A PILOT STUDY OF HYDROXYUREA MANAGEMENT IN KIDS: INTENSIVE VERSUS STABLE DOSAGE STRATEGIES (HUGKISS)

IND# 132032

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Protocol Summary

Protocol MNEMONIC and Title: HUGKISS – A pilot study of hydroxyurea management in kids: intensive versus stable dosage strategies
Sponsor Principal Investigator: Jeremie Estepp, MD
IND Holder: St. Jude Children's Research Hospital (SJCRH), IND #132032.
Brief Overview: This is a pilot study, single-blind, randomized, multicenter, therapeutic clinical trial designed to evaluate the feasibility of enrolling infants and toddlers (9 months to 36 months) with sickle cell anemia (SCA; HbSS or HbSβ^0thalassemia), regardless of disease severity, to a therapeutic trial. The BABY HUG trial demonstrated that a fixed dose (20 mg/kg/day) of hydroxyurea was safe and effective in decreasing SCA-related complications in very young children (9-18 months), and largely due to these findings, hydroxyurea is recommended to be offered to all children (≥9 months old) with SCA, independent of disease severity. Nevertheless, children in the treatment arm of BABY HUG continued to experience vaso-occlusive symptoms and to incur organ damage. In clinical trials of older children with SCA, intensification of hydroxyurea to a maximum tolerated dosage (MTD), defined by mild to moderate myelosuppression, may be associated with improved laboratory parameters compared to fixed lower-dosing, but the clinical benefits gained from dose intensification have not been described. Therefore, in the HUGKISS trial, children in the standard treatment arm will receive a fixed dose of hydroxyurea (20mg/kg/day), and participants in the experimental arm will receive hydroxyurea intensified to MTD, defined by a goal absolute neutrophil count (ANC) of 1500-3000 cells/μL. This trial aims to establish a multicenter infrastructure that will identify, enroll and randomize very young children (9-36 months) to receive fixed dose versus intensified-dose hydroxyurea in a single blinded manner, and to obtain prospective pilot data comparing the clinical and laboratory outcomes between the treatment arms to facilitate design of a definitive phase III trial.
Intervention: All participants will receive treatment with hydroxyurea, and they will be randomized to a fixed dose (20mg/kg/day) versus intensification to MTD.
Brief Outline of Treatment Plan: All participants will initiate hydroxyurea at a dose of ~20 mg/kg/day in an open label fashion for eight weeks (±2 week) prior to randomization. Participants will receive monthly medical evaluations (4 ± 2 weeks) where they will have height and weight measurements, medical history, physical examination, and medication adherence assessments. During these monthly visits complete blood counts with absolute reticulocyte count will be monitored. Hemoglobin electrophoresis, complete serum chemistries, urinalysis, lactate dehydrogenase and quality of life measurements will be obtained every 20 (±2) weeks. Transcranial Doppler (TCD) ultrasound velocities will be obtained at study entry (in participants ≥2 years of age) and exit. Participants randomized to receive hydroxyurea at MTD will have their dose increased by 5 mg/kg/day every 8 weeks, in the absence of toxicity, until a goal ANC of 1500-3000 cells/μL is achieved, up to a maximum of 35 mg/kg/day.
Study Design: Open label, single-blind, multicenter, randomized controlled trial comparing fixed dose (20mg/kg/day) to dose-escalation (goal ANC of 1500 – 3000 cells/μL) of hydroxyurea therapy.
Sample Size: Up to 65 participants
Data Management: Clinical sites will enter data via a secure, internet-based (electronic data capture) remote data capture system. Data management will be provided locally by the Clinical Trials Management Team within the Department of Hematology at SJRCH. Statistical analyses will be performed with the Departments of Biostatistics and Hematology at SJRCH.

Human Subjects: This pilot trial will involve a vulnerable population of children of any race (9-36 months) affected with SCA; however, we expect mostly children of African descent to be enrolled given the epidemiology of SCA in the United States. No nationality or gender exclusions are included in this trial. All participating sites will be following the same criteria for inclusion and exclusion on the study. All clinical sites chosen for the pilot trial will first undergo a careful checklist of "research readiness" to ensure proper clinical research can be safely conducted, including formal Human

Protocol MNEMONIC and Title: HUGKISS – A pilot study of hydroxyurea management in kids: intensive versus stable dosage strategies

Subjects Protection training to be completed by Clinical Investigators and staff members at each site. The risks to subject will be related to toxicity from hydroxyurea. Expected toxicity includes neutropenia, anemia, reticulocytopenia, and thrombocytopenia. The plan for dose reduction and toxicity monitoring is based on our safety data from BABY HUG[1]. Patients will be informed of any potential toxicity and other minor side effects during informed consent discussion. Adverse events (AEs) will be monitored and reported and treated appropriately. This study will be monitored by an independent Data and Safety Monitoring Board (DSMB).

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Acronyms used in the protocol

ACS: Acute Chest Syndrome

AEs: Adverse Events

ALT: Alanine Aminotransferase

ANC: Absolute Neutrophil Count

ARC: Absolute Reticulocyte Count

AUCINF: Area under the concentration versus time curve

BE: Baseline Evaluation

BPCA: Best Pharmaceuticals for Children Act

CBC: Complete Blood Count

CHOA: Children's Healthcare of Atlanta

CL/F/BW: Clearance, normalized for Body Weight

Cmax: Maximum observed plasma concentration

CPDMO: Central Protocol and Data Monitoring Office

CRF: Case Report Form

CRIS: Current Research Information System

CSSCD: Clinical Study of Sickle Cell Disease

CTCAE: Common Terminology Criteria for Adverse Events

CVA: Cerebrovascular Accident

DCC: Data Coordinating Center

DSMB: Data and Safety Monitoring Board

ES: Eligibility Screening

FDA: Food & Drug Administration

HbF: Fetal Hemoglobin

HbS: Sickle Hemoglobin

HPFH: Hereditary Persistence of Fetal Hemoglobin

IDE: Investigational Device Exemption

IMC: Internal Monitoring Committee

IND: Investigational New Drug

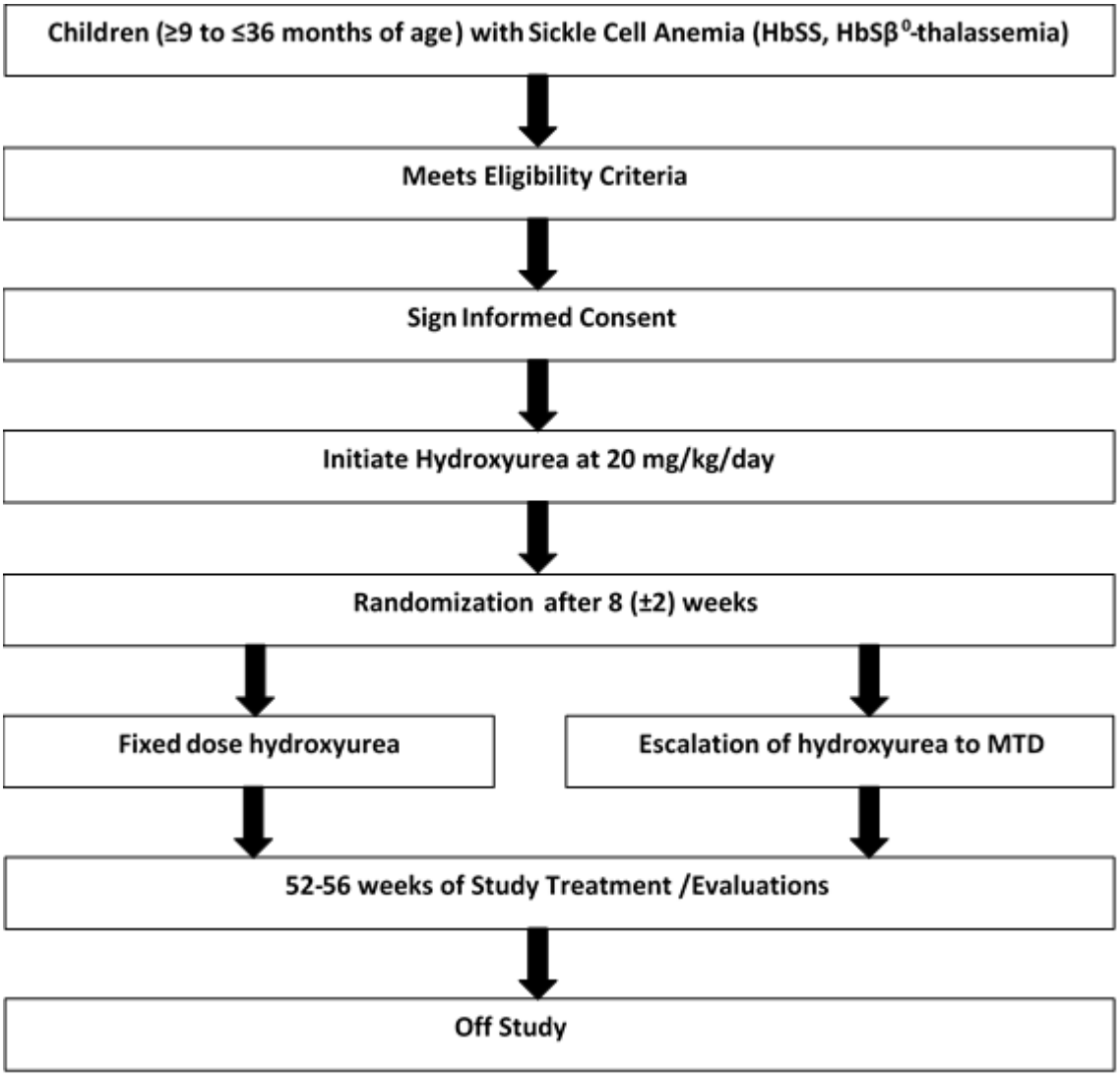
MAM: Medication Adherence Measure

MCC: Medical Coordinating Center

MCV: Mean Corpuscular Volume

MPR: Medication Possession Ratio
MSH: Multicenter Study of Hydroxyurea in Sickle Cell Anemia
MTD: Maximum Tolerated Dose
NHLBI: National Heart, Lung, and Blood Institute
OHRP: Office for Human Research Protections
PI: Principle Investigator
PK: Pharmacokinetics
PM: Project Manager
QOL: Quality of Life
RUQ: Right Upper Quadrant
SAE: Serious Adverse Events
SCA: Sickle Cell Anemia
SCD: Sickle Cell Disease
SJCRH: St. Jude Children's Research Hospital
TCD: Transcranial Doppler
TIA: Transient Ischemic Attack
Tmax: Time to maximum plasma concentration
UMMC: University of Mississippi Medical Center
URI: Upper Respiratory Infections
UTSW: University of Tennessee Southwestern
WBC: White Blood Count
WHO: World Health Organization

1.0 HUGKISS FLOW DIAGRAM



2.0 STUDY OBJECTIVES AND PURPOSE

2.1. Overview

Serious morbidity and mortality in sickle cell anemia (SCA) is well-described and begins early in life with the onset of the physiologic decline of fetal hemoglobin (HbF). The BABY HUG trial showed that 20mg/kg/day of hydroxyurea therapy is safe, blunts the decline of HbF, and is clinically beneficial for very young infants and children with SCA, and hydroxyurea therapy is now suggested to be offered to all very young children with SCA, whether or not they have clinical symptoms. Nevertheless, children treated with hydroxyurea continued to develop acute complications and acquired end organ damage.

The BABY HUG trial established the safety and efficacy of a fixed dosage of hydroxyurea in this very young group of children; however, it is now paramount to define the optimal dosing regimen in an attempt to maximize the benefits of therapy. Defining the clinical and hematologic impact of intensified dosages versus fixed dosing of hydroxyurea in this age group is crucial. The aims of this protocol are (1) to establish feasibility of enrolling and randomizing young children (9 to 36 months) with SCA (HbSS and HbSβ^0-thalassemia) to receive either a fixed or an intensified dose of hydroxyurea in a single blind manner, and (2) to obtain prospective pilot data comparing the clinical and laboratory efficacy of intensified versus fixed-dose hydroxyurea.

The HUGKISS pilot trial seeks to answer a basic question which remains after the publication of the BABY HUG study. That is, does escalation of hydroxyurea dose provide a clinical benefit in young children with SCA? Extrapolation from the adult literature suggests that escalation is beneficial, but the large reductions in adverse events observed in the fixed-dose BABY HUG trial call for this question to be addressed through a clinical trial. As such, the HUGKISS trial is the next logical step to potentially improve upon outcomes observed in the BABY HUG trial and advance the care of young children with SCA.

2.2. Primary and Secondary Objectives

2.2.1. Primary Objective

Demonstrate feasibility of enrolling, randomizing and administering young children (9 to 36 months) with SCA (HbSS and HbSβ^0-thalassemia) either a fixed or intensified dose of hydroxyurea therapy in a single blinded manner.

2.2.2. Secondary Objective(s)

1. Describe decision making process; i.e.; reasons for agreement or refusal to participate in HUGKISS for each family approached.

2. Develop an infrastructure and clinical monitoring plan that facilitates safe administration of hydroxyurea at a fixed dose and an intensified dose to MTD in a single-blinded manner in children.

3. Obtain pilot data on the laboratory effects, clinical outcomes and toxicities observed with fixed versus intensified hydroxyurea dosing.

4. Obtain pilot data on health related quality of life in children with SCA treated with a fixed dose and at MTD.

2.3. Hypothesis

The primary hypothesis is that a consortium of four large pediatric sickle cell centers will collaborate and demonstrate the feasibility of enrolling, randomizing and administering hydroxyurea for approximately 12 months in a single and blind manner. Demonstration of feasibility would support the design and implementation of a definitive comparison effectiveness trial (phase III) in this young population with SCA.

3.0 BACKGROUND AND RATIONALE

3.1. Background

3.1.1. Sickle Cell Disease (SCD)

SCA is a catastrophic hematologic disease that effects 300,000 newborn children worldwide every year[2]. A classic example of recessive Mendelian inheritance, SCA is caused by a mutation in the β globin gene that results in production of a mutant globin protein, sickle hemoglobin (HbS). In deoxygenated conditions, HbS polymerizes leading to the classic "sickle-shaped" erythrocytes. Clinically, children with SCA suffer from a wide spectrum of clinical manifestations including severe pain crises, acute chest syndrome, splenic sequestration, cholelithiasis, priapism and stroke with symptoms beginning in infancy. Without therapy, individuals with SCA experience recurrent acute vaso-occlusive complications and progressive organ damage that typically leads to a premature death in young adulthood[3].

3.1.2. Hydroxyurea Therapy for Children with SCA

In natural history cohorts of adults and children[4,5] with SCA, high levels of endogenous HbF ($\alpha_2\gamma_2$) correlate strongly with an improvement in clinical severity. For example, individuals with HbS mutations and concurrent hereditary persistence of fetal hemoglobin (HPFH), express very high HbF (mean 31.3%; SD, ±2.4; range, 27.1-36%) and exhibit a benign clinical course[6].

Hydroxyurea, a ribonucleotide reductase inhibitor[1,7], is a potent HbF inducer and antisickling agent[8] that was demonstrated to safely reduce vaso-occlusive events (pain and acute chest syndrome), hospitalizations and transfusion requirements compared to placebo in adults with SCA[9]. Subsequently, hydroxyurea was approved by the FDA in 1998 for the treatment of adult SCA patients with recurrent and severe crises.

In the 1990s, several small studies reported short-term safety and toxicity profiles for hydroxyurea therapy in children[10-12], but there was reluctance to use the drug in this population due to concerns of potential toxicities. Over the past several decades, a number of long-term studies have reported the benefits of hydroxyurea in preventing complications and reducing mortality in older children with SCA[13-16]. The HUG-KIDS phase I/II prospective trial was designed to identify the maximum tolerated dose (MTD) for hydroxyurea in children and treat them at that dose for one year. Eligible children 5 to 15 years old were initiated hydroxyurea at 15 mg/kg/day with escalation by 5 mg/kg/day every 8 weeks to MTD, defined as the dose 2.5 mg/kg below which 2 successive hematologic toxicities occurred or a maximal dose of 30 mg/kg, and was sustained without toxicity for 8 weeks. The mean (±SD) MTD observed was 25.6 (±6.2). Children treated with hydroxyurea at MTD showed significant hematologic

responses similar to those observed in adults (Table 1) and treatment was well tolerated without clinical AEs[17].

Table 1. Phase I/II hematologic values of children and adults being treated with hydroxyurea at MTD[17,18].

Hematologic value, mean (SD)	Pediatric Phase I/II				Adult Phase I/II
	Entry (n=84)	6 months (n=78)	12 months (n=77)	24 months (n=35)	(n=32)
Fetal Hemoglobin (%)	7.3(4.9)	14.9(6.4)	17.8(7.2)	15.5(7.3)	15(6)
Hemoglobin (g/dL)	7.8(1.0)	8.8(1.0)	9.0(1.4)	9.0(1.1)	9.7(1.8)
Mean Corpuscular Volume (fL)	85.9(6.6)	99.5(9.0)	101.3(10.2)	98.9(9.1)	117 (15)
Absolute Reticulocyte ($x10^9$/L)	354(144)	204(204)	191(100)	215 (92)	243 (73)
White Blood Cell ($x10^9$/L)	13.6(3.9)	9.3(3.0)	9.2(3.2)	9.2(3.0)	8.4 (1.4)
Absolute Neutrophil ($x10^9$/L)	7(3.0)	4.4(2.1)	4.4(2.2)	4.6(2.3)	4.6 (1.1)
Total Bilirubin (mg/dL)	3.6(2.6)	2.9(2.1)	2.5(2.0)	2.8(2.5)	1.9(1.2)
ALT (IU/L)	27(14)	28(20)	22(22)	27(12)	37(29)

All values at six months are significantly different from baseline (p<0.001). At 12 months MCV, HbF, and F-cells are higher than at six months (p <0.05).

The first pilot trial in infants with SCA (HUSOFT) demonstrated that hydroxyurea therapy was feasible, well-tolerated, and it had hematologic efficacy[19]. In 1999, following results from HUG-KIDS and based on recommendations of the NHLBI Sickle Cell Advisory Committee, the NHLBI released a competitive contract to conduct a trial to test whether hydroxyurea administered to infants for 2 years would inhibit cumulative organ damage. During planning of the Pediatric Hydroxyurea Phase III Trial (BABY HUG; NCT 00006400), many investigators advocated for aggressive hydroxyurea intensification, similar to the previous HUG-KIDS trial. However, the young age of participants (9-18 months) raised concern for toxicity and a lower fixed dose of 20 mg/kg/day was selected[20].

Between October, 2003 and September, 2009, BABY HUG[1] investigators from thirteen medical centers randomized very young (9-18 months) children with SCA to receive either placebo or hydroxyurea (20 mg/kg/day) and followed subjects for two years. The primary endpoints were splenic function (qualitative uptake of ^{99m}Tc spleen scan) and renal function (glomerular filtration rate by ^{99m}Tc-DTPA clearance). Secondary endpoints included hematologic parameters, biomarkers of splenic and renal function, transcranial Doppler (TCD) ultrasonography and growth. Of primary importance and contrary to some investigators' concerns[21], hydroxyurea was found to be safe in this young cohort (Table 2). Expectedly, mild to moderate myelosuppression occurred more frequently in those on hydroxyurea, but no episode was associated with an invasive bacterial infection. Severe anemia and thrombocytopenia occurred infrequently and were not associated with hydroxyurea therapy. No renal or hepatic toxicities were observed despite monitoring of bilirubin and creatinine[22]. Importantly, hydroxyurea did not adversely alter growth velocity[23]. Children treated with hydroxyurea had a normal

clinical response to pneumococcal polysaccharide vaccines and a normal immune state[24]. Children did not suffer from an increased frequency or a delayed incidence of splenic sequestration, and importantly, hydroxyurea did not appear to be mutagenic[25].

Table 2. Laboratory toxicities with 20 mg/kg/day of hydroxyurea versus placebo in the BABY HUG trial[1]

	Hydroxyurea (n=96)		Placebo (n=97)		Hazard Ratios (95% CI)	*p* value
	Events	Patients	Events	Patients		
ANC <500/μL^3	5	5	2	2	2.5 (0.5$^{I)}$- 12.9)	0.26
ANC 500–1250/μL^3	107	45	34	18	3.0 (1.7-5.1)	<0.0001
Thrombocytopenia	12	11	8	7	1.6 (0.6-4.1)	0.32
Severe Anemia	1	1	2	2	0.47 (0.04-5.2)	0.53

ANC, absolute neutrophil count; ARC, absolute reticulocyte count; ALT, alanine aminotransferase; thrombocytopenia: platelet count <80×10^3/μL^3; severe anemia: Hb <7.0 g/dL plus ARC <80×10^3/μL^3, creatinine elevation:≥2×baseline plus ≥10 mg/L

The benefits of hydroxyurea therapy at a fixed dose were clear and remarkable. Hydroxyurea therapy in infants/young children improved hematologic parameters, similar to what was observed in adults and in the HUG-KIDS trial, with elevated hemoglobin, mean corpuscular volume (MCV) and HbF and reduced white blood cell, neutrophil and reticulocyte counts (Table 3). Acute vaso-occlusive rates for the most common complications of SCA were significantly reduced in children treated with hydroxyurea versus placebo (Table 4). Pain was twice as frequent, dactylitis five times more common, and acute chest syndrome three times more likely to occur in the placebo group. Hospitalizations and transfusions were marginally increased in the placebo group.

Table 3. Mean hematologic parameters from BABY HUG trial at study entry and exit[1]

	Hydroxyurea (n=96)		Placebo (n=97)	
	Entry	Exit	Entry	Exit
White Blood Cells(x10^3/μL)	14.4	10.6	14.3	13.9
Hemoglobin (g/dL)	8.9	9.1	9.2	8.6
MCV (fL)	80.2	92.2	80	86.2
HbF (%)	25.6	22.4	27.1	17.1
ANC (x10^3/μL)	4900	4500	4200	5600

Exit versus entry mean differences all p<0.01 calculated via Student's *t* test

3.1.3. Fixed dose hydroxyurea at 20mg/kg/day did not prevent the development of organ dysfunction.

Qualitative spleen scans, measured by uptake of ^{99}Tc, were worse at exit than at entry in 19/70 (27%) patients on hydroxyurea and 28/74 (38%) of those receiving placebo (p=0.21). Additionally, renal function, measured by ^{99}mTc –DTPA clearance, did not differ in the two groups (p=0.84). However, quantitative measures of splenic function (red blood cell Howell-Jolly bodies and pit counts) and renal function (urine osmolality and specific gravity) suggested possible benefits of hydroxyurea therapy[26]. Also, the increase in time-averaged mean maximum velocity on TCD from baseline to exit was blunted in the hydroxyurea group (20 cm/sec versus 32 cm/sec, p=0.0002), suggesting reduced vasculopathy within the central nervous system[1].

Table 4. Adverse clinical events in BABY HUG trial[1]

	Hydroxyurea (n=96)		Placebo (n=97)		Hazard Ratios (95% CI)	*p* value
	Events	Patients	Events	Patients		
Pain	177	62	375	75	0.59 (0.42-0.83)	0.002
Acute Chest Syndrome	8	7	27	18	0.36 (0.15-0.87)	0.02
Hospitalization (all cause)	232	69	324	84	0.73 (0.53-1.0)	0.05
Transfusion	35	20	63	33	0.55 (0.32-0.96)	0.03
Dactylitis	24	14	123	42	0.27 (0.15-0.5)	<0.0001

Hazard ratio and 95% CIs generated via Cox model. p value calculated from log-rank life test

3.1.4. Children on fixed dose hydroxyurea continue to suffer from acute clinical manifestations.

Although we observed a substantial reduction in acute vaso-occlusive complications, children on 20mg/kg/day hydroxyurea continued to suffer from pain, acute chest syndrome and were still frequently hospitalized (Table 5).

Table 5: Vaso-occlusive complications observed in hydroxyurea and placebo arms in BABY HUG[1]

	Pain Events n (%)			Acute Chest Events n (%)			Hospitalizations n (%)		
	None	1	>2	None	1	>2	None	1	>2
Hydroxyurea	34(35)	29(30)	33(35)	89(93)	6(6)	1(1)	27(28)	22(23)	47(49)
Placebo	22(23)	15(15)	60(62)	79(82)	10(10)	8(8)	13(13)	16(17)	68(70)

3.1.5. Higher levels of HbF are associates with fewer SCA related complications in older children with SCA treated with hydroxyurea at MTD.

Several studies have shown that increased endogenous HbF (not drug-induced) was associated with reduced episodes of severe vaso-occlusive pain in SCA patients[27,28]. However, few (<10%) of the individuals studied had HbF >20%. The mean HbF of children randomized to fixed dose hydroxyurea in BABY HUG was 22.4% at study exit. The clinical benefit of further elevating HbF above this level is uncertain. To address this question, we evaluated 230 children who were enrolled in The Hydroxyurea Study of Long-Term Effects (HUSTLE, NCT00305175). These patients were older [age 9.6 (±5.0) years] when hydroxyurea was initiated and were intensified to an average dose of 26.7 (±4.6) mg/kg/day. In 610 patient-years of follow-up, there were 521 hospitalizations resulting in 0.85 hospitalizations per patient-year and a median of 1 (range, 0-43; mean 2.4, SD 4.5) hospitalization per child. When HbF levels were analyzed as a continuous variable, for every 5% decrease in HbF, the odds of being hospitalized during a three month interval were 1.2 (95% CI: 1.1-1.3), i.e., a 20% increase in the odds for each 5% decline in HbF. When HbF levels were categorized into 4 groups (<15%, 15 to ≤20%, >20 to ≤25%, and >25%), it was found that the odds of being hospitalized during any interval when HbF levels were ≤20% more than doubled (Table 6)[29]. Thus, our preliminary data suggested that higher HbF levels are associated with fewer SCD-related complications and support an intensified dosing strategy to maximize HbF levels. However, these results may not be generalizable to very young children and their clinical implications must be examined prospectively in young children.

Table 6. Fetal hemoglobin percentage and all hospitalizations or pain or acute chest syndrome

HbF, (%)	Subjects (*n*)	Intervals (*n*)	All Hospitalizations OR (95% CI)
<15	159	768	2.1 (1.3-3.3)
15 to ≤20	154	589	2.2 (1.3-3.6)
>20 to ≤25	131	509	1.0 (0.6-1.7)
>25	98	573	1

Odds ratios (OR) calculated with GEE model controlling for time. HbF Hemoglobin F, ACS acute chest syndrome

3.1.6. Hydroxyurea: Pharmacokinetics (PK) and Bioavailability

Despite safety and efficacy data from HUG-KIDS and BABY HUG and the NIH/NHLBI recent recommendation, limited data existed on the PK of hydroxyurea in children. The FDA offered a written request under the Best Pharmaceuticals for Children Act (BPCA) to specifically address this knowledge gap. In response, we performed the "Pharmacokinetics and Relative Bioavailability of a Liquid Formulation of Hydroxyurea in Pediatric Patients with Sickle Cell Anemia" trial

(HUB01; NCT01506544). Of relevance to the current proposal, we compared: 1) bioavailability of the liquid formulation of hydroxyurea administered in BABY HUG to capsules; and 2) hydroxyurea PK profiles in younger and older children. Liquid and capsule formulations were bioequivalent; hydroxyurea was rapidly absorbed with a mean (±SD) time to maximum plasma concentration (Tmax) of 0.86 (±0.53) hours and a half-life (t1/2) of 2.3 (±0.5) hours. Younger and older children had similar PK profiles for every parameter tested (Table 7)[30].

Table 7. Pharmacokinetics of hydroxyurea in young and old children

	Younger Children (n=17)	Older Children (n=22)
Age (y), mean (SD)	4.5 (1.7)	12.0 (3.6)
Cmax (µg/mL)	33.8 (8.3)	33.6 (8.2)
Tmax (hr)	0.86 (0.53)	0.97 (0.52)
AUCINF (µg·hr/mL)	114.1 (29.4)	116.3 (30.0)
t½ (hr)	2.3 (0.5)	2.3 (0.5)

Cmax, Maximum observed plasma concentration; Tmax, Time to maximum plasma concentration; AUCINF, Area under the concentration versus time curve calculated using the log-linear trapezoidal method from time 0 extrapolated to time infinity; t½, CL/F/BW, Clearance, normalized for Body Weight

3.1.7. Hydroxyurea Escalated to MTD for Young Children with SCA

A review of published literature for older children with SCA suggests that hydroxyurea intensified to moderate myelosuppression results in an average HbF of ~20%, compared to 15% when a lower fixed-dose is utilized[31]. We reported a cohort of children (median age 3.4 years, range, 2.6- 4.4 years) who had previously been treated with 20 mg/kg/day hydroxyurea for two years, followed by dose intensification, as per the HUG-KIDS protocol, to a mean (±SD) of 30 (±1.2) mg/kg/day[32]. This intensification was well tolerated and resulted in improved hematologic parameters (Table 8).

Table 8. Effects of hydroxyurea intensification in older children[32]

	Hydroxyurea Dose	
	20 mg/kg/d (n=21)	30 mg/kg/d (n=17)
White Blood Cells (x10^3/µL)	10.1	10.1
Hemoglobin (g/dL)	8.8	9.1*
MCV (fL)	90.0	95.1
HbF (%)	20.3	23.7
Reticulocyte Count (%)	8.6	8.2

*$p<0.05$

One important consideration in the HUGKISS study is that very young children are undergoing the physiological γ to β globin switch[33,34]. In children with SCA, levels of HbF at birth (60-90%) decline to a mean of 9% (5^{th}-95^{th} percentile; 3.3-21.9%) by 48-60 months of age as HbF is replaced by HbS[33]. This phenomenon may have profound implications on the therapeutic benefit of intensified hydroxyurea doses for very young children. In BABY HUG, HbF declined by 13% (=(0.256-0.225)/0.256) in children randomized to hydroxyurea from entry (HbF, 25.6%) to exit (HbF, 22.4%) compared to a decline of 37% (=(0.271-0.171)/0.271) (entry HbF, 27.1%; exit HbF 17.1%) in those randomized to placebo. We do not know what the effect of intensifying hydroxyurea therapy on HbF production would be in a population which, on average, has an endogenous starting HbF level of ~25%.

At SJCRH, 23 children (<3 years of age) initiated hydroxyurea, had dose intensification with a goal of ANC of 1500-3000 cells/μL and had one year of clinical follow up. At the time of hydroxyurea initiation, 7 were infants (younger than 18 months) (mean 12.1; SD ±3.2 years) and 16 were toddlers (18-36 months) (mean 26.4; SD ±2.7 months). Dose escalation was handled similarly between the infant and toddler groups and was similar after 18 months of therapy (27.0 mg/kg/day in infants versus 27.7 mg/kg/day in toddlers, p=0.84). Mean baseline HbF levels were 19% (±9%) in infants and 15% (±7%) in toddlers (p=0.25). After 18 months of intensification of hydroxyurea therapy, hematologic parameters improved (Table 8); specifically, there was a mean increase of HbF by 5% (±6%) (p=0.008). No participants in the infant group developed neutropenia, and only four toddlers experienced neutropenia. This preliminary data suggests that intensification of hydroxyurea in this very young age group does provide higher HbF levels, similar to the hematologic changes seen in older populations, but this relationship must be tested prospectively.

Table 9. Hematologic parameters of infants and toddlers at baseline and following 18 months of intensification of hydroxyurea therapy

Parameter; mean (SD)	Baseline n=22		18 months n=19	
Hemoglobin (g/dL)	8.2	(0.9)	9.1	(1.4)*
Mean Corpuscular Volume (fL)	86	(7.1)	100	(7.4)†
Fetal Hemoglobin (%)	16	(8)	24	(8)*
Absolute Reticulocyte (x10^9/L)	310	(80)	190	(90)†
White Blood Cell (x10^9/L)	16	(5.8)	10	(3.4)†
Absolute Neutrophil (x10^9/L)	5300	(2500)	3600	(1500)†

*p<0.01, †p<0.001; significance of difference from baseline.

3.2. Rationale

Mounting evidence supports the safety and clinical efficacy of hydroxyurea for the treatment of all children with SCA, however, the following have not been established: (1) the optimal dosage for infants and very young children, (2) whether hydroxyurea intensification provides further therapeutic benefit compared to standard low dose.

Therapeutic intervention prior to the onset of the clinical manifestations and irreversible organ injury during infancy may provide long-term benefits. In very young (9-18 months) children with SCD, daily fixed-dose hydroxyurea (20 mg/kg/day) safely and effectively raises HbF levels, improves hematological parameters and reduces clinical manifestations including pain, dactylitis and acute chest syndrome[1]. Nevertheless, children in the treatment arm of BABY HUG continued to experience vaso-occlusive symptoms[22] and to incur organ damage[1]. In older children and adults with SCD, dose-intensification of hydroxyurea to mild myelosuppression (ANC 1500-3000 cells/μL) may improve hematologic parameters[16,32] compared to fixed dosing, but intensification is not accepted as the standard of care globally because this approach, while promising, is not proven to improve clinical outcomes[35-38].

Our preliminary data demonstrate that the pharmacokinetic parameters of hydroxyurea in young children are predictable and similar to that observed in older children and adults[30], and that very young children exhibit a safe and robust hematologic response when hydroxyurea is intensified (unpublished). Together, these findings support our hypothesis that **dose intensification of hydroxyurea in very young children will be safe and produce superior results compared to fixed dose hydroxyurea, which was utilized in the BABY HUG trial.** Addressing this question is crucial for optimizing hydroxyurea therapy in young patients with SCA and represents the natural therapeutic extension to improve upon the BABY HUG study results.

A multicenter clinical trial is necessary to address our hypothesis. Here we describe plans to establish the feasibility and infrastructure for this important study.

4.0 RESEARCH PARTICIPANT ELIGIBILITY CRITERIA AND STUDY ENROLLMENT

4.1. Inclusion Criteria

1) Children with HbSS or HbS/β^0thalassemia
2) ≥9 to ≤ 36 months of age at study initiation
3) Enrollment will occur irrespective of clinical severity

4.2. Exclusion Criteria

Permanent:
1) Receiving chronic red blood cell transfusion therapy.
2) Condition or chronic illness, which in the opinion of the PI makes participation unsafe.

Transient (participants may be re-evaluated after ≥14 days):
1) Recent (<30 days) participation in another clinical intervention trial utilizing an IND/IDE agent.
2) Erythrocyte transfusion in the past 2 months.
3) Laboratory Assessments:
    a. Hemoglobin <6.0 gm/dL
    b. Absolute reticulocyte count <80 x 10^9/L if hemoglobin <9.0 mg/dL
    c. Absolute neutrophil count <1.5 x 10^9/L
    d. Platelet count <100 x 10^9/L
    e. Serum creatinine > twice the upper limit of normal for age
    f. ALT > twice the upper limit of normal

4.3. Research Participant Recruitment and Screening

Four institutions will collaborate in the proposed project: St. Jude Children's Research Hospital (SJCRH); University of Mississippi Medical Center (UMMC); UT Southwestern (UTSW) and Children's Healthcare of Atlanta (CHOA). Recruitment will begin in Year 1 and will be completed by the middle of Year 2 to ensure 52-56 weeks of study treatment/evaluations and 4 weeks of off-study follow-up. Universal newborn screening data will be utilized by each site to determine the number of potentially eligible children. We plan on approaching all available eligible participants until enrollment is completed. The study will be discussed with each potentially eligible family by the local PI and/or study coordinator in an unbiased basis until enrollment goals are met. During this recruitment phase, local sites will assess and document whether each eligible family verbally agreed to participate or not and will record the reason for the choice. Based on our previous experience in BABY HUG reasons for

initial affirmative responses will be coded based on common themes, such as: "perceive the child to be ill," "desire to aid research," "desire for closer follow-up," "hope that the child would be randomized to receive higher dose hydroxyurea," and "other." Conversely, reasons for declining participation will be grouped into the categories: "fear of research," "transportation problems," "excessive clinic visits and/or lab draws with hydroxyurea therapy," "perception that the child was not ill," "not willing to initiate hydroxyurea therapy due to concerns for toxicity," and "other."

4.4. Enrollment on Study at SJCRH

A member of the study team will confirm potential participant eligibility as defined in Sections 3.1 and 3.2, and complete the "Participant Eligibility Checklist." The completed checklist will be scanned and emailed to the Central Protocol and Data Monitoring Office (CPDMO): EligibilityCoordinators@STJUDE.ORG.Eligibility will be reviewed and entered into the PPM system. A research participant-specific consent form will be generated when applicable. The protocol and consent document will be delivered to the area designated on the checklist. The entire signed informed consent must be scanned and emailed to the CPDMO to complete the enrollment process.

The CPDMO is staffed 7:30 am-5:00 pm CT, Monday through Friday. A staff member is on call Saturday, Sunday, and holidays from 8:00 am-5:00 pm. Enrollments may be requested during weekends or holidays by calling the CPDMO on call cell phone [redacted] or referencing the "On Call Schedule" on the intranet.

4.5. Procedures for Identifying and Randomizing Research Participants

All children will be initiated on hydroxyurea (100 mg/mL) at a dose of ~20 mg/kg/day in an open label fashion for eight weeks (±2 weeks) prior to randomization. Participants without toxicity (defined in section 6.4), which requires discontinuation of hydroxyurea during those eight weeks (±2 weeks), will be randomized to receive "standard" or "intensive" therapy in a 1:1 ratio. Randomization will be stratified by clinical center and by baseline age of the participant (9 to <24 months and 24 to 36 months) because of the natural physiologic decline of HbF with increasing age.

4.6. Enrollment Instructions for Collaborating Sites

Collaborating Site research participants should be registered at SJCRH within 24 hours of enrollment at the site. A Project Manager (PM) will serve as a Medical Coordinating Center (MCC) Staff Member. The PM will serve as liaison to all collaborating sites to ensure compliance with protocol prescribed interventions and evaluations, answer questions and provide clarifications. The PM will maintain regulatory documents from all collaborating sites, maintain documentation of training for all site personnel, receive

and process all AE's, send reports to PI and governing bodies regarding study as indicated.

The completed Eligibility Checklist and entire signed Informed Consent should be scanned and emailed to the Central Protocol and Data Monitoring Office (CPDMO): EligibilityCoordinators@STJUDE.ORGThe Protocol Eligibility Coordinator will then register the research participant in the PPM system and assign a study ID number.

5.0 TREATMENT PLAN

5.1. Hydroxyurea Therapy

All participants randomized to the standard treatment (20 mg/kg/day) and investigational treatment (escalation to MTD) will undergo the same number of study visits and laboratory tests, which include monthly visits with clinical evaluations (every 4 ± 2 weeks), laboratory tests, and experimental evaluations (see Table 10). The frequency of toxicity monitoring is based on recent guidelines for the management of SCD[39].

For all participants, hydroxyurea will be taken once daily, ideally at a regular dosing time either in the morning or before bedtime. The starting dose of hydroxyurea is 20 mg/kg/day. Given the developmental age of this population, hydroxyurea will be provided as a liquid formulation (100 mg/mL). Dosing flexibility of up to +/- 2.5 mg/kg/day is permitted to allow for rounding to available easily measurable volumes. Liquid hydroxyurea will be formulated by KP Pharmaceutical Technology, INC. Using IND Number 111866, which is held by Kathleen Neville, a pediatric hematologist at Arkansas Children's Hospital, for the liquid formulation of hydroxyurea used in the HUBO1 trial (ClinicalTrials.gov identifier: NCT01506544) and a separate liquid formulation which was used by BABYHUG, held by NHLBI, IND number 67,289. SJCRH has an IND from the FDA to use hydroxyurea in the HUGKISS trial, IND Number 132032.

5.2. Standard Treatment Arm (20 mg/kg/day)

The plan for dose reduction and toxicity monitoring for the standard treatment arm is based on safety data and toxicity monitoring from BABY HUG[1].

The starting dose of hydroxyurea will be 20 (+/- 2.5) mg/kg/day. If toxicity occurs (defined in section 6.4), treatment will be paused and blood counts will be checked every seven days until they return to non-toxic values. Transient toxicity will not cause a dose reduction or termination of therapy, but prolonged (defined as ≥21 days) or repeated toxicity will. If a toxicity resolves within 21 days, treatment will be resumed at the previous dose. If the toxicity recurs, hydroxyurea will again be held for an additional 7 days. If toxicity resolves, hydroxyurea will resume at a daily dose 2.5 mg/kg lower than

the previous dose. If that dose does not cause toxicity for eight weeks, then the lower dose will be assigned as the stable dose. If hematologic toxicities occur while a participant is being treated with an established stable dose, treatment will be stopped until toxicity resolves and then treatment will resume using the previously established stable dose. Repeated toxicity in a 12-week period will reduce the stable dose by 2.5 mg/kg for the remainder of the study. Based on previously published experience with hydroxyurea therapy in young subjects with sickle cell disease[40], discontinuation of hydroxyurea therapy due to persistent or intolerable myelosuppression is extremely unusual.

5.3. Investigational Arm (Escalation to MTD)

The goal of the investigational arm of HUGKISS will be to achieve a neutrophil count of 1500-3000 cells/μL. Hydroxyurea will be titrated according to myelosuppression, and will be increased to the MTD as defined by achieving the goal neutrophil count without toxicity. Escalation to MTD of hydroxyurea usually takes 6-9 months. Hydroxyurea dosing will commence at 20 (+/- 2.5) mg/kg/day. Hydroxyurea dose escalation will occur in 5 mg/kg/day increments, adjusting every 8 weeks unless hematological toxicity (defined in section 6.4) is achieved. Most pediatric subjects require hydroxyurea doses of 20-30 mg/kg/day to reach the target ANC. After reaching MTD, minor dose adjustments will be made as necessary based on weight changes and blood counts, to maintain the desired laboratory effects. If the ANC rises above the target range on two consecutive interval visits, compliance will be reinforced and the dose may be adjusted as needed by 2.5 mg/kg/day at eight week intervals to a maximum of 35 mg/kg/day.

Hydroxyurea will be temporarily discontinued if toxicity occurs (defined in section 6.4), treatment will be paused and blood counts will be checked every seven days until they return to non-toxic values. Transient toxicity will not cause a dose reduction or termination of therapy, but prolonged (defined as ≥21 days) or repeated toxicity will. If a toxicity resolves within 21 days, treatment will be resumed at the previous dose. If the toxicity recurs, hydroxyurea will again be held for an additional 7 days. If toxicity resolves, hydroxyurea resume at a daily dose 2.5 mg/kg lower than the previous dose. If that dose does not cause toxicity for eight weeks, then the lower dose will be assigned as the stable dose. If hematologic toxicities occur while a participant is being treated with an established stable dose, treatment will be stopped until toxicity resolves and then treatment will resume using the previously established stable dose. Repeated toxicity in a 12-week period will reduce the stable dose by 2.5 mg/kg for the remainder of the study.

Complications of hydroxyurea therapy (excessive myelosuppression, severe anemia or thrombocytopenia, or drug non-compliance) will be collected at each interval visit on the interval visit case report form (CRF) and as part of AE reporting.

5.4. Treatment Modifications for Collaborating Sites

All collaborating sites will follow the study design as written in the protocol.

5.5. Concomitant Therapy

A concomitant medication is defined as any prescription or over-the-counter preparation, including vitamins and supplements. In the interests of subject safety and acceptable standards of medical care the Investigator will be permitted to prescribe treatment(s) at his/her discretion. Almost all concomitant medications will be allowed, such as antibiotics, anticonvulsants, and asthma therapy. For all enrolled subjects, all administered concomitant medications from signing the informed consent until 30 days after the subject's last dose of study drug, will be recorded in the subjects' CRF (medication, dose, treatment duration and indication). All reported prior and concomitant medications will be coded using the current version of the World Health Organization (WHO) Drug Dictionary.

No subject can be currently participating in other therapeutic clinical trials or taking other agents for SCD, such as hydroxyurea, arginine, decitabine, magnesium, nitric oxide, etc. Eligible subjects taking any therapeutic agent for SCD will require a washout period of at least three months prior to study enrollment.

Clinically-indicated erythrocyte transfusions may need to be given during the study if complications from SCA arise, such as a severe episode of acute chest syndrome or splenic sequestration.

5.6. Subject Compliance

Interval visit CRFs and study medication logs will be completed by the site study personnel at each interval visit to capture medication adherence with hydroxyurea. Study visits are scheduled every 4 (± 2) weeks, allowing flexibility for family or center scheduling needs. Subjects should bring the previously dispensed medication bottles each interval visit for review with study personnel. In addition, the Medication Adherence Measure (MAM) will be administered at each interval visit.

5.7. Duration of Treatment

Subjects in both treatment arms will be followed for a maximum of 56 weeks regardless of the actual number of study visits. Upon completion of the 56 weeks, patients will be followed for an additional 30 days for toxicities and will then be taken off study.

5.8. Blinding

All participants enrolled in HUGKISS will be treated with hydroxyurea. A double-blind would be logistically challenging and could result in a significant safety risk to participants. The population eligible for enrollment will require liquid formulation and randomization will dictate a dosage of ~20 mg/kg/day or escalation to ~25-35 mg/kg/day.

A double-blind design would require either higher concentrations of hydroxyurea suspension for participants undergoing dose escalation or for participants to ingest two aliquots of study drug or study drug/placebo combinations. Currently, the standard formulation of hydroxyurea is 100 mg/mL, and obtaining antimicrobial stability testing for other concentrations would incur significant expense and added burden to the local pharmacies; furthermore, these formulations would not provide meaningful clinical benefit in the future. Requiring participants and/or care givers to administer two aliquots of study drug (hydroxyurea + hydroxyurea, or hydroxyurea + placebo) would place a burden on participants, likely reduce medication adherence, and be a potential source for medication error.

Although both of the double-blind designs were contemplated, they would place significant burden on families and/or pharmacies, and it was felt that the objectives of HUGKISS could be safely and more efficiently obtained with a single-blind design, in which primary investigators and medical providers would remain blinded, and patients would have their volume of medication assigned.

5.8.1. HUGKISS Enabled Local Provider for Evaluating Response (HELPER)

For clinical decisions regarding dose escalation, drug toxicity and/or management of clinical illness, there will be a local clinical provider, not affiliated with HUGKISS, who will provide interpretation of laboratory data.

The integrity of the HUGKISS trial will depend upon providing study treatment that is blinded to investigators by randomization and delivery of unbiased clinical care for the duration of the trial. The MCC will maintain records of each patient's drug assignment and current dosage. The staff of the MCC will have access to individual patient treatment assignment codes and current dose on a "need to know" basis. The Clinical Center PIs must agree to avoid seeking information that may unblind them to an individual participant's treatment assignment. This requires an "honor system" approach, which was successful in the BABY HUG trial. However, as the HUGKISS study requires dose intensification of hydroxyurea based on an ANC goal of 15003000 cells/μL, real-time knowledge of local CBC results is essential for Clinical Center study staff. As such, a HELPER will be utilized. The HELPER will be an unblinded local medical provider, unaffiliated with the steering committee or data analysis, who

is knowledgeable about SCA. These individuals will be charged with transmitting endpoint data and monitoring toxicities and adherence in real time with the goal of optimizing clinical care of study patients. The HELPER (unblinded) study member will perform these clinical responsibilities and that the site PI will attest that they will avoid clinical duties that may unblind them to participant treatment status. This will be similar to the infrastructure of the BABY HUG trial.

5.8.2. Emergent Unblinding

Every family will be given an identification card describing the child's participation in the HUGKISS study, listing emergency study telephone numbers (e.g., the Clinical Center Principal Investigator's (PI) telephone number and the Central Study Emergency Contact's telephone number). The Central Study Emergency Contact's number will be answered by a pediatric SCA consultant to the MCC at all times.

If HUGKISS children become ill, treating physicians will be urged to call the Clinical Center PI before altering the child's study regimen. In an emergency, family members will be instructed to call a telephone number that will be manned by a pediatric hematologist 24 hours a day. Arrangements will be made so that the child's medication can be disclosed to the Clinical Center PI after consultation between the Clinical Center PI and the pediatric SCA consultant to the MCC.

Reasons for unblinding are limited and are based on clinical grounds. Unblinding must be initiated by the Clinical Center PI. Reasons for unblinding include overdose of the study medication, accidental ingestion of the study medication by another person, development of infection or bleeding that could be due to reduced white blood cell or platelet counts and for which management might be changed if the nature of the study drug were known. Examples of clinical situations when information on study treatment could be useful include severe thrombocytopenia calling for a decision to use prednisone versus platelet transfusion or severe neutropenia calling for a decision on choice of antibiotics. If a child's therapy is unblinded, the Clinical Center PI or staff member who unblinded the treatment must send a report to the MCC.

5.8.3. Randomization and Treatment Allocation

Children who meet all criteria for enrollment will be randomized to single-blind treatment at week 8. Randomization will be stratified by baseline age obtained during the baseline evaluation obtained on week 0 and Clinical Center. Assignment to treatment groups will be determined by a computer-generated random sequence housed within the Department of Biostatistics. Treatment allocation will then be communicated to the local unblinded study personnel (HELPER and pharmacy) via encrypted email regarding treatment allocation.

6.0 DRUG INFORMATION

6.1. Source and Distribution

One form of hydroxyurea will be used in this study, a standardized liquid formulation containing 100 mg/mL hydroxyurea will be prepared using a commercial source of hydroxyurea, USP and Syrpalta® (without color), USP. Hydroxyurea will be purchased from Spectrum Pharmacy Products to be used as the source drug substance for the liquid formulation of hydroxyurea. The Syrpalta® (without color), USP will be purchased from Humco Compounding to be used as a suspension for hydroxyurea. Hydroxyurea, USP will be hand filled into 4 ounce amber bottles with 11g ± 5% (10.45-11.55g) by KP Pharmaceutical Technology (KPPT), INC. Sharp Packaging Services will provide 24 hour monitored storage of the bottles and shipment to study sites.

6.2. Formulation and Stability

Because of the limited stability of the liquid formulation of hydroxyurea, hydroxyurea will be formulated into liquid at 100 mg/ml with assistance of local pharmacies. Purified water (60 mL) will be added to a bottle pre-filled hydroxyurea, USP (11 gm/bottle). Syrpalta® (without color) will be added to the hydroxyurea-water suspension in a sufficient quantity to achieve a final volume of 110 mL. The final concentration of the hydroxyurea solution is 100 mg/mL. The whole bottle will be dispensed to a participant. Release testing and specifications for hydroxyurea will include appearance, assay, related substance and microbial limits. Release testing results and Certificates of Analysis for Hydroxyurea, USP bottle will be performed by KPPT and submitted to the FDA prior to shipping of drug to any clinical sites.

Stability and microbial limit testing of this liquid formulation has already been performed during the previous "Pharmacokinetics and Relative Bioavailability of a Liquid Formulation of Hydroxyurea in Pediatric Patients with Sickle Cell Anemia" trial and submitted to the FDA (see attached Letter of Cross Reference for IND). The stability of the liquid formulation of hydroxyurea has been demonstrated for up to 6 months[41].

6.3. Dosage and Route of Administration

Hydroxyurea (100 mg/mL) will be administered orally at a dose of 20 mg/kg/day for eight weeks (±2 week) prior to randomization. Participants randomized for dose intensification will receive hydroxyurea escalated to 25-35 mg/kg/day.

6.4. Toxicity

1) Neutropenia: absolute neutrophil count of less than 1.0 x 10^9/L or
2) Anemia: Hemoglobin less than 6.0 g/dL that occurs in the clinical setting of an absolute reticulocyte count of less than 80 x 10^9/L. or
3) Reticulocytopenia: absolute reticulocyte count less than 80 x 10^9/L, associated with a hemoglobin level of less than 9.0 g/dL. or
4) Thrombocytopenia: Platelet count less than 80 x 10^9/L

7.0 REQUIRED EVALUATIONS, TESTS, AND OBSERVATIONS

7.1. Pre-Study Evaluations

Eligibility screening (ES) and baseline evaluations should be performed prior to initiation of hydroxyurea. Eligibility screening may be performed the same time as baseline evaluations. A new hemoglobin identification only needs to be performed at eligibility screening if a previous result is not already available in the subject's medical record.

7.2. Clinical Events

All patients will be monitored for the occurrence and severity of clinical events. These will be identified through the use of standardized history questions administered at each clinical encounter. At each visit, parents will be asked to describe any illnesses experienced since the last visit. If illness is reported, a directed history will be obtained to allow its characterization. All events will be reported utilizing standardized definitions (See Appendix III) on an Events Form.

Documentation (discharge summaries, clinic/emergency department records, local laboratory values or radiology reports) for all fatal or life threatening events will be collected by Clinical Center staff for review. Events of interest include bacteremia with known pathogens, meningitis, splenic sequestration, acute chest syndrome, stroke or transient ischemic attacks, osteomyelitis, all hospital admissions and any event for which the patient receives a transfusion.

Table 10. Schedule of HUGKISS Evaluations*

Evaluation	ES[1]	BE	Weeks												Exit
	-4 to 0	0	4	8	12	16	20	24	28	32	36	40	44	48	52-56
Informed Consent	X														
Weight, Height & Vital Signs	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
History, Physical Exam	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Prior/concomitant Medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Adverse Events/Study Endpoints		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Hydroxyurea Initiation		X													
Randomization				X											
CBC with WBC Count	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Reticulocyte Count	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
CMP[5]	X	X					X					X			X
Lactate Dehydrogenase		X					X					X			X
Hemoglobin Identification	X[2]	X					X					X			X
Urinalysis		X					X					X			X
TCD Examination[3]		X													X
Quality of Life Assessment[4]		X					X					X			X
MAM[6]			X	X	X	X	X	X	X	X	X	X	X	X	X
Study Drug Accountability			X	X	X	X	X	X	X	X	X	X	X	X	X

*Ideal visits are every 4 weeks ±2 weeks, except for ES,BE, and the Exit visit. ES, eligibility screening; BE, baseline evaluation, which should be performed prior to initiation of hydroxyurea. [1]Eligibility screening may be performed the same time as baseline evaluations. [2]A new hemoglobin identification only needs to be performed at eligibility screening if a previous result is not already available in the subject's medical record. [3]To be performed on participants >2 years of age. [4]Age appropriate screening tool. [5]CMP, Comprehensive Metabolic Panel, Chemistries include: Sodium, Potassium, Chloride, CO2, Glucose, BUN, Creatinine, Calcium, Albumin, Bilirubin Total, Alk, Phos, ALT, AST, Protein Total. [6]MAM, Medication Adherence Measurement.

8.0 EVALUATION CRITERIA

8.1. Toxicity Evaluation Criteria

The plan for dose reduction and toxicity monitoring is based on our safety data from BABY HUG[1]. The starting dose of hydroxyurea will be 20 mg/kg. If toxicity occurs (defined in section 6.4), treatment will be stopped and blood counts will be checked every seven days until they return to non-toxic values. Transient toxicity will not cause a dose reduction or termination of therapy, but prolonged or repeated toxicity will. If a hematologic toxicity is identified, therapy will be discontinued for seven days. If counts recover, treatment will be resumed at the previous dose. If the toxicity persists, treatment will continue to be held for an additional 7 days and treatment will resume at a daily dose 2.5 mg/kg lower than the previous dose once the toxicity is resolved. If that dose does not cause toxicity for eight weeks, then the lower dose will be assigned as the stable dose. If hematologic toxicities occur while a participant is being treated with an established stable dose, treatment will be stopped until toxicity resolves and then treatment will resume using the previously established stable dose. Repeated toxicity in a 12-week period will reduce the stable dose by 2.5 mg/kg for the remainder of the study.

9.0 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF-STUDY CRITERIA

9.1. Off-study Criteria

1) Initiation of chronic blood transfusion therapy
2) Stroke
3) Death
4) Lost to follow-up
5) Request of the Patient/Parent
6) Discretion of the Study PI, such as the following
   a. The researcher decides that continuing in the study would be harmful
   b. A treatment is needed that is not allowed on this study
   c. The participant misses so many appointments that the data cannot be used in the study
   d. New information is learned that a better treatment is available, or that the study is not in the participant's best interest
   e. Study evaluations are complete

9.2. Collaborating Sites Notifications

Collaborating sites will be required to notify the PM with any participant information that will affect treatment criteria on this protocol. The PM will send reports to PI and governing bodies regarding the study.

10.0 SAFETY AND ADVERSE EVENT REPORTING REQUIREMENTS

10.1. Sickle Cell Related Events

In HUGKISS, clinical event definitions will be applied for consistency with other important NHLBI-sponsored clinical studies of sickle cell anemia such as the Clinical Study of Sickle Cell Disease (CSSCD), the Multicenter Study of Hydroxyurea in Sickle Cell Anemia (MSH), and BABY HUG.

These clinical events are defined in Appendix III.

10.2. Reporting Adverse Experiences and Deaths to SJCRH Institutional Review Board (IRB)

The Common Terminology Criteria for Adverse Events (CTCAE) version 4 will be used to grade adverse events. Only "unanticipated problems involving risks to participants or others" referred to hereafter as "unanticipated problems" are required to be reported to the SJCRH IRB promptly, but in no event later than 10 working days after the investigator first learns of the unanticipated problem. Regardless of whether the event is internal or external (for example, an IND safety report by the sponsor pursuant to 21 CFR 312.32), only AEs that constitute unanticipated problems are reportable to the SJCRH IRB. As further described in the definition of unanticipated problem, this includes any event that in the PI's opinion was:

- Unexpected (in terms of nature, severity, or frequency) given (1) the research procedures that are described in the protocol-related documents, such as the IRB approved research protocol and informed consent document, as well as other relevant information available about the research; (2) the observed rate of occurrence (compared to a credible baseline for comparison); and (3) the characteristics of the subject population being studied; and
- Related or possibly related to participation in the research; and
- Serious; or if not serious suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Unrelated, expected deaths do not require reporting to the IRB. Though death is "serious," the event must meet the other two requirements of "related or possibly related" and "unexpected/unanticipated" to be considered reportable.

Deaths meeting reporting requirements are to be reported immediately to the local IRB and sponsor (SJCRH), but in no event later than 48 hours after the investigator first learns of the death.

The following definitions apply with respect to reporting adverse experiences:
Serious Adverse Event (SAE): Any AE temporally associated with the subject's participation in research that meets any of the following criteria:

- Results in death;
- Is life-threatening (places the subject at immediate risk of death from the event as it occurred);
- Requires inpatient hospitalization or prolongation of existing hospitalization;
- Results in a persistent or significant disability/incapacity;
- Results in a congenital anomaly/birth defect; or
- Any other AE that, based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition (examples of such events include: any substantial disruption of the ability to conduct normal life functions, allergic bronchospasm requiring intensive treatment in the emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse), a congenital anomaly/birth defect, secondary or concurrent cancer, medication overdose, or is any medical event which requires treatment to prevent any of the medical outcomes previously listed.

Unexpected AE:

- Any AE for which the specificity or severity is not consistent with the protocol related documents, including the applicable investigator brochure, IRB approved consent form, IND or Investigational Device Exemption (IDE) application, or other relevant sources of information, such as product labeling and package inserts; or if it does appear in such documents, an event in which the specificity, severity or duration is not consistent with the risk information included therein; or
- The observed rate of occurrence is a clinically significant increase in the expected rate (based on a credible baseline rate for comparison); or
- The occurrence is not consistent with the expected natural progression of any underlying disease, disorder, or condition of the subject(s) experiencing the AE and the subject's predisposing risk factor profile for the AE.

Internal Events: Events experienced by a research participant enrolled at a site under the jurisdiction of SJCRH IRB for either multicenter or single-center research projects.

External Events: Events experienced by participants enrolled at a site external to the jurisdiction of the SJCRH IRB or in a study for which SJCRH is not the coordinating center or the IRB of record.

Unanticipated Problem Involving Risks to Subjects or Others: An unanticipated problem involving risks to subjects or others is an event which was not expected to occur and which increases the degree of risk posed to research participants. Such events, in general, meet all of the following criteria:

- Unexpected;
- Related or possibly related to participation in the research; and
- Suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized. An unanticipated problem involving risk to subjects or others may exist even when actual harm does not occur to any participant.

Consistent with FDA and OHRP guidance on reporting unanticipated problems and AEs to IRBs, the SJCRH IRB does not require the submission of external events, for example IND safety reports, nor is a summary of such events/reports required; however, if an event giving rise to an IND safety or other external event report constitutes an "unanticipated problem involving risks to subjects or others" it must be reported in accordance with this policy. In general, to be reportable external events need to have implications for the conduct of the study (for example, requiring a significant and usually safety-related change in the protocol and/or informed consent form).

Although some AEs will qualify as unanticipated problems involving risks to subjects or others, some will not; and there may be other unanticipated problems that go beyond the definitions of serious and/or unexpected AEs. Examples of unanticipated problems involving risks to subjects or others include:

- The theft of a research computer containing confidential subject information (breach of confidentiality); and
- The contamination of a study drug. Unanticipated problems generally will warrant consideration of substantive changes in the research protocol or informed consent process/document or other corrective actions in order to protect the safety, welfare, or rights of subjects or others.

10.3. Reporting to the Sponsor and/or Federal Agencies

10.3.1. Notification of Federal Agencies by Investigator

Study coordinator will assist PI with protocol submissions for review by federal or institutional committees as applicable.

10.3.2. Recording AEs and SAEs

The HUGKISS trial will use a Data Safety and Monitoring Board (DSMB) comprised of 5-6 persons with expertise in clinical trials who are not directly involved in the study or based at a participating site. These members will include a hematologist with sickle cell research experience, pharmacist, statistician, bioethicist, and patient advocate/representative. The DSMB will be appointed by the SJCRH and will receive regular reports of SAEs and study data

10.4. Process for Reporting AEs from the sites to SJCRH, and to the rest of the Collaborating Sites/Affiliates

For the purposes of this protocol, hospitalizations due to expected sickle cell related complications (Appendix III) will not be counted as AEs but will be captured as study related endpoints.

AEs will be reported to the local IRB by the respective clinical center's PI and study staff. In addition, each clinical center will be responsible for reporting AEs to the DCC via electronic data entry. The DCC will be responsible for submitting AE reports to the DSMB. All serious, unexpected events will be reported within 24 hours to the DSMB. SJCRH will assume responsibility for ensuring that AEs are reported to the FDA within the appropriate time period, and that DSMB summary reports are distributed to study investigators for submission to their local IRBs.

11.0 DATA COLLECTION, STUDY MONITORING, AND CONFIDENTIALITY

11.1. Data Collection

Data will be captured during the study visits in a timely manner, as specified in Appendix I. The Departments of Biostatistics and Hematology at SJCRH has experience in designing and implementing secure databases for multisite clinical trials involving SCA in children. Data monitoring of the study will be performed by the CPDMO office at SJCRH.

For HUGKISS, a secure Current Research Information System (CRIS) database will be established that will facilitate remote data entry with electronic data transfer. All data entered into CRIS must have verifiable source documents in the patient chart and should be reproducible at site monitoring and audit visits. The sites will complete the data entry as it is outlined in the protocol and Manual of Operations. Data entered in the CRIS database will be monitored and queried by the protocol coordinator for protocol and regulatory compliance.

11.2. Data Collection Instructions for Collaborating Sites

All data will be collected by the participating sites and sent via electronic data capture to the study database.

11.3. Study Monitoring

All Clinical Centers will submit the HUGKISS Protocol and the Informed Consent Form as modified for their local IRB for initial review before any patient can be screened for eligibility. Centers will submit to their local IRB updates for annual review, protocol amendments, study-wide reports such as DSMB recommendations, and any other information that may be requested by the IRB. Clinical Centers will comply with local IRB requirements regarding reporting serious AEs, whether or not related to study treatment. Clinical Centers will provide the MCC with documentation of approval from the IRB. The approved local consent form will be submitted to the DSMB Chair for review to assure that it conforms to the requirements for protection of human subjects.

Monitoring of this protocol is considered to be in the high-risk 3 category. Protocol and regulatory compliance will be assessed as well as the accuracy and completeness of all data points for 100% of study enrollees semiannually. Accrual will be tracked

continuously, and the appropriateness of SAE reporting will be assessed on all participants.

The study team will meet at appropriate intervals to review case histories or quality summaries on participants.

The Eligibility Coordinators will verify 100% of the informed consent documentation on all participants and verify 100% of St. Jude participants' eligibility status within 5 working days of the completion of enrollment.

The Clinical Research Monitor will track accrual continuously and verify 100% of all data points on all study participants semiannually to assess overall study conduct, Human Subjects Protections, and the accuracy of database entries. Essential regulatory documents and all study documents including medical records, electronic media database entries, study worksheets, and case report forms will be reviewed for recording and reporting of Adverse Events/Serious Adverse Events (SAEs) to include type, grade, attribution, duration, timeliness and appropriateness. Study documents are also reviewed for participant status, eligibility, the informed consent process, demographics, staging, study objectives, subgroup assignment, treatments, evaluations, responses, participant protocol status, off-study and off-therapy criteria, and for all other specifics as detailed in the protocol. The Monitor will generate a formal report which is shared with the Principal Investigator (PI), study team and the Internal Monitoring Committee (IMC). This will include verification of appropriate documentation of consent. Monitoring of timeliness of serious adverse event reporting will be done as events are reported in TRACKS.

St. Jude affiliates and domestic collaborating study sites will be monitored on-site by a representative of St. Jude at intervals specified in the Data and Safety Monitoring Plan. Monitoring may be conducted more frequently if deemed necessary by the CPDMO or the IMC.

11.3.1. Composition and Responsibilities of the DSMB

The HUGKISS pilot trial will use a DSMB comprised of 5-6 independent experts, including a hematologist, pharmacist, statistician, bioethicist and patient advocate/representative. The DSMB will be responsible for monitoring the clinical centers individually and in the aggregate for unexplained lag in recruitment, missed visits, missed specimen collection, missed scheduled procedures, missing expected forms, agreement between treatment dose recommended and dose dispensed, study treatment not accounted for, medical events required to be reported, and routine AEs. Weekly lists of expected visits, blood specimens, and scheduled procedures will be generated and reviewed by the Data Coordinating Center (DCC; chaired by Guolian Kang) at SJCRH. The DSMB will meet regularly to review cumulative study data. In

addition to the routine performance reports, they will review data according to the assigned groups. Forms and procedures expected, but not reported within five days will be queried in a weekly cumulative delinquency report, and all cases of AEs and laboratory alert values will be reviewed in detail. The DSMB will make recommendations to the SJCRH regarding recruitment goals, study performance, and patient safety. Specific topics for data monitoring are discussed below:

11.3.1.1. Eligibility

Status regarding eligibility criteria will be entered into the database for all consented patients. Aggregate reports will be generated weekly throughout recruitment showing, according to Clinical Center, the numbers of patients who are eligible, those disqualified and reasons for such, those qualified, those randomized and enrolled, and those in follow-up. Steering Committee members will monitor these accrual reports and make recommendations to improve performance or change procedures.

11.3.1.2. Follow-Up Clinic Visits

At every follow-up visit, a standardized data capture form regarding specific events and any unusual conditions or occurrences will be collected. The site PIs will be required to review this and provide an assessment of whether an AE has occurred. Since this is an IND monitored study, the usual definitions of AE may be implemented, such as the occurrence of death or a life-threatening condition or an event that causes or prolongs hospitalization. For the purposes of this protocol, hospitalizations due to expected sickle cell related complications will not be counted as AEs but will be captured as study-related endpoints.

11.3.1.3. Study Treatments

Study forms will require complete accounting of study treatments to verify that each patient received his/her correct bottle with the correct dose, and that treatment was administered daily. Local laboratory results must be entered into the study database for the study participant to continue taking prescribed study treatment. If laboratory results obtained to monitor for myelosuppressive toxicities are not entered into the database within five days, then an immediate stop-treatment order will be issued automatically. At every visit, unused study drug is expected to be returned, and participants will be assessed for compliance by measurement of residual drug returned. Treatment errors will be reported to the Study Chairman immediately and will be counted cumulatively. If these errors represent significant protocol violations, then study policies regarding remedial action for repeated treatment regimen violations will be implemented.

11.3.1.4. Data Entry and Database Quality Assurance

Because most data entry will be performed at remote Clinical Center sites, data quality assurance will be evaluated and maintained through site visits that will include complete audits of the local data against the center's medical records. Periodically, copies of original forms may be requested from Clinical Centers for independent data entry and review in the MCC and DCC to verify the accuracy of the local center data entry.

Given the multisite design (Figure 1.) of HUGKISS, oversight of all study related activities will be crucial. Specific topics regarding assuring data quality are discussed below:

1. Clinical Centers will be visited and audited during the study to assure quality of data collection. The MCC will generate computer printouts of remotely entered data for comparison to actual patient charts. If significant discrepancies are noted, the Study Chairman, and the MCC staff will visit the Clinical Center and provide a site visit report to the DSMB for recommendation on corrective action. Treatments dispensed by the Clinical Center (Investigational) Pharmacies will be checked for agreement with the treatment kit number assigned to the child, as well as the recommended prescription. The Clinical Center PI will be notified immediately of any discrepancies. Confirmed discrepancies will be tallied cumulatively. Pharmacies accruing unacceptable numbers of discrepancies also will be reported to the Study Chairman for immediate action.

2. At the DCC, the randomization schedule will assure the appropriate blocking and balance of treatment groups for clinically important patient characteristics within each Clinical Center. The final schedule will be reviewed to assure that randomization is balanced by age and Clinical Center.

3. Within the MCC, laboratory results, treatment assignments, and study drug records will be monitored. Local laboratory parameters will be monitored by the HELPER, which will allow for real-time assessment of values. All laboratory results will be entered into the HUGKISS database within 5 days, which will facilitate a second safety evaluation of individuals across the entire study. Laboratory value outliers will trigger safety alerts, will be verified, and will be reviewed by the Study Chairman. Extreme values that constitute an AE will be managed according to specified procedures. Abnormal values that do not exceed levels will be used in formulating treatment recommendations. The HUGKISS study team will follow standard dose adjustments for hydroxyurea therapy and these recommendations and records of treatment will be reviewed along with the laboratory results by the designated medical staff at the MCC.

Confirmed treatment recommendations will be forwarded to the Clinical Center electronically. All misreported laboratory results will be cumulated and reported, as well as all alert values, and routine dose changes. MCC staff will review reported toxicities and their resolution and consequent dose adjustments to see if the frequency of adjustments is reasonable or if patients are being stopped too frequently. Tabulations of the time patients are not maintained on assigned treatment will also be reviewed.

11.3.1.5. Safety and Patient Monitoring

For the purposes of this protocol, hospitalizations due to expected sickle cell related complications (Appendix III) will not be counted as AEs but will be captured as study-related endpoints.

All AEs, regardless of attribution to study treatment or SCD will be reported to the MCC. Any event meeting the definition of a serious AE will be reported to the DSMB Chair, Executive Secretary of DSMB, and the FDA. The Clinical Centers will report all serious AEs at their institution according to their local IRB requirements. In the event of an emergency in which the Clinical Center PI or local treating physician requires knowledge of the patient's HUGKISS treatment assignment is necessary for determining patient management, the PI or treating physician will telephone the HUGKISS Study Chairman, or the SJCRH site PI in the event the Study Chairman is unavailable, to discuss the need for unblinding. An automated system will be available to obtain unblinding information 24 hours/day as deemed necessary.

The staff of Clinical Centers and Core Laboratories, the Endpoint Evaluation Committee members, and the MCC personnel share in responsibility for patient safety and the scientific integrity of HUGKISS. With the assistance of oversight provided by the DSMB and the local IRBs, all matters of protocol implementation and data reporting will be subject to review. Procedures for monitoring of scientific integrity and quality assurance in multicenter trials have been promulgated by the Society for Clinical Trials[42]. Violations of the protocol will be investigated promptly to maintain patient safety.

In order to prevent Major Protocol Violations or reporting of incorrect data, we will utilize training and certification, site visits, audits, statistical checks, edit checks, and careful follow-up of any abnormalities that could result in study-wide suspension of randomization or treatment stoppage. Specifically, we will ensure the following:

1. Data collection by non-certified personnel will be prevented by training and certification (including education that misuse of certification number is a

scientific integrity issue) and by review of study forms all of which require signature, certification number and date. If this occurs, it will trigger a clinic wide audit, deletion of erroneous data, and a corrective action plan from the Clinical Center PI to keep the problem(s) from recurring.

2. Requesting enrollment (randomization) for a child who does not meet eligibility criteria will be prevented by training and certification, detected by review of eligibility data collection forms, and acted upon with suspension of randomization.

3. Requesting enrollment (randomization) for a child for whom informed consent has not been obtained, or whose family has been noted by the Patient/Family advocate to have reservations will be prevented by training and certification, detected on site visit, and acted upon with suspension of randomized study treatment and randomization.

4. Failure to act to implement a treatment stop order within 72 hours will be prevented by training and certification, detected by review of study treatment data collection forms and Patient/Family advocate follow-up and acted upon with suspension of randomized study treatment and randomization.

5. Failure to report a serious AE within 24 hours of learning of the event will be prevented by training and certification, detected on review of study treatment data collection forms, and acted upon with suspension of randomized study treatment and randomization.

11.4. Confidentiality

All clinical information will be collected by the participating sites and sent via electronic data capture to the study database. Data will be encrypted during the internet transfer process in order to protect against loss of confidentiality. Access to the HUGKISS database will be restricted to the DCC staff and will be password-protected. Each study subject will have a study identification number to protect patient identity. Each site will maintain a list to allow linkage to subject identity; this list will be kept locally at each site and its access will be restricted to local site personnel, and to entities that may need access to verify accuracy and completeness of data, including the NHLBI, the HUGKISS DCC, and the DSMB.

12.0 STATISTICAL CONSIDERATIONS

12.1. Design and Sample Size Justification

HUGKISS is a prospective, randomized, multi-site, single-blinded, therapeutic, pilot trial aimed at demonstrating the feasibility of enrolling and randomizing young children (9 to 36 months) with SCA (HbSS and HbSβ^0-thalassemia) to receive either fixed or intensified dose of hydroxyurea therapy in a single blinded manner and at collecting pilot clinical and laboratory efficacy data (i.e., estimates of the effect size and variability for future studies) to design a future phase III trial. The study is planned to enroll 65 children with SCA to get 50 randomized in a 27-month period. All eligible participants who consent will be enrolled on the study. The duration of the study is based on sample size of 50 patients randomized and/or 27-month period, whichever comes first. If we randomize 50 eligible individuals within 27 months of study initiation, we will halt enrollment of new participants. If we are unable to randomize 50 patients in a 27 month period, then we will conclude that this pilot study is not feasible in terms of the feasibility definition below. The patients who are randomized will be evaluable to assess the feasibility below. The analysis will be limited to the patients who meet the feasibility criteria 2) below.

Given previous experience of the BABY HUG trial, we anticipate a 20% dropout rate (which includes removal for noncompliance). Feasibility of the overall study will be determined by two points, 1) in terms of enrollment and randomization, that we are able to randomize 50 eligible individuals in the 27-month duration; and 2) in terms of the feasibility of conducting a randomized trial, that is, after randomization, per each arm, 80% (40 individuals in total) of randomized individuals will have $\geq$ 80% chronic medication compliance, and will have the %HbF collected at baseline and at study exit. Chronic medication compliance is defined based on medication possession ratio (MPR), a measure of the percentage of time that a patient has access to medication. Each participant MPR is calculated as [(days medication in family's possession/days prescribed medication)$\times$100]. The design of this study will conclude that this pilot HUGKISS trial is feasible if both of the feasibility criteria in terms of the enrollment and randomization and of conducting a randomized trial are satisfied, that is, for both arms, $\geq$ 40 out of 50 randomized patients in the 27-months and have $\geq$ 80% chronic medication compliance and have the total HbF collected at baseline and at study exit after randomization to design a future phase III trial.

This is a pilot study and no formal sample size justification needed. A sample size of 50 (randomized patients) is based on our enrollment capacity (see next paragraph). However, based on our definite phase III trial, we will expect both a significant decrease in the rate of hospitalization and a significance increase in %HbF. The two component (hospitalization and %HbF) hypotheses and the overall composite hypotheses to be tested are expressed as follows: Let μA and μC denote the mean %HbF at study exit for the alternative (intensified dose) and standard treatment (fixed dose) arms, respectively. The null and alternative hypotheses for the total HbF component of the primary endpoint are Hh0: $\mu A - \mu C = 0 \leftrightarrow$ Hh1: $\mu A - \mu C > 0$. The mean %HbF (SD) at study exit (two years) in the standard treatment arm is assumed to be 22.4% (8.7%) based on

the results from BABY HUG. Using design parameters and 20 individuals per each arm, μA-μC = 5% (based on data from HUSOFT and the SJRCH internal data on infants), the power will be 0.56 and 0.70 based on a one-sided t test statistic at a significance level of $\alpha = 0.05$ and 0.1, respectively. Denote pA and pC as the true proportions of individuals with hospitalization events during the two year study period in the alternative and standard treatment arms, respectively. The null and alternative hypotheses for the clinical hospitalization component of the primary endpoint are Hc0: pA = pC ↔ Hc1: pA < pC. Based on data from BABY HUG, 69 out of 96 participants experienced at least one hospitalization within a two year therapy period (pC = 0.72). Using design parameters and 20 individuals per each treatment arm, pA = 0.52, the power will be 0.32 and 0.45 based on a one-sided exact test at a significance level of $\alpha = 0.05$ and 0.1, respectively. This power calculation is only for illustrative purposes due to the nature of the pilot study.

We estimate we will need to approach approximately 125 eligible participants to enroll 65 patients to get at least 50 randomized. Only the patients who sign the consent and enroll on the study but go off study before randomization will be replaced. Once the patient is randomized, the patient will be evaluable to assess the feasibility criteria 2) above. At St. Jude, we will have approximately 30 children who meet eligibility criteria of this study per year; we anticipate that the other three sites will identify about 130 of eligible patients per year. Among these eligible patients, we estimate that there are approximately half of patients with age 9 to <24 months and half of patients with age 24 to 36 months. This supports the expectation of an 27-month duration to complete enrollment.

In addition to monitor the trial for feasibility, we will closely monitor the excess toxicity in terms of invasive infection. If the alternative treatment arm has 3 or more patients experienced invasive infection that occurs during excessive myelosuppression from hydroxyurea, we will stop the trial for excess invasive infection in the alternative treatment arm.

Randomization:
Eligible patients will be consented and will be initiated on HU (100 mg/mL) at a dose of ~20 mg/kg/day in an open label fashion for eight weeks (±2 weeks) prior to randomization. Participants without toxicity (defined in section 6.4), or with toxicity which requires discontinuation of HU but resolved and participant continues HU during those eight weeks (±2 weeks), will be randomized to receive "standard" or "intensive" therapy based on a block randomization. Randomization will be stratified by clinical center (four centers: St. Jude, UMMC, UT Southwestern, and Children's Health Care of Atlanta) and by baseline age of the participant (9 to <24 months and 24 to 36 months) because of the natural physiologic decline of HbF with increasing age. Block randomization with a block size of 4 will be used in each stratum. Randomization will be performed in DCC based on the registration identifiers, using the randomization

program developed by the Department of Biostatistics. Once a patient is randomized, all related randomization information will be frozen in the Biostatistics randomization database and cannot be changed.
This study is a single blinded randomization study and will need unblinding in some emergent cases (Section 5.8.2). The DCC and MCC will document and track participants who are unblinded either in an emergent or incidental fashion. The rate of unblinding of participants will be described by the percentage of overall participants and the percentage of study related visits.

12.2. Statistical Analyses

12.2.1. Describe reasons provided for agreement or refusal for participation in HUGKISS for each family approached.

Descriptive statistics will be provided including the number and frequency.

12.2.2. Develop an infrastructure and clinical monitoring plan that facilitates safe administration of hydroxyurea at a low-fixed dose and an intensified dose to MTD in a single-blinded manner to children.

No analytical plan is needed for this objective.

12.2.3. Obtain pilot data on the laboratory effects, clinical outcomes and toxicities observed with fixed versus intensified hydroxyurea dosing.

Upon the completion of the study, summary statistics including mean, SD, median and range for continuous variables and frequency for categorical variables for each treatment arm will be provided and will be compared between two treatment arms using two sample t-test or exact Wilcoxon Rank Sum test depending on the normality of the data tested by the Shapiro-Wilk test. The comparison of time to first event defined below will also be compared between two treatment arms using the Kalbfleisch-Prentice method[43]. Death is the competing risk event. The analysis will be implemented using SAS macro (bmacro252-Excel2007\cin) available in the St. Jude Department of Biostatistics. Longitudinal analysis based on Generalized Estimating Equation[44] will also be assessed, but it is exploratory analysis due to the small sample size. The laboratory effects of hydroxyurea will be characterized by description of white blood cell counts, hemoglobin, hematocrit, mean corpuscular volume, platelet count, absolute neutrophil count, absolute reticulocyte count, and fetal hemoglobin concentrations. The clinical complications observed in participants will be characterized by description of sickle cell related events as described in Appendix III.

12.2.4. Obtain pilot data on health related quality of life in children with SCA treated with a low-fixed dose and at MTD.

For quality of life (QOL), the measurements will be scored on a 0–100 nominal scale, with 0 the worst and 100 the best composite outcome score. Changes in QOL scores from baseline to study exit will be summarized for each treatment arm using descriptive statistics including mean, SD, median and range and will be compared between two treatment groups by the exact Wilcoxon -Mann–Whitney test. The subscores for each domain, including physical, emotional, social and school functioning will be analyzed as well using the same methods. Because PedsQL 4.0, the instrument for QOL assessment, contains two components of child self-report and parent proxy-report, it is expected that for young patients who cannot fill their self-report, we will have the parent proxy-report data only. The analyses for the two components will be separated using the same methods.

12.3. Anticipated Completion Dates

Anticipated Primary Completion Date: Aug 31, 2020
Anticipated Study Completion Date: Aug 31, 2021

13.0 OBTAINING INFORMED CONSENT

13.1. Informed Consent Prior to Research Interventions

This trial will be designed and implemented with great concern for the welfare of subjects. Subjects will be recruited from all participating centers by local clinical staff. Because this study will involve the participation of minors, the child's guardian will be approached during regular clinic visits and invited to have his/her child participate in the HUGKISS pilot study. Approach during hospitalization will be avoided due to the vulnerability and stress associated with hospital admissions. If a child is eligible, the family will be informed about the study, and detailed information about the trial will be given, including the risks and benefits of study participation. An informed consent session will take place in which patients and guardians will have the opportunity to ask questions regarding participation in the study, and to learn the risks and benefits of participation. After detailed discussion of the protocol, they will be given a copy of the informed consent document for review. Participation will be completely voluntary and parents may withdraw their child from the study at any time. Families will receive no payment for participation in the study other than the costs of medical care performed as part of the study and modest reimbursement for parking, food, and travel, so there will be no undue financial incentive for participation. Subjects and families will be informed of any information that becomes available during the trial that might impact their continued participation.

13.2. Consent at Age of Majority

Not applicable due to study participant's age and the planned duration of the study.

13.3. Consent When English is Not the Primary Language

When English is not the patient, parent, or legally authorized representative's primary language, the Social Work department will determine the need for an interpreter. This information will be documented in the participant's medical record. Either a certified interpreter or the telephone interpreter's service will be used to translate the consent information. The process for obtaining an interpreter and for the appropriate use of an interpreter is outlined on the Interpreter Services, OHSP, and CPDMO websites.

13.4. Collection of Collaborating Institution Consent Forms

Signed collaborating institution's consent forms will be scanned and emailed to the Central Protocol And Data Monitoring Office (CPDMO): Eligibilitycoordinators@stjude.org

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APPENDIX I: SCHEDULE OF EVALUATIONS

Event	ES[1]	BE	Weeks												Exit
	-4 to 0	0	4	8	12	16	20	24	28	32	36	40	44	48	52-56
Informed Consent	X														
Weight, height & vital signs	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
History, physical exam	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Prior/concomitant medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Adverse events		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Hydroxyurea initiation		X													
Randomization				X											
CBC with WBC count	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Reticulocyte count	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
CMP[5]	X	X					X					X			X
Lactate Dehydrogenase		X					X					X			X
Hemoglobin identification	X[2]	X					X					X			X
Urinalysis		X					X					X			X
TCD examination[3]		X													X
Quality of life assessment[4]		X					X					X			X
MAM[6]			X	X	X	X	X	X	X	X	X	X	X	X	X
Study Drug Accountability			X	X	X	X	X	X	X	X	X	X	X	X	X

*Ideal visits are every 4 weeks ±2 weeks except for ES and BE. ES, eligibility screening; BE, baseline evaluation which should be performed prior to initiation of hydroxyurea. [1]Eligibility screening may be performed the same time as baseline evaluations. [2]A new hemoglobin identification only needs to be performed at eligibility screening if a previous result is not already available in the subject's medical record. [3]To be performed on participants ≥2 years of age. [4]Age appropriate screening tool. [5]CMP, Comprehensive Metabolic Panel, Chemistries include: Sodium, Potassium, Chloride, CO2, Glucose, BUN, Creatinine, Calcium, Albumin, Bilirubin Total, Alk, Phos, ALT, AST, Protein Total. [6]MAM, Medication Adherence Measurement.

APPENDIX II: RESEARCH TESTS

Research Test	**Time Points**
Hemoglobin identification	All
Quality of life assessment	All
MAM	All
Study Drug Accountability	All

APPENDIX III: CLINICAL EVENT DEFINITION

Introduction

In HUG KISS, clinical event definitions will be applied for consistency with other important NHLBI-sponsored clinical studies of sickle cell anemia such as the Clinical Study of Sickle Cell Disease (CSSCD), the Multicenter Study of Hydroxyurea in Sickle Cell Anemia (MSH), and BABY HUG.

Definitions

Anemia: A reduction of hemoglobin level by at least 30% from the steady-state level ***OR*** a reduction by at least 20% accompanied by an acute increase in spleen size. Acute anemic events should be classified into one of the following categories.

1. Splenic sequestration crisis: The event is characterized by an increase in spleen size and firmness, reduction of hemoglobin level by at least 20% and may include drop in platelet or white counts. Splenic sequestration is defined in HUG KISS by the findings of a palpable, large spleen with hemoglobin less than 5 gm/dL for nonfatal occurrences.

2. Aplastic Crisis: This event is characterized by a substantial decrease in reticulocyte count to below 1.5 % before or concurrent with a reduction in hemoglobin level to a level greater than 30% below the steady-state level. Characteristically results from acute infection with parvovirus B19. Check the patient's parvovirus IgM titer; usually it should be positive.

3. Other anemia: Reduction of hemoglobin because of blood loss, transfusion reaction or hyper-hemolysis will be classified as another anemia. A hyper-hemolytic episode is characterized by normal or increased reticulocyte counts and nucleated red cell count during an episode of falling hemoglobin associated with an increase in indirect bilirubin level over the usual value. The latter finding is important to allow discrimination from a recovering aplastic crisis.

Aplastic Crisis: See Anemia.

Arthritis (septic): See Infection (other).

Aseptic Necrosis: Presentation in the age range of patients in BABY HUG would be unusual. Patients encounter pain in their hip or shoulder, often of a different character than their usual crisis pain. The pain persists indefinitely, long after the expected duration of crisis pain. Early

radiographic findings are subepiphyseal lucency and widening of the joint space. Late changes include flattening of the epiphysis and sclerosis with fragmentation.

Bacteremia: Febrile illness with blood culture positive for bacteria. Organism must be specified.

Cerebrovascular Accident (CVA): Acute neurologic syndrome secondary to occlusion of an artery or hemorrhage with resultant ischemic and neurologic symptoms and signs.

1. Stroke, hemorrhagic: Injury to brain tissue resulting from disturbance of blood supply to the brain due to hemorrhage. The area of the hemorrhage should also be reported (e.g., subarachnoid, subdural, intracerebral, aneurysm).
2. Stroke, infarctive: Injury to brain tissue consistent with occlusion of vessel(s) by thrombus or embolus which results in neurologic abnormalities on physical examination that persist beyond 24 hours.
3. Transient Ischemic Attack (TIA): Temporary interference with blood supply to the brain. The symptoms include neurologic signs that clear within 24 hours (48 hours if basilar system is involved). After the attack, no evidence of residual neurologic damage remains on physical examination.

Chest Syndrome: Also known as acute chest syndrome (ACS). A clinical syndrome that includes at least 3 of the following symptoms: chest pain, temperature elevation over 38.5^{o}C/101.5^{o}F, tachypnea, wheezing or cough. A new pulmonary infiltrate must be present on x-ray involving at least one complete lung segment to be consistent with alveolar consolidation instead of atelectasis.

Cholangitis: See Right Upper Quadrant Syndrome

Cholecystitis: See Right Upper Quadrant Syndrome

Cholelithiasis: See Right Upper Quadrant Syndrome

Dactylitis: Pain and tenderness with or without swelling in hands and/or feet. Also known as Handfoot syndrome.

Fever without Focus: Elevation of temperature greater than 38.5^{o}C/101.5^{o}F (regardless of route, oral, axillary or rectal) not associated with a positive culture from any source or with any other special event. Will be characterized as an event managed with blood culture (no growth) and empiric parenteral antibiotics or by parental history (no blood culture, no empirical parenteral antibiotics).

Hematuria: Blood in the urine (greater than 5 red blood cells per high power field), usually suggested by history, confirmed by urinalysis.

Hepatitis: See Right Upper Quadrant Syndrome

Infection (other): Inflammation, caused by a pathogenic agent, which may or may not be accompanied by a fever. Sepsis, meningitis, osteomyelitis, hepatitis and urinary tract infections are ***NOT INCLUDED*** in this category since they are categorized elsewhere. If encountered, the type of infection, site, and organism if known should be specified.

1. **Abscess/Cellulitis:** Infection of skin or deeper tissues.
2. **Gastroenteritis:** Inflammation of the stomach and intestinal tract. Signs include nausea, vomiting and diarrhea lasting at least 8 hours.
3. **Lymphadenitis/Lymphangitis:** Infection in regional lymph nodes or channels draining the primary site of infection.
4. **Mastoiditis:** Infection of the mastoid bone.
5. **Orbital Cellulitis:** Infection of the orbit and tissues posterior to the eye.
6. **Otitis Media:** Infection of the middle ear associated with ear pain and erythema of eardrum, bulging or decreased mobility of eardrum with loss of landmarks.
7. **Periorbital or Preseptal Cellulitis:** Infection of the eyelids causing erythema and swelling.
8. **Pharyngitis:** Pain in the pharynx associated with redness of pharyngeal and tonsillar mucosa with or without exudates. Indicate if streptococcal culture positive.
9. **Septic Arthritis:** Bacterial infection of a joint. The causative agent should be listed if known.
10. **Upper Respiratory Infections (URI):** An imprecise term for almost any kind of infectious process involving the nasal passages, pharynx and bronchi. Often called a "cold."
11. **Varicella Infection**: Clinical syndrome of skin lesions, fever associated with varicella zoster virus infection.

Liver Sequestration: See Right Upper Quadrant Event 5. Liver Sequestration.

Mastoiditis: See Infection (other).

Meningitis: Inflammation of the membranes of the spinal cord or brain usually caused by and infectious agent, as demonstrated by lumbar puncture abnormalities and culture. The causative agent should be listed if known.

Orbital Cellulitis: See infection (other).

Osteomyelitis: Bacterial infection of bone requiring long-term antibiotics. The causative agent should be listed if known.

Other Event Not Specified: Includes any event that is not included in the list of the events specified on this form.

Painful events: Pain in the extremities, back, abdomen, chest or head for which no other explanation can be found and which is not classified as one of the other special events. The pain shall have lasted for at least 2 hours and for which medication either narcotic or non-steroidal, antiflammatory agent is taken. In a young child, pain or tenderness on palpation will be considered appropriate evidence of event.

Pancreatitis: See Right Upper Quadrant Event 6. Pancreatitis.

Pneumonia: See Chest Syndrome.

Priapism: A painful erection of the penis lasting for more than 2 hours.

Proteinuria: Presence of 1+ protein or more on urinalysis.

Renal Complications (not specified): A renal condition not categorized as hematuria, urinary tract infection, proteinuria or renal insufficiency. The type of complication should be sought and specified.

Renal Insufficiency: At least a two-fold increase in serum creatinine to a level greater than or equal to 1.0 mg/dl.

Right Upper Quadrant (RUQ) Event: Defined as any two of the following; pain only in the right upper quadrant of the abdomen, twofold increase in total bilirubin over baseline to a level exceeding the upper limit of normal, two centimeter increase in liver size over baseline or twofold increase in ALT over baseline to a level exceeding the upper limit of normal.

1. **Cholangitis**: Inflammation of the bile ducts. Diagnosis is usually made by abdominal ultrasound.
2. **Cholecystitis**: Inflammatory condition of the gallbladder causing RUQ pain that may or may not be associated with gallstones. Diagnosis is usually made by abdominal ultrasound.
3. **Cholelithiasis**: Formation or presence of calculi or bile stones in the gallbladder or common bile duct, with minimal or no symptoms.
4. **Hepatitis**: An inflammation of the liver. The causative agent if known, should be specified.
5. **Liver Sequestration/Intrahepatic Sequestration:** Jaundice and pain in the liver not due to gallstones. May include intrahepatic sickling crisis.
6. **Pancreatitis**: Inflammation of the pancreas. May be related to medication, infection or gallstone blockage of the Ampulla of Vater.

Seizure: A paroxysmal disorder of cerebral function characterized by sudden, transient attack of altered consciousness, motor activity or sensory phenomena. The type of seizure (e.g., petit mal, psychomotor, grand mal) and etiology if known should be specified.

Sepsis: Severe febrile illness with unstable vital signs or shock associated with positive blood culture. Organism must be specified. Positive blood culture in stable patients reported as bacteremia.

Splenic Sequestration: See anemia.

Splenomegaly: without acute sequestration: Spleen is palpable in abdomen with stable hemoglobin (within 1 gm/dl of baseline), and platelet counts > 100,000/cu mm.

Surgery: Any operative procedure will be listed.

Transient Ischemic Attack (TIA): See Cerebrovascular Accident (CVA).

Transfusion: The provision of red blood cells to correct anemia. The reason for the transfusion should also be specified. Simple or Exchange transfusion should be specified.

Upper Respiratory Infection (URI): See Infection (other).

Urinary Tract Infection: A clinical event which may or may not be associated with fever and symptoms which is associated with a positive urine culture.