research use will occur only after appropriate human subject protection and institutional approval such as prospective NIH IRB review and approval or an exemption from the NIH Office of Human Subjects Research (OHSR). Data will not be sent outside the NIH without IRB notification and an executed MTA or CTA.

11.0 DATA MANAGEMENT PLAN

11.1 Data collection

The PI will be responsible for overseeing entry of these data into an in-house password protected electronic system and ensuring data accuracy, consistency, and timeliness. The principal investigator and associate investigators, research nurses and/or a contracted data manager will assist with the data management efforts.

All human subjects personally identifiable information (PII) as defined in accordance to the Health Insurance Portability and Accountability, eligibility and consent verification will be recorded. Primary data obtained during the conduct of the protocol will be kept in secure network drives or in approved alternative sites that comply with NIH security standards. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant, (e.g., study-specific identifying number [SSPIN]) or other unique code, or minimum PII required for subject identification. PII will not be contained within the computer program; instead, study identifiers will be used to keep track of HU dosing information, weight, blood counts, and kidney function.

12.0 BIOSTATISTICAL CONSIDERATIONS

12.1 Sample size

The sample size is estimated based on comparison of the HbF change from baseline to year 1 with no change. Based on data from our previous studies, the mean absolute change in HbF (measured in percentage) at months 8, 10, and 12 from baseline is about 11% with a standard deviation of change of about 10% for patients with standard HU dosing. With the assumption of HbF change for this study being of a similar magnitude and using a paired t-test, a sample of 9 will be needed for this single arm study to achieve 83% power at a significance level of 0.05 on testing the null hypothesis that there is no significant difference between the baseline and year 1 HbF after the algorithm-based dosing. The sample size calculation was performed in SAS 9.3, using the power procedure.

We assume that up to 30% of patients will be noncompliant (defined as missing >20% of pills within one month on at least 2 occasions). Further, because red blood cell transfusion may suppress HbF, frequent transfusions may interfere with HU response. Based on our current cohort of 71 patients that we follow consistently, up to 20% of patients receive more than 2 transfusions per year. Therefore, we assume that up to 20% of patients will receive more than 2 transfusions per year. So the total dropout rate is expected to be up to 60%. To account for this, a sample size of 24 patients would be needed. Those patients who receive more than 2 transfusions per year or who are noncompliant as described above will be discontinued from the protocol.

Parameters to be followed:

- a. HbF, F-cells, F-reticulocytes
- b. ANC, Hb, MCV, platelet count, ARC
- c. Cr, AST, ALT, total bilirubin, direct bilirubin, GGT, LDH, EPO
- d. GFR, cystatin C
- e. Urine osmolality, urine protein/creatinine ratio, urine albumin/creatinine ratio
- f. Ejection fraction, TRV, BNP
- g. 6 minute walk distance, oxygen saturation at baseline and after walking for 6 minutes
- h. Frequency of analgesics used at home and comprehensive pain score
- i. Quality of life
- j. Frequency of hospitalization for pain crises and acute chest syndrome
- k. Transfusion requirement
- l. Medication compliance, frequency of medication refills, and compliance with clinic visits
- m. RBC deformability

12.2 Primary endpoint

The difference between the mean HbF calculated at baseline (from the 3 values obtained during the run-in period (at the first visit and then again 1 and 2 months later)) as compared to the mean HbF calculated from 3 values obtained at months 8, 10, and 12.

12.3 Secondary endpoints

When stated below, baseline is defined as an average of the values collected over the 2 months during the run-in period. Mean baseline laboratory values will be compared to a mean of the laboratory values collected at months 8, 10, and 12. If only one set of data was collected at baseline, those data (each a single measurement) will be compared to data collected one year after starting/adjusting HU (each a single measurement).

- a. Number of hospitalizations for pain crises and acute chest syndrome as compared to the 1 year prior to HU initiation or dose increase.
- b. Total hemoglobin level as compared to baseline.
- c. Home analgesic use and comprehensive pain score as compared to before starting or adjusting HU.
- d. The percentage of patients that reach MTD.
- e. Quality of life as compared to before starting or adjusting HU.
- f. Markers of organ function (proteinuria, urine osmolality, creatinine, GFR, cystatin C, alkaline phosphatase, direct bilirubin, BNP) as compared to baseline.
- g. Ejection fraction and TRV as compared to before starting or adjusting HU.
- h. Overall survival (the percentage of patients who are alive one year after starting/adjusting HU).
- i. Transfusion requirement as compared to the 1 year prior to starting or adjusting HU.
- j. 6 minute walk distance and oxygen saturation as compared to before starting or adjusting HU.
- k. Concordance of the HU dose as determined by the computer algorithm as compared to the manual dose calculated.
- l. Frequency of noncompliance.

m. RBC deformability as related to HbF response to HU

12.4 Study Analysis

Primary Response Variable

The primary response variable is the absolute change of HbF (measured in percentage) from baseline to the mean level achieved from months 8, 10, and 12 with algorithm-based HU dosing. The baseline of HbF is the mean of the 3 HbF levels measured during the run-in period. Descriptive analysis will be performed on the primary outcome variable. Comparison of baseline and months 8, 10, and 12 values will be performed using a paired t-test, and a 95% confidence interval of the change will be reported.

Secondary Response Variables

For secondary response variables that are to be compared with their baselines, paired t-test will be used for continuous variables (appropriate transformation will be applied where distribution is deviated from normal), and McNemar test will be used for categorical variables. For secondary response variables that are not being compared with their baselines, descriptive analysis will be performed, and mean, standard deviation, 95% confidence interval, and frequencies will be calculated, where appropriate.

Model Analysis

Regression analysis of primary and selected secondary response variables on the following factors of interest will be performed:

1. Final hydroxyurea dose
2. Hydroxyurea status at baseline
3. Rate of noncompliance
4. Number of transfusions
5. Baseline hemoglobin
6. Baseline ARC⁵⁰
7. Baseline creatinine⁵⁰
8. Baseline HbF⁵⁰
9. Baseline total bilirubin⁵⁰
10. Baseline body mass index⁵⁰
11. RBC deformability after starting or increasing HU

Linear Model or Analysis of variance will be the primary method for model analysis in this study. The response variables of interest will be regressed as a linear function of the target factors and covariates.

Other Issues in Statistical Analysis

Missing values

Subjects will only be included in the final analyses if data were collected 3 times during the run-in period and at months 8, 10, and 12. For the secondary endpoints where only one set of data were collected, analyses of those data will only be performed if data were collected at baseline and after one year of therapy. Subjects with missing measurements will be excluded from the analysis.

12.5 Stopping Rules

The study will be monitored to ensure that the occurrence of a specified set of treatment-related serious adverse events (TRSAEs) within 24 weeks (+/- 7 days) of the treatment period does not substantially exceed an anticipated rate. The following TRSAEs will be monitored for early stopping of the study:

- Death considered to be probably or definitely related to hydroxyurea.
- Any grade IV toxicity excluding readily reversible metabolic or laboratory abnormalities which are considered to be probably or definitely related to hydroxyurea.

The study will be monitored using the stopping rules as outlined below for early stopping if the number of subjects in the study who have developed one or more of the above specified TRSAEs is over a pre-specified threshold value. TRSAEs are those attributed as definitely or probably related to hydroxyurea. From the literature involving long-term follow-up of patients with SCD who administer hydroxyurea,^{14,15} we anticipate the rate of developing at least one of the above specified TRSAEs for this patient population to be 10% or less, with estimated SD of about 0.2. Following Geller,⁵² our stopping rule is determined by a Bayesian approach. The stopping boundary for the study is reached if the Bayesian posterior probability that the true probability of developing one or more of the above specified TRSAEs exceeds this benchmark rate of 10% is at least 90%. We take our prior distribution to be a beta distribution with the sum of the two beta parameters to be 3, i.e. the parameters of the beta prior distribution are 0.30 and 2.70. These parameters are chosen to approximate the prior information about the rate of 0.1 with SD of about 0.2, with at least 3 patients enrolled before starting the monitoring. The following table summarizes the threshold numbers for stopping the study:

Number of subjects enrolled	Stop if the number of subjects who develop any of the above specified TRSAEs reaches:
≤ 5	2
6-11	3
12-18	4
19-23	5

The monitoring plan was evaluated by simulation. If the true TRSAE rate is 0.1, we will stop about 18% of the time. True TRSAE failure rates of 0.05, 0.15, 0.2 and 0.3 entail stopping about 3%, 41%, 62% and 91% of the time respectively.

12.6 Off study criteria

Patients may withdraw from study at their request. The risks of withdrawing will be discussed, as will alternative treatment options. During the run-in period subjects may be re-enrolled at their request, and at the discretion of the PI. This will not require re-consenting and subjects may retain their original study ID number. Should any of the following events occur during the 1 year study period, hydroxyurea will be discontinued permanently and the subject will be removed from the study:

- Life threatening acute hypersensitivity reaction.

- Any grade IV toxicity excluding readily reversible metabolic or laboratory abnormalities which are considered to be probably or definitely related to hydroxyurea.
- Pregnancy or unwillingness to use acceptable forms of contraception.
- More than 2 transfusions per year while on study.
- HU non-compliance (missing >20% of pills within one month on at least 2 occasions).

Subjects who experience an event referenced above will be followed until resolution of the event. Labs will be monitored through 30 days after the final study visit or until he/she recovers to baseline, whichever is longer.

Subjects at or near MTD of HU during the run-in period will be taken off study per the discretion of the PI. Alternative treatment options will be discussed with the subject.

All subjects will be followed for 30 days following their last study visit, at which time the subject's participation on this study will be considered complete. Subjects may continue to take hydroxyurea after their participation in this study ends. Subjects will then be followed by their primary hematologist.

13.0 DATA AND SAFETY MONITORING PLAN

13.1 Data and Safety Monitoring

Principal Investigator: Accrual, efficacy, and safety data will be monitored by the principal investigator Dr. Courtney Fitzhugh. The PI, Dr. Matthew Hsieh, and/or Anna Conrey will also ensure manually that the HU doses calculated by the computer program are appropriate.

NHLBI's IRB: Prior to implementation of this study, the protocol and the proposed patient consent and assent forms will be reviewed and approved by the properly constituted Institutional Review Board (IRB) operating according to 45 CFR 46. This committee will approve all amendments to the protocol or informed consent and conduct continuing annual review so long as the protocol is open to accrual or sample and/or data analysis continues. Accrual and safety data will also be monitored and reviewed annually by the IRB.

Quality assurance and control monitoring will be consistent with the NHLBI Division of Intramural Research Clinical Research Quality Assurance and Quality Control Policy.

Safety Monitoring/Recording/Reporting of Events:

Serious Events

Reports to the IRB and CD:

The PI must report serious UPs, and serious PDs, to the IRB and CD as soon as possible but not more than 7 days after the PI first learns of the event.

Reports to the IRB Chair and CD:

The PI must report all SAEs that do not meet the definition of UP to the IRB chair and CD not more than 14 days after the PI first learns of the event.

Non-serious Events

Reports to the IRB and CD:

The PI must report all UPs that are not serious to the IRB and CD, and PDs that are not serious to the IRB, not more than 14 days after the PI first learns of the event.

Deaths

The PI must report all deaths (that are not UPs) to the CD as soon as possible, but not more than 7 days after the PI first learns of the event.

Reports at the time of continuing IRB review:

At continuing review, the PI will provide to the IRB a summary of:

- All UPs
- All PDs (except for those granted a waiver of reporting)
- All AEs (except for those granted a waiver of reporting or any AEs occurred before the initiation of the study drugs)
- If, while preparing the continuing review, the PI identifies a greater frequency or level of severity of expected adverse events than was previously identified in the protocol or investigational brochure (IB), these should be reported separately as a UP. If such an observation occurs before the time of continuing IRB review, it should be reported to the IRB and CD as a UP in the time frames noted above, and summarized at the time of continuing review.

We request a waiver from reporting inpatient hospitalization or a prolongation of an existing hospitalization for vaso-occlusive pain crisis which, in the opinion of the investigator, is attributable to the subject's sickle cell disease (hospitalization is not uncommon for the subjects in this study based on the nature of their illness) to the IRB unless it reaches the threshold of a UP.

13.1.1 Adverse Events:

Adverse Events (AEs) are defined as any unfavorable and unintended diagnosis, symptom, sign (including an abnormal laboratory finding), symptom, or disease which either occurs during the study participation, having been absent at baseline or if present at baseline, appears to worsen.

The Principal Investigator will be responsible for assessing AEs. Information on AEs will be solicited from subjects through questions from study personnel and information volunteered by the subject.

All AEs (serious and non-serious) will be recorded from start of study treatment through final study visit and reported to the IRB at the time of continuing review.

All subjects will be expected to experience significant myelosuppression, and only myelosuppression that leads to SAEs will be reported to the IRB. HU is also expected to lead to megaloblastic erythropoiesis in all subjects. Hyperpigmentation and nail discoloration is expected to occur in a minority of subjects (<15%) and will only be reported to the IRB if this anticipated rate is exceeded. Further, the GI toxicity described above is expected to occur in a minority of subjects (<30%), and will only be reported to the IRB if the GI toxicity leads to an SAE or if the anticipated rate is exceeded and not controlled by anti-emetics or by dividing the daily dose of HU into 2 portions.

Suspected adverse reaction: Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of safety reporting, ‘reasonable possibility’ means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

Unexpected adverse reaction: An adverse event or suspected adverse reaction is considered “unexpected” if it is not listed in the package insert or is not listed at the specificity or severity that has been observed; or is not consistent with the risk information described in the protocol or elsewhere in the current application. “Unexpected”, also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

Adverse events used to evaluate the safety of this protocol regimen will be collected to include any unfavorable and unintended signs, symptoms or diseases which either occurs during the study, having been absent at baseline or if present at baseline appear to worsen. The AEs will be attributed (unrelated, unlikely, possibly, probably or definitely) to study medication and/or disease and graded by severity utilizing CTCAE version 4.0. A copy of the criteria can be down-loaded from the CTEP home page at <http://ctep.cancer.gov/reporting/ctc.html>.

Adverse event recording will start after the initial dose of the drug is administered. All AEs, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until satisfactory resolution. AEs should be reported up to 30 days following the last study visit. An abnormal laboratory value will be considered an AE if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization
- Is judged by the Investigator to be of significant clinical impact
- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient’s outcome.

13.1.2 Grading of adverse events:

Grade	Category	Description
1	Mild	Mild; asymptomatic; clinical or diagnostic observations only; intervention not indicated
2	Moderate	Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL

3	Severe	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL
4	Life threatening	Life-threatening consequences; urgent intervention indicated
5	Death	Death related to AE

13.1.3 Attribution of Adverse Events:

Relationship	Attribution	Description
Unrelated to investigational agent/intervention ¹	Unrelated	The AE <i>is clearly NOT related</i> to the intervention
	Unlikely	The AE <i>is doubtfully related</i> to the intervention
Related to investigational agent/intervention ¹	Possibly	The AE <i>may be related</i> to the intervention
	Probably	The AE <i>is likely related</i> to the intervention
	Definitely	The AE <i>is clearly related</i> to the intervention

¹NOTE: AEs listed as 'possibly, probably, or definitely' related to the investigational agent/intervention are considered to have a suspected 'reasonable causal relationship' to the investigational agent/intervention (ICH E2A).

Serious Adverse Events:

A Serious Adverse Event (SAE) is defined by federal regulation as any AE that results in any of the following outcomes: death, life-threatening AE, requires inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect.

If the serious adverse event is thought to be due to the experimental component of the protocol, accession to the protocol may be stopped until a full discussion with the IRB has been held.

Treatment related SAEs (TRSAEs): Those attributed as definitely, or probably, related that will be monitored and considered for early stopping of the study according to statistically determined criteria. These include death and any grade IV toxicity considered to be probably or definitely related to study medication.

Unanticipated Problems and Protocol Deviations:

An unanticipated problem is any incident, experience, or outcome that is:

1. unexpected in terms of nature, severity, or frequency in relation to:

- a) the research risks that are described in the IRB-approved research protocol and informed consent document, Investigator's Brochure or other study documents, and
- b) the characteristics of the subject population being studied, and

2. related or possibly related to participation in the research, and
3. places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized. (An AE with a serious outcome will be considered increased risk.)

A protocol deviation is any change, divergence, or departure from the study design or procedures of an IRB-approved research protocol. Since study drug non-compliance is an expected occurrence, we request an exemption from reporting a deviation for less than 20% non-compliance in any given month.

Hospitalizations for administrative issues (for example for transfusion) or upgrading to ICU for routine monitoring will not be reported as an SAE.

a. Reporting of pregnancy

Subjects who become pregnant during the study will discontinue the study drug immediately. The investigator, or his/her designee, will collect pregnancy information on any subject who becomes pregnant while participating in this study. An unanticipated problem will be submitted to the Clinical Director and the IRB should pregnancy occur during the course of this study.

14.0 HUMAN SUBJECT PROTECTION

14.1 Rationale for Subject Selection

Study population: The study will be open to all eligible subjects based on inclusion and exclusion criteria and who provide informed consent. No patient will be excluded from participation based on gender, race, or ethnicity. Patients may self-refer, be recruited through the NIH office of recruitment, and may include patients participating on NIH Clinical Center Protocols and NIH employees and/or children of NIH employees. If subjects are NIH employees, recruitment, enrollment and compensation of NIH employee subjects will be consistent with NIH Manual Chapter 23s00-630-3, "Leave Policy for NIH Employees".

14.2 Participation of children

Hydroxyurea is FDA-approved only for adult patients with SCD. However, HU has been widely used safely in the pediatric population, and multiple clinical trials have been published.^{28,53-55} Further, younger patients with SCD are less likely to have evidence of organ damage, and the damage that may be present is more likely to be reversible. Therefore, pediatric patients as young as 15 years of age who will be able to understand well the risks of the study including the importance of adhering to 2 forms of contraception and benefits of the study will be enrolled.

14.3 Rationale for the Exclusion of Pregnant Women

Hydroxyurea can cause fetal harm when administered to a pregnant woman. Hydroxyurea has been demonstrated to be a potent teratogen in a wide variety of animal models, including mice, hamsters, cats, miniature swine, dogs, and monkeys at doses within 1-fold of the human dose given on a mg/m² basis. Hydroxyurea is embryotoxic and causes fetal malformations (partially ossified cranial bones, absence of eye sockets, hydrocephaly, bipartite sternebrae, missing lumbar vertebrae) at 180 mg/kg/day (about 0.8 times the maximum recommended human daily dose on a mg/m² basis) in rats and at 30 mg/kg/day (about 0.3 times the maximum recommended human

daily dose on a mg/m² basis) in rabbits. Women of childbearing potential should be advised to avoid becoming pregnant. Women who are pregnant or lactating are excluded due to the risk to the developing fetus or nursing babies.

14.4 Participation of NIH Staff

- NIH staff may voluntarily participate in this protocol.
- Recruitment, enrollment and compensation of NIH staff subjects will be consistent with the Guidelines for the Inclusion of Staff in NIH Intramural Research Studies (December 2015) (SOP 14F, Appendix C) and NIH Policy Manual Chapter 2300-630-3, "Leave Policy for NIH Employees Participating in NIH Medical Research Studies"
- If the individual requesting to participate in the protocol is a co-worker, the consent from the employee (co-worker) will not be obtained by the research coordinator or the employee's direct supervisor but by another research staff member approved for obtaining informed consent.
- Neither participation nor refusal to participate as a subject in this protocol will have an effect, either beneficial or adverse, on the participant's employment or position at NIH.
- The consenting staff member will make the NIH Information Sheet on Employee Research Participation available to employees who are considering enrolling in research. (SOP 14F, Appendix A)
- Staff subjects' privacy and confidentiality will be respected by protocol and consenting staff the same as for all subjects participating in research protocols. However, all subjects will be made aware that there are limits to these protections.

14.5 Risks and Discomforts

14.5.1 Hydroxyurea

Toxicities: Toxic effects are primarily bone marrow suppression and rarely gastrointestinal disturbances (nausea and vomiting). These effects are dose-dependent and are reversible with interrupted therapy. Hydroxyurea is not known to be carcinogenic, although there is a theoretical risk of secondary malignancies based upon experience in the myeloproliferative disorders. However, no definite predilection to secondary malignancies has been observed despite repeated clinical use. There is no proof of increased incidence of leukemia or other malignancies among patients enrolled in the randomized polycythemia vera trials or the multi-center HU trials for SCD.^{56,57}

Hematologic: Myelosuppression will be expected based on the doses of HU that will be used in this study. Patients' blood counts will be followed closely, and the doses adjusted as necessary per the dosing algorithm. Patients will also be expected to develop macrocytosis and megaloblastic erythropoiesis.

Gastrointestinal Disturbances: Anorexia, nausea, vomiting, diarrhea and constipation have all been reported with moderate frequency in patients taking HU. Such disturbances have, rarely, been the cause for terminating therapy. The daily dose will be divided into two portions as necessary in order to attempt to improve GI toxicity as described above. Less frequently, patients have experienced stomatitis and rarely pancreatitis. Concurrent treatment of HU and antiretroviral

agents (including stavudine and didanosine) leads to a higher risk of potentially fatal pancreatitis and hepatic failure.

Reproductive: Animal studies suggest that HU may be teratogenic, necessitating caution in administering it to females of reproductive age;⁵⁸ although the number of birth defects does not appear to be increased in offspring of women who administered HU during pregnancy.⁴² HU should be used with caution in women of child-bearing age and in men contemplating conceiving a child while on this medication. Subjects will be excluded from the study if they do not agree to use two forms of contraception throughout the period of HU administration.

Malignant potential: Long term HU leukemogenesis is extrapolated from myeloproliferative disorders. In vitro, there is evidence of copy number changes at specific cytogenetic locations in cultured cells. Long term HU therapy is frequently prescribed in polycythemia vera, a condition which carries an inherent risk of leukemia. Cases of leukemia have been reported in this population; however it is difficult to quantify the leukemogenic potential of HU in this disease. There are no studies to date which report an increased risk of malignancy in patients who administer HU.

Hyperviscosity: Since HU may increase hemoglobin levels, patients may experience symptoms of hyperviscosity. These symptoms include headache, dizziness, visual impairment, paresthesias, muscle weakness, and somnolence. Phlebotomy will be initiated and/or HU doses will be adjusted as above if hemoglobin levels increase to $\geq 12\text{g/dL}$ in order to decrease the risk of hyperviscosity.

Other reported side effects: Alopecia, edema, hyperpigmentation, nail atrophy and discoloration, rashes, renal disturbances, abnormal transaminases, and neurological symptoms (drowsiness, dizziness, disorientation, hallucinations, seizure, peripheral neuropathy and headache) have all been reported infrequently. Acute diffuse pulmonary infiltrates and pulmonary fibrosis have been reported rarely.

14.5.2 Phlebotomy and blood draws

The inconvenience and discomfort associated with phlebotomy relate to the intravenous catheter placement and blood removal. The risks associated with intravenous catheter placement include brief but relatively minor pain, bleeding, localized tissue damage, bruising, and induction of a small clot in a superficial blood vessel. If patients have permanent access devices such as a mediport, the permanent catheter will be used if possible. Risks associated with phlebotomy and/or blood draws include symptoms related to a fear of blood drawing, temporary lowering of blood pressure, dizziness, lightheadedness, fainting, and rarely seizures. Patients will be encouraged to drink plenty of fluids prior to phlebotomy, and intravenous fluids will be administered as necessary.

14.5.3 Quality of life questionnaire and pain diary

The only anticipated adverse consequences associated with the quality of life questionnaires and pain diaries will be the time required for the participants to complete each questionnaire and pain diary.

14.5.4 Echocardiogram

There are no known adverse consequences related to the ultrasound waves.

14.6 Risks in Relation to Benefits

For adult participants:

Risk/Benefit Analysis:

The benefits to the patients include potentially decreasing the frequency of some of the acute complications associated with sickle cell disease and improving quality of life as well as possibly preventing or decreasing organ dysfunction and improving survival. Potentially, transfusions and hospitalizations could also be avoided or postponed. Further, the knowledge gained should help contribute to the design of future trials. Therefore, this research involves greater than minimal risk to subjects with the prospect of direct benefit (45 CFR 46.102).

For pediatric participants aged 15-18:

The inclusion of children satisfies the criteria set forth in 45 Code of Federal Regulations 46, Subpart D as follows:

(a) the risk is justified by the anticipated benefit to the subjects:

Trials involving pediatric patients have also shown a decrease in the frequency of some of the acute complications associated with sickle cell disease, and this will likely lead to an improvement in quality of life. Further, enrolling patients at a young age before significant organ damage occurs may help to prevent the development of organ damage and potentially improve survival. Potentially, transfusions and hospitalizations could also be avoided or postponed.

(b) the relation of the anticipated benefit to the risk is at least as favorable to the subjects as that presented by available alternative approaches:

The alternatives are hydroxyurea dosing by a primary provider which may not lead to an optimal fetal hemoglobin response, no sickle-specific therapy and treating acute complications conservatively with hospitalization as necessary, or transfusion therapy with the associated risks of alloimmunization, hemolytic transfusion reaction, and iron overload; and

(c) adequate provisions are made for soliciting the assent of the children and permission of their parents or guardians, as set forth in 46.408.

Therefore for children participating in this study, the risk is consistent with 45 CFR 46.405, Children's risk category Assignment 2: the research involves greater than minimal risk but presents the prospect of direct benefit to the individual subjects.

14.7 Informed Consent Processes and Procedures

All subjects will read and sign the informed consent document prior to enrollment. Members of the protocol team will describe the protocol and the risks and benefits of each to the individual signing the consent. The principal investigator or the associate investigators designated to obtain informed consent may consent the research subject and sign the consent documents after the case has been discussed with the PI and/or the primary clinical team.

If the subject is a minor, the parent who signs the consent for the minor must be a legally recognized parent or guardian. The child will also be included in all discussions about the trial and a minor's assent will be obtained. The parent or guardian will sign on the designated line on

the informed consent attesting to the fact that the child had given assent. We will inform the minor during the assent process that for safety, we need to do a pregnancy test. She will also be told that if it is positive, we will counsel her and help her tell her parents. If the minor does not want to proceed she will be advised not to sign the assent and her enrollment on this protocol will end.

When a pediatric subject reaches age 18, continued participation will require re-consenting of the now adult with the standard protocol consent document to ensure legally effective informed consent has been obtained. Should sample or data analysis continue following completion of active participation and the subject has reached 18 years of age, we will attempt to contact the subject using the last known contact information to obtain consent for continued use of data or samples collected during their prior visit. Given the length of time that may have transpired for some of the subjects since their last visit for this study, we request waiver of informed consent for those individuals who after good faith efforts to contact them, we are unable to contact.

Requirements for Waiver of Consent consistent with 45 CFR 46.116 (d), each of which must be addressed in relation to the protocol:

- (1) The research involves no more than minimal risk to the subjects;
 - a) Analysis of samples and data from this study involves no additional risks to subjects.
- (2) The waiver or alteration will not adversely affect the rights and welfare of the subjects;
 - a) Samples and data will be kept in secure locations in the laboratory of Dr. Young. Retention of samples or data does not affect the welfare of subjects.
- (3) The research could not practicably be carried out without the waiver or alteration; and
 - a) Considering the length of time between a minor's enrollment and their age of majority, it is possible that more than a few subjects may be lost to follow up. A significant reduction in the number of samples analyzed could impact the quality of the research.
- (4) Whenever appropriate, the subjects will be provided with additional pertinent information after participation.
 - a) We only plan to request a waiver of re-consent for those subjects who have been lost to follow-up.

If the individual obtaining consent is a supervisor of the employee requesting to participate in the protocol, there will be independent monitoring of the consent process to minimize the risk of undue pressure on the employee. If the individual requesting to participate in the protocol is a co-worker, there will be independent monitoring of the consent process unless this is waived by the IRB. Independent monitoring will be provided by the Clinical Center Department of Bioethics Consultation Service.

If at any time during participation in the protocol, new information becomes available relating to risks, adverse events, or toxicities, this information will be provided orally or in writing to each enrolled or prospective patient. Documentation will be provided to the IRB and if necessary the informed consent amended to reflect relevant information.

Informed Consent of Non-English Speaking Research Participants:

If there is an enrollment of a research participant for which there is no translated extant IRB approved consent document, the principal investigator and or those authorized to obtain informed

consent will use the Short Form Oral Consent Process as described in MAS Policy M77-2, 45 CFR 46.117 (b) (2). The summary that will be used is the English version of the extant IRB approved consent document.

We request prospective IRB approval of the use of the short form for up to a maximum of five participants and we will notify the IRB at the time of continuing review of the frequency of the use of the Short Form. Should we reach the threshold of five, we will notify the IRB of the need for an additional use of the Short Form and that we will have the consent document translated into the given inherent language.

Exclusion of participants unable to provide informed consent:

All participants must be capable of providing informed consent. Participants not capable of providing informed consent, needing a legally authorized representative, will be excluded from participating in this study. The rationale for exclusion is while there may be direct benefit from participation, this is a pilot study with greater than minimal risk and this justifies requiring that each participant be able to provide informed consent.

14.8 Conflict of Interest

The Principal Investigator assured that each associate investigator listed on the protocol title page received a copy of the NIH's Guide to preventing conflict of interest. No initial or subsequent members of the research team reported a potential conflict of interest.

15.0 PHARMACEUTICALS

15.1 Hydroxyurea

Names:

Hydroxyurea, Hydrea, Droxia®

Supply:

Commercially available

Product description:

Hydroxyurea is available for oral use as capsules providing 200 mg, 300 mg, and 500 mg of hydroxyurea. The 200 mg, 300 mg, and 500 mg capsules are stocked by the NIH Clinical Center Pharmacy.

Storage and stability:

Oral capsules should be stored at controlled room temperature 15-30°C (59-86°F).

Route of administration:

Oral administration.

Regulatory Status:

OFF Label Usage

Hydroxyurea will be used in this study beyond what is indicated in the package insert. Hydroxyurea is approved by the FDA to prevent pain crises in adults with SCD; however this protocol will enroll subjects

as young as 15 years old. The use of this drug meets the requirements for an exemption from the IND regulations, 21 CFR 312, specifically:

1. The investigational drug is lawfully marketed in the United States
2. The investigation is not intended to be reported to the FDA as a well-controlled study in support of a new indication for use of the drug product
3. The investigation is not intended to support a significant change in advertising to an existing lawfully marketed prescription drug product
4. The investigation does not involve a route of administration or dosage level or use in a patient population or other factor that significantly increases the risks (or decreases the acceptability of the risks) associated with the use of the drug product.
5. The investigation will be conducted in compliance with the requirements for institutional review set forth in FDA regulations 21 CFR 56, and requirements for informed consent as set forth in FDA regulations 21 CFR 50
6. The investigation will be conducted in compliance with FDA regulations 21 CFR 312.7: Promotion and charging for investigational drugs.

Advice to Patients:

Hydroxyurea is a medication that must be handled with care. People who are not taking hydroxyurea should not be exposed to it. If a caregiver must handle the medication for you, hands must be washed after handling. If the powder from the capsule is spilled, it should be wiped up immediately with a damp disposable towel and discarded in a closed container, such as a plastic bag. The medication should be kept away from children and pets. Women of childbearing potential will be advised to avoid becoming pregnant.

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APPENDIX A: NIH INFORMATION SHEET ON STAFF RESEARCH PARTICIPATION (DECEMBER 2015)

As an NIH employee, contractor, Special Volunteer, Guest Researcher, or trainee, you may participate in intramural research studies unless it is prohibited by your Institute or Center (IC), or if you are excluded by the criteria of the protocol in which you want to enroll. The inclusion of NIH staff in a particular protocol must also be approved by the IRB. You may be motivated by altruism, a commitment to research in your own or related fields, or want access to clinical trials of potential direct therapeutic benefit.

When deciding, you should make an informed decision about participation. This information sheet offers some points to consider for NIH staff who are considering research participation at NIH.

First, similar to any individual who is considering research participation, you should seek adequate information about the study purpose, what is required of you in terms of procedures, interventions and time, and the potential risks and benefits of participation. For more information, see the NIH Clinical Center's public website "Are Clinical Studies for You?" at <http://www.cc.nih.gov/participate/studies.shtml>.

When you are thinking about participation in a research study that is being conducted by your supervisor, or others with whom you work closely in your laboratory, branch, or unit, you should consider some additional factors:

A. Possible bias: Are you confident that you can be unbiased about reporting answers, side effects, or other information that could influence the study outcome or risk to you?

B. Confidentiality: Are you comfortable sharing your medical history (including, for example, mental health history or STDs) and your social history (e.g. substance use) with study investigators who may be your coworkers, or with the possibility of them discovering something about your health during the study (e.g. pregnancy status or a new diagnosis)? Although every effort will be made to protect your information and keep it private and confidential, your information will be available in medical records and it will be available to authorized users outside of the study team, both in an identifiable and unidentified manner.

C. Pressure: Do you perceive any pressure or expectations from your supervisor or colleagues regarding participation? Could that pressure influence your decision or make it difficult for you to choose whether or not to participate? Remember that it is your choice whether or not to participate and that your decision to participate or not should not have an effect, either beneficial or adverse, on your position at NIH.

D. Time and Compensation: Can you take time off from work to complete the study requirements or participate solely during non-duty hours? Can you receive compensation for your participation in this study? Will your supervisor give you permission to participate during work hours? See the NIH Policy Manual 2300- 630-3 *Leave Policy for NIH Employees Participating in NIH Medical Research Studies*.

E. Consent Process: Is the person obtaining your consent for the study your supervisor, a subordinate, or co-worker? If so, is there an independent person monitoring the consent process? If the study PI is a supervisor and intends to obtain consent from you, an independent person (e.g., through Bioethics or the NIMH Human Subjects Protections Unit [HSPU], or others as approved by the IRB) must monitor the consent process. If the person obtaining consent from you is a co-worker then an independent person (e.g., through Bioethics or the NIMH HSPU, or others as approved by the IRB) may be required to monitor the consent process, as determined by the IRB for the specific study.

If you are thinking of enrolling as a subject at the NIH Clinical Center and you have any questions or concerns, please contact the Office of Human Subjects Research Protections (OHSRP) at 301-402-3444 and/ or the Patient Representative if you are thinking of enrolling as a subject at the NIH Clinical Center on 301-496-2626. If you are at a NIH site outside the Clinical Center then please contact local site leadership.