CLINICAL PROTOCOL

PROTOCOL TITLE:	Effectiveness of Hydroxyurea and Magnesium Pidolate Alone and in Combination in Hemoglobin SC Disease: A Phase II Trial
CSCC PROTOCOL NUMBER:	Version 6.1
DATE:	December 13, 2007
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1 SYNOPSIS

Title of the Protocol: Effectiveness of Hydroxyurea and Magnesium Pidolate Alone and in Combination in Hemoglobin SC Disease: A Phase II Trial

CSCC Protocol Number: December 13, 2007; Version 6.1

Overview: Hemoglobin (Hb) SC disease is characterized by dense red blood cells, but treatment approaches aimed at its pathophysiology have been limited in scope. Furthermore, combination drug therapy in sickle cell disease has not been adequately tested. We will examine two oral therapies, hydroxyurea and magnesium, given by themselves and in combination and compare them to placebo in a double-blinded Phase II study to determine their efficacy in reducing red cell density in Hemoglobin SC in children and adults. We will also examine their effects on other red cell characteristics and on the frequency of clinical vaso-occlusive events. To accomplish this, 188 subjects will be assigned randomly to one of four treatment groups (see below) and followed for one year after beginning treatment.

CSCC Protocol Chair: Winfred Wang, MD

Intervention: Individuals meeting entry criteria will be randomized to receive either hydroxyurea (20 mg/kg/day), magnesium pidolate (0.6 mEq/kg/day), hydroxyurea (20 mg/kg/day) + magnesium pidolate (0.6 mEq/kg/day), or placebo.

IND Holder: National Heart, Lung, and Blood Institutes (NHLBI), # 76,348

Objectives:

Primary Objective(s): To compare the effectiveness of hydroxyurea (HU) alone, magnesium pidolate (Mg) alone, and hydroxyurea + Mg in combination, and placebo in reducing the density of HbSC erythrocytes.

Secondary Objective(s): To examine the effect of the individual and combination treatments on:

- hematologic parameters and Hb F levels in HbSC disease;
- other biologic measures of erythrocyte activity and red cell-endothelial interactions in HbSC disease;
- and prevention of vaso-occlusive episodes in HbSC disease.

Additionally, we will examine the safety of HU + Mg in combination.

Hypotheses/Estimates: We will be testing the null hypothesis of no difference in change-from-baseline percent of RBCs > 41 g/dL after two months of treatment among the four treatment groups using 3 comparisons of active treatment to placebo. These tests will be performed using an F-test from an analysis of covariance, controlling for baseline percent of RBCs > 41 g/dL. A test of interaction between the two treatments will be completed as a secondary hypothesis. This test will be performed using an F-test from a two-way ANCOVA, controlling for baseline percent of RBCs > 41 g/dL.

Criteria for Evaluation:

Efficacy:

Primary Endpoint: The change-from-baseline density of hemoglobin SC red cells (percent of RBCs with density greater than 41 g/dL) measured 2 months after initiation of treatment.

Secondary Endpoint: Change-from-baseline in:

- Standard hematologic parameters [hemoglobin level, mean cell volume (MCV), reticulocyte count, white blood cell count, platelet count, absolute neutrophil count (ANC)].
- Hemoglobin S, C, and F levels.
- Red cell metabolic studies: K-Cl co-transport activity, Gardos channel activity, Na-Mg exchanger activity, red cell cation content, intracellular Mg.
- Plasma total Mg and ionized Mg (iMg) levels.
- Adhesion studies to laminin, thrombospondin, and endothelial cells; expression of red cell receptors and phosphatidylserine; adhesive response to epinephrine.

Also the frequency of clinical vaso-occlusive events (pain events, acute chest syndrome).

Safety: Adverse events and blood chemistry, hematology, and urinalysis laboratory measurements.

Study Design: This double-blinded, placebo-controlled phase II multi-center trial will consist of two parts. In Part I, the safety of magnesium and HU will be evaluated (Safety Pilot) in all four treatment arms after 10 subjects

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per arm have been enrolled and had a minimum of 2 months follow-up time. If warranted on safety grounds, the second part of the trial will consist of enrolling another 37 subjects per arm, continuing the follow-up of the 40 subjects enrolled in Part I, and comparing the efficacy and safety of the 4 treatment arms.
Study Population: Subjects with HbSC disease, 5 years of age and older with at least one vaso-occlusive event (pain, acute chest syndrome) in the previous 12 months.
Sample Size: 188 subjects will be enrolled into this study. We assume: • a 20% decrease in red cell density due to HU or Mg pidolate therapy; • a mean percent of RBCs > 41 g/dL of 7.5% in the placebo/placebo group; • an equal standard deviation of 2.12 (corresponding to a correlation of 0.75); • a two-sided alpha of 0.0167 (to adjust for 3 multiple comparisons); • a positive interaction effect (implying no benefit of combination therapy); • and, use of an analysis of covariance to generate our test statistics. We will need 47 subjects per treatment group to achieve 85% power to detect the 20% difference between each of the treatment groups compared to placebo.
Randomization: Patients will be randomized in a 1:1:1:1 allocation across the four treatment arms using an adaptive randomization procedure, stratified by center and age group (5-15 years old vs. ≥ 16 years old). This will be performed centrally through the SDMC, using an interactive randomization system.
Data Analyses: **Part I:** The Safety Pilot will be used to evaluate the safety of magnesium after 10 subjects per treatment arm have been enrolled and had a follow-up time of at least 2 months. This will not be a formal interim analysis, as safety results will be evaluated and no hypothesis will be tested. Safety endpoints will be summarized by age and treatment groups and analyzed for differences across treatment group using a Fisher's Exact Test as the data allow. **Part II:** The effect of treatment on the primary outcome of change-from-baseline percent of RBCs > 41 g/dL to the percent at two months will be analyzed using analysis of covariance, controlling for baseline percent of RBCs > 41 g/dL. As a secondary analysis, a two-way ANCOVA controlling for baseline will be used to test for an interaction between treatments. Also, as a secondary analysis, the change-from-baseline percent of RBCs > 41 g/dL measured at two, four, six and twelve months will be analyzed using a generalized linear mixed model, with treatment group, time, and baseline percent of RBCs as fixed effects and subject as a random effect. Secondary laboratory parameters will be analyzed in a similar manner. Clinical endpoints and adverse events will be summarized by treatment and age group and analyzed for differences across treatment group using a Fisher's Exact Test as the data allow.

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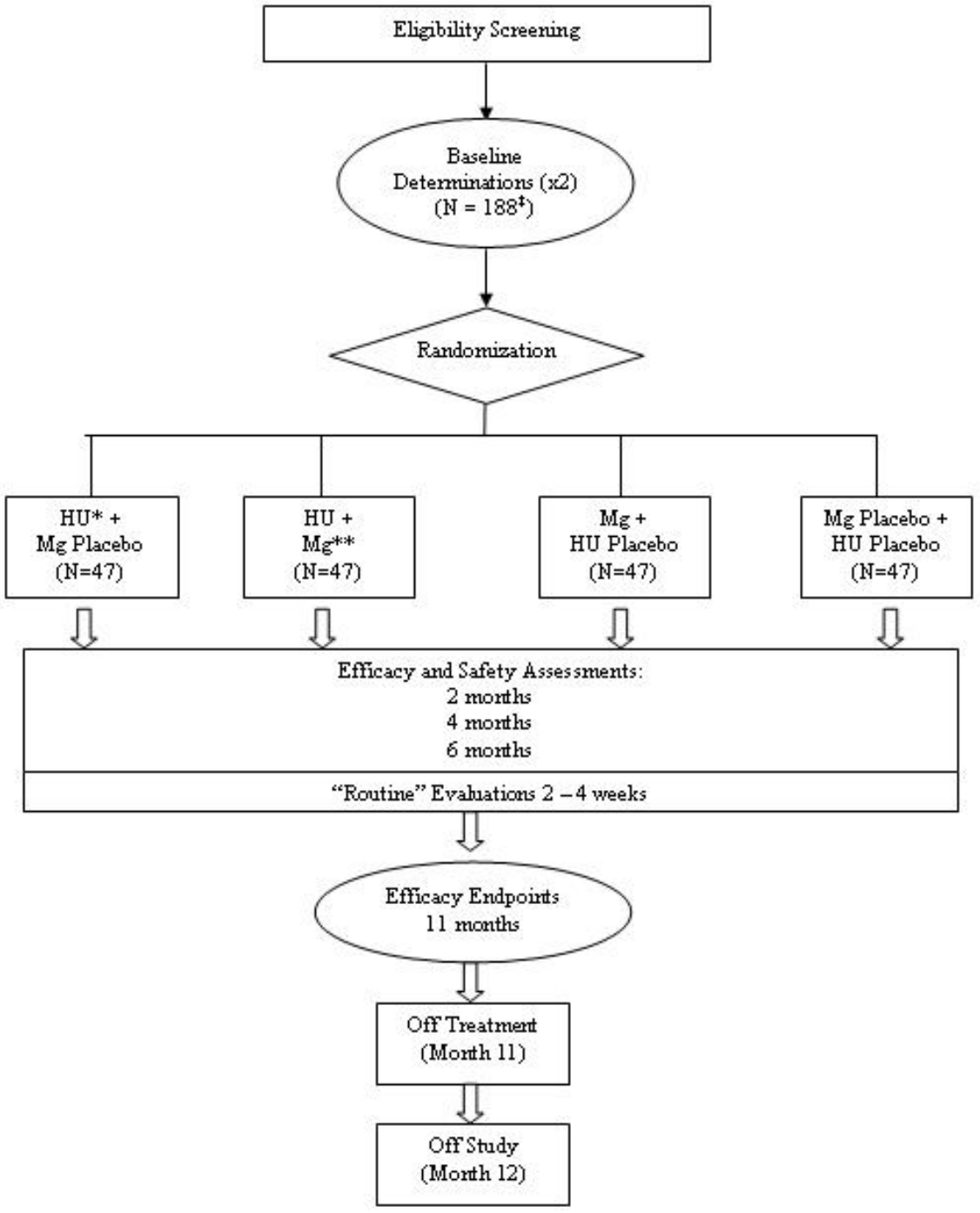
2 SUBJECT FLOW DIAGRAM



‡ first 40 patients to be evaluated in a Safety Pilot Study
* dose of HU: 20 mg/kg/day
** dose of Mg pidolate: 0.6 mEq/kg/day in 2 divided doses

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4 ABBREVIATIONS

ACS	Acute Chest Syndrome
AE	Adverse Event
ANC	Absolute Neutrophil Count
Ca	Calcium
CAP/CLIA	College of American Pathologists/Clinical Laboratory Improvement Amendments
CBC	Complete Blood Count
CH	Corpuscular Hemoglobin Content
CHCM	Cell Hemoglobin Concentration Mean
CHr	Reticulocyte Hemoglobin Content
CLIA	Clinical Laboratory Improvement Act
CRA	Clinical Research Associate
CRF	Case Report Form
CSCC	Comprehensive Sickle Cell Centers
CTCAE	Common Toxicity Criteria for Adverse Events
DSMB	Data Safety Monitoring Board
ESR	Erythrocyte Sedimentation Rate
FET	Fisher's Exact Test
FDA	Food and Drug Administration
GCP	Good Clinical Practice
Hb	Hemoglobin
HbC	Hemoglobin C
HbF	Fetal Hemoglobin
HbS	Sickle Hemoglobin
HbSC	Sickle Cell/Hemoglobin C Disease
HbSS	Sickle Cell Anemia
Hct	Hematocrit
HDW	Hemoglobin Concentration Distribution Width
HPLC	High Performance Liquid Chromatography

HU	Hydroxyurea
iCa	Ionized Calcium
ICH	International Conference on Harmonisation
iMg	Ionized Magnesium
IND	Investigational New Drug
IRB	Institutional Review Board
ISE	Ion-Selective Electrode
IVRS	Interactive Voice Randomization System
JCAHO	Joint Commission on Accreditation of Healthcare Organizations
K	Potassium
MCH	Mean Cell Hemoglobin
MCHC	Mean Cell Hemoglobin Concentration
MCV	Mean Cell Volume
MedDRA	Medical Dictionary for Regulatory Activities
MFC	Mean Fluorescent Channel
Mg	Magnesium
MSH	Multi-Center Study of HU in Sickle Cell Anemia
MTD	Maximum Tolerated Dose
Na	Sodium
NCI	National Cancer Institute
NHLBI	National Heart, Lung, and Blood Institute
PI	Principal Investigator
PRC	Protocol Review Committee
QA	Quality Assurance
QC	Quality Control
RBC	Red Blood Cell Count
RDW	Red Cell Volume Distribution
RhoFED	Rho Federal Systems Division, Chapel Hill, NC
SAE	Serious Adverse Event
SCD	Sickle Cell Disease

SDMC	Statistics and Data Management Center, located at Rho Federal Systems Division, Chapel Hill, NC
SGPT	Serum Glutamic Pyruvic Transaminase
SAP	Statistical Analysis Plan
TSP	Thrombospondin
U/A	Urinalysis
WBC	White Blood Cell Count

5 BACKGROUND AND RATIONALE

At birth, about 1 in 1200 African Americans has sickle cell/hemoglobin C disease (HbSC). These subjects have the same vaso-occlusive complications as sickle cell anemia (HbSS)--only less often. Sickle cell anemia is considered an "orphan disease," but HbSC disease is even further ignored as far as studying available treatment options. Clinical descriptions of HbSC disease abound and substantial insights into its distinct pathophysiology have been gained (1). Nevertheless, few published trials of its treatment exist. Perhaps this is due to the mistaken perception that HbSC disease is benign or that its rarity precludes conclusive therapeutic trials. We submit that: HbSC disease complications often merit acute treatment; a safe preventive treatment might forestall the disease complications that develop with age; novel means of reversing the pathophysiology of this disorder are available; and sufficient patients exist to carry out a successful therapeutic trial.

HbSC disease exhibits a characteristic erythrocyte dehydration compared with sickle cell trait or hemoglobin C (HbC) trait erythrocytes. Cell dehydration plays a crucial role in the pathophysiology of HbSC disease because it allows the intracellular sickle hemoglobin (HbS) to reach concentrations that induce clinically significant HbS polymerization and cell sickling. K-Cl co-transport is highly expressed in HbSC erythrocytes and determines their characteristic microcytosis and dehydration. Thus, HbSC disease represents an ideal target for preventing K-Cl co-transport-mediated cell dehydration (1). Despite this, clinical studies have been extremely limited.

Hydroxyurea (HU) can effect changes in HbSC disease erythrocytes that may be independent of any change in fetal hemoglobin (HbF). In a study of 6 adults with HbSC disease, low dose hydroxyurea was associated with sustained erythrocyte volume increases and a fall in both absolute reticulocyte counts and the number of high-intensity staining or "stress" reticulocytes (2). While hematocrit (Hct) increased slightly, this is not necessarily a desirable goal for the treatment of HbSC disease since much of the pathology of this disorder may result from increased blood viscosity caused by dense cells. After treatment, density gradients showed a decrease in the number of the densest

cells. Reticulocyte and erythrocyte volume increased, and there was a fall in the number of red cells and reticulocytes with a (CHCM) >38 g/dL. Also, a fall in serum bilirubin suggested a decline in hemolysis. In another study, 6 pediatric subjects were selected for severe disease (3). In this uncontrolled trial, all subjects had clinical improvement. Laboratory results from the two studies described above conflict somewhat. The adult study (2) showed a significant increase in hemoglobin but not in HbF levels, whereas the pediatric study (3) showed a significant increase in HbF; both studies showed significant increases in mean cell volume (MCV). In total, 25 subjects with HbSC disease treated with hydroxyurea have been reported. Thus, even a moderate-sized clinical trial of hydroxyurea in subjects with HbSC disease remains to be performed.

Several drugs block cation-transport in the sickle red cell and can restore normal cellular cation content and density. Clotrimazole, an anti-fungal agent, reduced cellular dehydration *in vitro* in a transgenic mouse model of sickle cell disease and also when given to subjects with sickle cell anemia (4, 5). Recent studies in subjects with HbSS showed that cell density changes are achievable with well-tolerated doses of this drug. However, the reduction in cell density was modest and less than that observed following hydroxyurea treatment of HbSS. Derivatives of this agent that are not associated with some of its undesirable effects are now in clinical trials.

Magnesium (Mg) salts also interfere with cation-transport and cause cell rehydration in HbSS and in sickle transgenic mice. In the SAD mouse strain, oral Mg supplementation restored red cell Mg and K contents, and reduced K-Cl co-transport activity, MCHC and cell density (6). Mg pidolate at a dose of 0.6 mEq/kg/day was used as oral Mg supplementation in 10 subjects with sickle cell anemia (7). Four weeks of treatment induced an increase in red cell Mg and K content and a decrease in the activity of K-Cl co-transport. There were no laboratory or clinical signs of hypermagnesemia; mild, transient diarrhea was the only reported side effect. A pilot study, using Mg pidolate for 6 months, confirmed the beneficial effects on red cell dehydration of oral Mg supplementation and demonstrated a 58% reduction in the number of painful days (8). These results represent a promising therapeutic approach for preventing red cell dehydration in sickle cell disease. However, no trials of Mg in subjects with HbSC

disease have been reported. Our proposed study would allow definitive data collection regarding magnesium's effect on HbSC red cell density as well as providing preliminary information on clinical efficacy.

In addition, this HU/Mg study will examine the effect of combination chemotherapy in a manner that has not previously been applied to subjects with sickle cell disease. Historically, combination chemotherapy has been extremely successful in the treatment of cancer, HIV, tuberculosis, and other conditions, but has not been utilized in sickle cell disease except in small pilot studies involving combinations such as hydroxyurea and erythropoietin. Because HU and Mg have different primary effects on sickle red cells (increased HbF, blockage of K-Cl co-transport), they may have additive or even synergistic effects on the HbSC erythrocyte. Furthermore, hydroxyurea and Mg have different toxicities (primarily neutropenia and diarrhea, respectively) and neither drug is likely to cause severe or life-threatening toxicity (9).

Our hypothesis is that oral Mg and HU will increase intracellular Mg, block K-Cl co-transport, prevent cell dehydration, decrease red cell-endothelial interaction, and reduce vaso-occlusive complications. Accordingly, we propose a double-blinded, placebo-controlled trial to examine the effectiveness of HU, Mg pidolate, and HU + Mg pidolate in reducing cell density in HbSC disease. The cellular effects of hydroxyurea and magnesium should favorably modulate the course of this disorder and the results of this study will provide the framework for a definitive efficacy trial.

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6 STUDY OBJECTIVES AND PURPOSE

6.1 Primary Objective

To compare the effectiveness of hydroxyurea (HU) alone, magnesium pidolate (Mg) alone, and HU + Mg in combination to placebo in reducing the density of HbSC erythrocytes.

6.2 Secondary Objectives

To examine the effect of the individual and combination treatments in subjects with HbSC disease on:

a. hematologic parameters and HbF levels;
b. other biologic measures of erythrocyte activity and red cell-endothelial interactions;
c. and prevention of vaso-occlusive episodes.

Additionally, we will examine the safety of HU + Mg in combination.

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7 STUDY DESIGN

7.1 Primary and Secondary Endpoints

The primary endpoint for the study is the change-from-baseline density of hemoglobin SC red cells (the individuals' percentage of RBCs with density greater than 41 g/dL) measured 2 months after initiation of treatment.

Secondary endpoints for this trial are change-from-baseline in:

1. Standard hematologic parameters [hemoglobin level, mean cell volume (MCV), reticulocyte count, white blood cell count, platelet count, absolute neutrophil count (ANC)].
2. Hemoglobin S, C, and F levels.
3. Red Cell Metabolic Studies: K-Cl co-transport activity, Gardos channel activity, Na-Mg exchanger activity, red cell cation content, intracellular Mg.
4. Plasma total Mg and ionized Mg (iMg) levels.
5. Adhesion Studies: Adhesion of red cells to laminin, thrombospondin, and endothelial cells; expression of receptors for laminin, thrombospondin, and endothelial cells; red cell surface phosphatidylserine; adhesive response to epinephrine.

Additionally, the frequency of clinical vaso-occlusive events (pain events, acute chest syndrome) will be assessed.

The primary and secondary endpoints will be evaluated at baseline, 2, 4, 6, and 11 months after the initiation of treatment.

7.2 Overall Design

This is a double-blinded, placebo-controlled phase II multi-center study of subjects with HbSC disease, 5 years of age and older. Individuals meeting entry criteria will be randomized to receive either HU, Mg pidolate, HU + Mg pidolate, or placebo. HU will be administered at a dose of 20 mg/kg/day, and adjusted for toxicity. Magnesium, 0.6 mEq/kg/day, will be given as Mg pidolate. HU + Mg will be given as HU, 20 mg/kg/day + Mg pidolate, 0.6 mEq/kg/day. All treatment arms will be adjusted for toxicity when necessary.

The first part of this phase II trial will be a Safety Pilot study to evaluate the safety of the two study drugs in all four treatment arms when 10 subjects per arm have been enrolled and had a minimum of 2 months follow-up time on study drug. Since there is relatively little information about treatment with Mg in Hb SC patients alone or in combination with HU, the Safety Pilot will also specifically look for any unexpected side effects from Mg. If warranted on safety grounds, the trial will be continued. The subjects enrolled during the Safety Pilot will continue their follow-up and 37 new subjects per treatment arm will be enrolled and followed for 1 year.

As in most trials involving HU, subjects will be monitored through clinic visits every 2 to 4 weeks. At these visits, a standard interim history, physical examination, and set of laboratory evaluations will be conducted and data will be recorded on Case Report Forms (CRFs). At these visits, any history of adverse events, particularly vaso-occlusive episodes, and any symptoms suggestive of toxicity, such as diarrhea, will be ascertained. Standard laboratory data will include a complete blood count (CBC) at every study visit and a chemistry profile at Visits 1 and 2, and then every 8 weeks while the subject is on study. The dose of HU/placebo will be reduced in response to laboratory evidence of toxicity such as neutropenia. Because standard hematologic values are not expected to be markedly influenced by either HU (in the HbSC subject) or Mg, routine laboratory studies will be performed by local labs rather than a central lab which might otherwise be necessary to insure blinding. A possible limitation of this approach relates to an increase in MCV, which may occur in HbSC red cells of subjects receiving HU. However, clinical site investigators must agree not to see any results of red cell indices in order to avoid potential unblinding.

Specialized research laboratory determinations will be utilized to measure the biologic effects of HU and Mg on relevant red cell properties. Red cell density will be measured twice in the baseline period and subsequently at 2 months (8 weeks), 4 months, 6 months and 11 months after randomization. When the maximal effects of these drugs will take place and how long the effects will be sustained in HbSC subjects are unknown, but substantial changes in red cell properties are anticipated by the 2-month time point. Serial laboratory measurements taken throughout the one-year study should ensure

capture of adequate data to address the primary objective. Collection of data over a one year period would also provide preliminary information regarding frequency of vaso-occlusive acute events in the 4 groups. For example, if the placebo/placebo group continued to have an estimated baseline rate of 1.5 events/year, they would accumulate approximately 60 events over the course of a year. The group receiving hydroxyurea alone might be expected to have approximately half the number of events or about 30 over the course of a year. In the 2 groups receiving magnesium event numbers would be less predictable but of great interest. Although the overall study is not powered to answer questions of clinical efficacy, nevertheless a finding of, for example, 60 events versus 30 events in the placebo/placebo versus HU/placebo group, would be extremely valuable in determining a sample size for a future Phase III trial to investigate optimal long-term intervention for patients with HbSC disease. Important information about the long-term safety of HU and Mg will also be obtained by following the subjects for a year.

Excessive toxicity in the form of diarrhea from Mg could potentially unmask subjects on this agent. However, in preliminary experience in children in Memphis and Paris and in adults reported by Dr. Brugnara's group, diarrhea related to Mg pidolate has been limited (and could easily be controlled by decreasing the dose of Mg pidolate).

8 SELECTION AND WITHDRAWAL OF SUBJECTS

8.1 Inclusion Criteria

1. Diagnosis of HbSC disease.
2. ≥ 5 years of age or older.
3. Hb level 8 - 12.5 g/dL for children and adults.
4. At least one vaso-occlusive event (pain, acute chest syndrome) in the previous 12 months. Definitions of a pain event and an episode of acute chest syndrome are based on those utilized by the Cooperative Study of Sickle Cell Disease (CSSCD). An episode of pain is defined as the occurrence of pain in the extremities, back, abdomen, chest, or head that lasts at least 2 hours; requires a visit to a hospital, Emergency Room, clinic, or provider's office; and is not explained except by sickle cell disease. Acute chest syndrome is defined as a new pulmonary infiltrate on chest x-ray associated with a fever (> 38.5° C), tachypnea, wheezing, cough, or chest pain.
5. Participant has signed the informed consent/assent.
6. Regular compliance with comprehensive care.
7. Subject is in steady state and not having an acute complication of sickle cell disease [i.e., no hospitalization, pain event, or episode of acute chest syndrome within the past 4 weeks (28 days)].

8.2 Exclusion Criteria

1. Previous transfusion with remaining Hb A > 10%.
2. Previous treatment with HU within the past 3 months.
3. Previous treatment of magnesium within the past 3 months (including vitamins containing magnesium).
4. Poor compliance with previous treatment regimens.
5. Hepatic dysfunction (SGPT > 2x upper limit of normal) within the past month.
6. Renal dysfunction (creatinine ≥ 1.0 mg/dL, < 18.0 years of age; ≥ 1.2 mg/dL, ≥ 18.0 years of age) within the past month.
7. Pregnancy.
8. Hospital admissions (≥ 10) for pain in last 12 months or daily use of narcotics.
9. Treatment with any investigational drug in last 3 months.
10. < 3 percent RBCs with density > 41 g/dL (as measured by the ADVIA 120).
11. Positive HIV test.
12. Other chronic illness or disorder other than SCD that could adversely affect the subject's performance in the study (e.g., tuberculosis).

8.3 Subject Discontinuation

Participation in this protocol will not mean that standard care for Hb SC disease will be compromised, delayed, or withheld. Subjects may decide to discontinue participation at any time during the study. Investigators may discontinue any subject at their discretion if, in their professional opinion, the subject's health, safety, and/or well being is threatened by continued participation in the study. The following circumstances require discontinuation of study drugs by subjects:

1. Decline in Hb level to < 5 g/dL.
2. Increase in Hb level to > 13.5 g/dL (because of viscosity concerns).
3. Initiation of chronic transfusion.
4. Hepatic dysfunction (SGPT > 2x upper limit of normal).
5. Renal toxicity (creatinine ≥ 1.2 mg/dL < 18.0 years of age; ≥ 1.4 mg/dL, ≥ 18.0 years of age).
6. Pregnancy.
7. Stroke.
8. Pulmonary failure requiring intubation.
9. Grade 3 or 4 toxicity lasting longer than two weeks.
10. Decision of the PI (e.g, subject is non-compliant).

Subjects who receive a single transfusion can continue receiving study drugs, but laboratory efficacy measurements will not be assessed until their HbA decreases to less than ten percent. All other efficacy measures and all safety measures will continue to be gathered on these subjects. Adverse events caused by participation in the study may necessitate discontinuation of a subject. Specific known adverse events associated with the use of HU and Mg can attempt to be resolved by adjusting the dosage before withdrawing the subject (see Section 9). Subjects who are randomized but are determined to be ineligible after the first screening visit, but before randomization (e.g., positive HIV test, < 3 percent RBCs with density > 41 g/dL) and subjects who discontinue study participation prior to Visit 6 (Month 2) WILL be replaced. Subjects who discontinue after Visit 6 WILL NOT be replaced. Subjects will be discontinued for hepatic dysfunction or renal toxicity only if either one is not resolved with dose reduction(s) in the study drug (HU) that is associated with the toxicities. If the site PI has concerns with the level of either the SGPT or the creatinine of the subject, the Operations

Committee for the CHAMPS study will be contacted and each situation will be evaluated on a case-by-case basis.

Subjects who discontinue prematurely from the study for any reason, including those subjects determined to be ineligible, will be encouraged to complete a safety follow-up visit.

However, a subject will be considered "off study" for the following reasons:

1. Consent is withdrawn by the subject or the subject's parent/guardian.
2. Subject is lost to follow-up.
3. Subject is deceased.

9 TREATMENT OF SUBJECTS

9.1 Hydroxyurea: Study Drug and Dosing Regimen

Hydroxyurea is an anti-metabolite, initially utilized in the treatment of chronic myelogenous leukemia and other malignancies. It prevents DNA replication by inhibition of ribonucleotide diphosphate reductase, the enzyme that catalyzes the reductive conversion of ribonucleotides to deoxyribonucleotides (10). It is specific for the S-phase of the cell cycle and causes arrest of cells at the G1-S interface.

Pharmacokinetics of HU have been described primarily in adults (11). HU is rapidly absorbed from the gastrointestinal tract and reaches peak plasma values in 1-2 hours. The plasma half-life is approximately 2 hours. It is excreted intact through the kidney, and about 80% of the drug is recovered in urine within 12 hours. Reversible dose-related myelosuppression, particularly neutropenia is the main toxic effect of hydroxyurea. Mild gastrointestinal disturbances (nausea, vomiting), hair loss, skin rash, and fever have been reported, but were not increased compared with controls in the Multi-Center Study of HU in Sickle Cell Anemia (MSH)(12). Transient and reversible hepatitis and renal toxicity (including renal tubular dysfunction) and abnormal renal test findings have also been reported as adverse effects of hydroxyurea.

Hydroxyurea is known to be teratogenic in rats, cats, and monkeys, but teratogenicity has not been documented in humans (13-15). There is concern that long-term HU treatment may be carcinogenic or leukemogenic. In older adults with polycythemia vera, essential thrombocythemia, or myelofibrosis (conditions associated with increased risk for development of malignancy), the risk of acute leukemia after treatment with HU may or may not have been significantly different (16). In children with erythrocytosis due to cyanotic congenital heart disease, treatment with HU for 2-15 years did not result in cancer or leukemia (17). Long term results of HU use among adults with sickle cell disease enrolled on the MSH revealed no leukemia cases and 3 cases of solid malignancies: one breast cancer, one endometrial cancer, and one cervical cancer case (18). Whether HU had any role in the pathogenesis of these neoplasms remains uncertain, as their low incidence may be similar to that observed in the general

population. Because the continued use of HU could increase the risk of acute leukemia, induce chromosomal breaks, and/or provoke undesirable reproductive health outcomes, extended follow-up for these potential toxicities remains a necessity.

As described in the background section, experience with HU in HbSC disease is extremely limited. In the pediatric trial, the HU was started at a dose of 15 mg/kg/day and no subjects were able to reach the proposed maximum tolerated dose (MTD) of 30 mg/kg/day (3). In the adult trial, subjects received 500-1,000 mg/day (2). In this trial, a "moderate" hydroxyurea dose of 20 mg/kg/day will be given, and the dose will be modified for toxicity in a similar manner to that of MSH and HUG-KIDS trials (19, 20). Subjects will be given hydroxyurea in 200mg and 500mg capsules. Subjects who are obese (BMI > 30 kg/m²) will receive a modified dose of hydroxyurea -- 15 mg/kg/day.

Side effects of HU are mainly hematologic. Hematologic toxicity will be defined as one or more of the following: absolute neutrophil count (ANC) < 1000/ mm^3, platelet count < 75,000/ mm^3, ≥ 20% decrease in Hb concentration from the baseline value, or a total Hb concentration < 5 g/dL. Hepatic toxicity will be defined as a SGPT value greater than twice the upper limit of normal. Renal toxicity will be defined as a creatinine value of ≥ 1.4 mg/dL in adults and 1.2 mg/dL in children. If a subject experiences a toxicity, such as neutropenia, it may be presumed to be associated with hydroxyurea (20/mg/kg/d), but it could also be coincident with placebo. In either case, hydroxyurea/placebo will be discontinued for one week. Then if the toxicity has resolved, hydroxyurea/placebo will be resumed at a dose of 17.5 mg/kg/d (2.5 mg/kg/d less than the original dose). If the toxicity has not resolved, hydroxyurea/placebo will continue to be "held" and repeat evaluations will be performed on a weekly basis until the toxicity has resolved. At that point, the subject will resume hydroxyurea/placebo at a dose of 17.5 mg/kg/d. Subsequently, if the patient again experiences toxicity, the same steps will be followed until the toxicity resolves. The subject will then resume hydroxyurea/placebo at a dose of 15 mg/kg/d. Further toxicities will be managed in a similar manner (Appendix V).

9.2 Magnesium Pidolate: Study Drug and Dosing Regimen

Magnesium pyrrolidone carboxylate or magnesium pidolate was chosen as the Mg salt to be used due to the accumulated experience in sickle cell subjects to date (7, 8). Mg pidolate is absorbed passively by the gastrointestinal (GI) tract at a rate of approximately 50%. The excretion is mainly untransformed by the kidneys. Both tablet and powder formulations are available over the counter in many countries. The magnesium pidolate will be provided to the pharmacist in liquid form.

The dose of Mg pidolate will be 0.6 mEq/kg/day, divided into two daily oral administrations given approximately 12 hours apart. This daily dose is slightly higher in elemental Mg than doses shown to increase erythrocyte Mg without consequences (32.4 mEq of Mg daily for 4 weeks) (21). The primary toxicity of Mg pidolate is expected to be diarrhea, which will likely occur within the first few weeks if it does occur. If the diarrhea is grade 3 or 4, if it persists for more than 72 hours, if there are signs of dehydration from the diarrhea, or if the subject has abdominal pain severe enough to interfere with daily activities, Mg pidolate/placebo will be interrupted.

Diarrhea toxicity for this study will be measured according to the National Cancer Institute's Common Toxicity for Adverse Events v3.0 (CTCAE), as follows:

Adverse Event	**Grade**				
	1	**2**	**3**	**4**	**5**
Diarrhea	Increase of < 4 stools per day over baseline	Increase 4-6 stools per day over baseline; IV fluids indicated < 24 hours; not interfering with activities of daily life (ADL)	Increase of ≥ 7 stools per day over baseline; incontinence; IV fluids ≥ 24 hours; hospitalization; interfering with ADL	Life-threatening consequences (e.g., hemodynamic collapse)	Death

In a recent clinical trial conducted by Dr. Brugnara's group in adults with sickle cell disease in Europe, the only significant toxicity found during 6 months of treatment was diarrhea, which led to withdrawal from the study of 2 of 20 subjects (8). However, Dr. Brugnara has found that minimizing this side effect by modest reduction of the dose of Mg is usually possible. Diarrhea from Mg (0.6 mEq/kg/d) is most likely to occur within the first few weeks of the study. If the diarrhea is severity grade 3 or 4, if it persists for

more than 72 hours, if there are signs of dehydration from the diarrhea, or if the subject has abdominal pain severe enough to interfere with daily activities, Mg/placebo will be discontinued for one week. If symptoms have resolved at that time, Mg/placebo will be restarted at 75% of the original dose (i.e., 0.45 mEq/kg/d). If the patient has no recurrence of toxicity, Mg/placebo will be continued at 75% of the original dose. If the subject has recurrent toxicity while receiving a dose of 0.45 mEq/kg/d, the dose will be further decreased to 50% of the original dose (0.3 mEq/kg/d). If significant toxicity again occurs, the patient will discontinue Mg/placebo treatment (Appendix VI).

Mg pidolate will be supplied by PAT Products (Brad Fairweather, Bangor, Maine), the supplier of Mg pidolate for several studies in the United States. It is estimated that the total amount of Mg pidolate required for this study will be approximately: 45 kg (assumed average weight of pediatric and adult subjects) x 94 subjects receiving Mg x 0.6 mEq/kg/day x 365 days x 0.15 g/mEq =138,956 g = approximately 139 kg. The cost of Mg pidolate is approximately $39/kg. Mg pidolate will be formulated as a liquid at a concentration of 2 mEq/ml by Xcelience (Tampa, FL). Mg pidolate and placebo will be distributed in 16-ounce bottles.

9.3 Packaging, Labeling, Blinding, and Return of Study Drugs

The two companies manufacturing each of the study drugs (Xcelience and UPM Pharmaceuticals) will also package, label, and distribute study drug to each of the sites. All unused study drugs will be returned to the local pharmacy and destroyed appropriately.

9.4 Randomization and Blinding

A total of 188 subjects will be enrolled over approximately 16 sites and randomized centrally through the Statistics and Data Management Center (SDMC) into 4 treatment arms within 2 age strata (5 to 15 years old versus greater than 16 years old). As the number of stratification factors is rather large (16x4x2), many strata may have fewer subjects than the block size, and a good balance can no longer be guaranteed for all of them (40). As a result, the use of a traditional stratified, blocked randomization schedule could lead to significant imbalance in treatment allocations, both across the study and

within the various strata. In order to increase the likelihood of balance in treatment allocations, subjects will be randomized using the standardized range variation (41) of the sequential allocation algorithm of Pocock and Simon (42), a minimization method. This method attempts to achieve treatment balance on several subject characteristics (i.e. age group in this study) simultaneously – not within separate strata. Minimization consists of biasing the treatment allocation so as to minimize the total imbalance between the treatment groups on some scale (43). The order of entry of the subjects to the various centers and in the various prognostic groups, is assumed to be random. As minimization is a dynamic method that uses information on subjects already entered to allocate treatment to the next subject, and thus a continuous updating of the information related to previous treatment allocations is required, a centralized randomization system will be used. Pharmacists at each center will be unblinded and will utilize information from the interactive randomization system (IRS) at the SDMC to obtain a new treatment assignment for a subject and provide each subject with appropriate dosing of both therapies. The IRS system has the capability of providing unblinding information in case of a medical emergency where the exact study drug is needed in order to appropriately treat a subject.

9.5 Prior and Concomitant Therapy

Subjects enrolled in this trial will receive all therapy and monitoring considered standard of care for subjects with HbSC disease. Subjects will not be eligible for the study if they have received prior HU or Mg treatment within the last 3 months. If they have been taking a vitamin preparation, it should be determined that Mg is not included or had been discontinued 3 months prior to the start of the study.

9.6 Subject Compliance

Good subject compliance/adherence will be critical to the success of the study and will be monitored in several ways. Subjects will be required to return their bottles of HU/placebo and containers of Mg pidolate/placebo every 4 weeks when seen in clinic in order to receive their new supply of "treatment." Each site will count the number of pills and measure the amount of liquid returned in order to monitor compliance. In addition,

subjects will be instructed to maintain a study drug log in which they record when they took their daily doses of drugs/placebo.

9.7 Study Procedures

Subjects will have study evaluations at two-week and one-month intervals. The two-week visits must occur within ± 4 days of the scheduled appointment, whereas the monthly visits must occur within ± 8 days.

9.7.1 Visit 1

At Visit 1 (1 week before baseline determinations), inclusion and exclusion criteria will be assessed and standard evaluations will be performed. These clinical evaluations will include a routine physical exam, standard CBC measurements and a chemistry panel, a pregnancy test for all female subjects of childbearing potential, and an assessment of clinical outcomes. All subjects will receive a hemoglobinopathy diagnosis at this visit to confirm that the subject has Hemoglobin SC. The standard CBC measurements will include: Hb, Hct, RBC, WBC, MCV, MCHC, Platelet and Reticulocyte count, and ANC. The chemistry panel will include the following assessments: Na, K Cl, CO_2, Ca, BUN, Cr, total protein, albumin, total bilirubin, SGPT/ALT, alkaline phosphatase, and LDH. The clinical outcomes collected will include pain crises, ACS, hospital admissions, transfusions, clinical stroke, cancer, recent neuroimaging, and acute splenic sequestration.

In addition to the standard hematologic evaluations, biologic measures of red blood cell activity will also be taken at Visit 1. Evaluation of red blood cell activity will include the following efficacy assessments: Hemoglobin F, S, and C levels, the proportion of red blood cells with CHMC > 41 g/dL, K-Cl co-transport activity, Gardos channel activity, Na-Mg exchanger activity, and red cell cation content. Plasma total magnesium and ionized Mg levels will also be measured. Visit 1 will include *in vitro* measurements to assess red cell-endothelial interactions. Assays of red cell adhesion to laminin, thrombospondin, endothelial cells, red cell receptor and phosphatidyl serine expression, and the response to epinephrine will provide a qualitative picture of the red cell surface. Genomic DNA will be extracted from a blood sample and α-globin gene sequencing will occur at Visit 1 only.

In order to determine subject eligibility, the central labs will verify each subject's red cell density, which must be > 3% "Hyper" cells with density > 41 g/dL. The study coordinator will be informed within one week's time whether or not the subject's value for this test is within the study's inclusion criterion. The study coordinator will also perform an HIV test for all subjects at this visit. If the subject is found to be HIV-positive, he/she will not be eligible for the study.

9.7.2 Visit 2 (Baseline)

At Visit 2 (baseline), study subjects will be assigned randomly to one of four treatment groups. The clinical evaluations that occur at Visit 2 will include a routine physical exam and the standard CBC and chemistry panel measurements, a urinalysis (U/A), and a pregnancy test for all subjects of childbearing potential. In addition to lab results, clinical outcomes, concomitant medications, and adverse events that occurred since the previous visit will be recorded on the CRF. The same efficacy assessments and *in vitro* measurements that were conducted in Visit 1 will also occur at baseline. Thus the primary endpoint, red cell density, will be measured twice in the pre-treatment period. Enough of the study drugs for one month, along with a study drug log (a tool used for recording when study drug is taken) will be given to the subject at this visit.

9.7.3 Visit 3

At Visit 3 (week 2), subjects will have been on their randomly assigned treatment for 2 weeks. The clinical evaluations that occur at Visit 3 will include a routine physical exam, the standard CBC measurement, and a pregnancy test for all female subjects of childbearing potential. In addition to these results, clinical outcomes, concomitant medications, and adverse events that have occurred since the previous visit will be recorded on the CRF. Hematologic toxicity will be checked throughout the study using the following measurements: ANC, platelet count, and hemoglobin. Renal and hepatic toxicity will be monitored at specified visits and if required for evaluation of an ongoing toxicity using measurement of creatinine and SGPT, respectively.

9.7.4 Visit 4

At Visit 4 (week 4), the subjects will have been on their randomly assigned treatment for 4 weeks. The clinical evaluations that occur at Visit 4 include a routine physical exam, the standard CBC measurement, and a pregnancy test for all female subjects of childbearing potential. In addition to these lab results, clinical outcomes, concomitant medications, and adverse events that occurred since the previous visit will be recorded on the CRF. Study drug toxicity will also be checked at this visit. Subjects should return the study drug log and remaining study drugs for study drug pill counts and liquid measurements by the study coordinator. Another month's supply of study drugs and a study drug log will be given to the subject at Visit 4.

9.7.5 Visit 5

At Visit 5 (week 6), the subjects will have been on their randomly assigned treatment for 6 weeks. The clinical evaluations that will occur at Visit 5 will include a routine physical exam, the standard CBC measurement, and a pregnancy test for all female subjects of childbearing potential. In addition to these results, clinical outcomes, concomitant medications, and adverse events that occurred since the previous visit will be recorded on the CRF. Study drug toxicity will also be checked at this visit.

9.7.6 Visit 6

At Visit 6 (week 8), the subject will have been on their assigned treatment for 2 months. The clinical evaluations that will occur at Visit 6 will include a routine physical exam, the standard CBC and chemistry panel measurements, and a pregnancy test for all female subjects of childbearing potential. The same efficacy assessments and *in vitro* measurements that were conducted at Visit 1 will also occur at Visit 6. In addition to these results, clinical outcomes, concomitant medications, and adverse events that occurred since the previous visit will be recorded on the CRF. Study drug toxicity will also be checked at this visit. Subjects should return the study drug log and remaining study drugs for pill counts and liquid measurements. Another month's supply of study drugs and a study drug log will be given to the subject at Visit 6.

9.7.7 Visit 7

At Visit 7 (month 3), the clinical evaluations will include a routine physical exam, the standard CBC measurement, and a pregnancy test for all female subjects of childbearing potential. In addition to these results, clinical outcomes, concomitant medications, and adverse events that occurred since the previous visit will be recorded on the CRF. Study drug toxicity will also be checked at this visit. Subjects should return the study drug log and remaining study drugs for pill counts and liquid measurements. Another month's supply of study drug and a study drug log will be given to the subject at this visit.

9.7.8 Visit 8

At Visit 8 (month 4), the clinical evaluations will include a routine physical exam, the standard CBC and chemistry panel measurements, and a pregnancy test for all female subjects of childbearing potential. The same efficacy assessments and *in vitro* measurements that were conducted at Visit 1 will also occur at Visit 8. In addition to these results, clinical outcomes, concomitant medications, and adverse events that occurred since the previous visit will be recorded on the CRF. Study drug toxicity will also be checked at this visit. Subjects should return the study drug log and remaining study drugs for pill counts and liquid measurements. Another month's supply of study drugs and a study drug log will be given to the subject at this visit.

9.7.9 Visit 9

At Visit 9 (month 5), the clinical evaluations will include a routine physical exam, the standard CBC measurement, and a pregnancy test for all female subjects of childbearing potential. In addition to these results, clinical outcomes, concomitant medications, and adverse events that occurred since the previous visit will be recorded on the CRF. Study drug toxicity will also be checked at this visit. Subjects should return the study drug log and remaining study drugs for pill counts and liquid measurements. Another month's supply of study drugs and a study drug log will be given to the subject at this visit.

9.7.10 Visit 10

At Visit 10 (month 6), the clinical evaluations will include a routine physical exam, the standard CBC and chemistry panel measurements, a urinalysis (U/A), and a pregnancy test for all female subjects of childbearing potential. The same efficacy assessments and *in vitro* measurements that were conducted at Visit 1 will also occur at Visit 10. In addition to these results, clinical outcomes, concomitant medications, and adverse events that occurred since the previous visit will be recorded on the CRF. Study drug toxicity will also be checked at this visit. Subjects should return the study drug log and remaining study drugs for pill counts and liquid measurements. Another month's supply of study drugs and a study drug log will be given to the subject at this visit.

9.7.11 Visit 11

At Visit 11 (month 7), the clinical evaluations will include a routine physical exam, the standard CBC measurement, and a pregnancy test for all female subjects of childbearing potential. In addition to these results, clinical outcomes, concomitant medications, and adverse events that occurred since the previous visit will be recorded on the CRF. Study drug toxicity will also be checked at this visit. Subjects should return the study drug log and remaining study drugs for pill counts and liquid measurements. Another month's supply of study drugs and a study drug log will be given to the subject at this visit.

9.7.12 Visit 12

At Visit 12 (month 8), the clinical evaluations will include a routine physical exam, the standard CBC and chemistry panel measurements, and a pregnancy test for all female subjects of childbearing potential. In addition to these results, clinical outcomes, concomitant medications, and adverse events that occurred since the previous visit will be recorded on the CRF. Study drug toxicity will also be checked at this visit. Subjects should return the study drug log and remaining study drugs for pill counts and liquid measurements. Another month's supply of study drugs and a study drug log will be given to the subject at this visit.

9.7.13 Visit 13

At Visit 13 (month 9), the clinical evaluations will include a routine physical exam, the standard CBC measurement, and a pregnancy test for all female subjects of childbearing potential. In addition to these results, clinical outcomes, concomitant medications, and adverse events that occurred since the previous visit will be recorded on the CRF. Study drug toxicity will also be checked at this visit. Subjects should return the study drug log and remaining study drugs for pill counts and liquid measurements. Another month's supply of study drugs and a study drug log will be given to the subject at this visit.

9.7.14 Visit 14

At Visit 14 (month 10), the clinical evaluations will include a routine physical exam, the standard CBC and chemistry panel measurements, and a pregnancy test for all female subjects of childbearing potential. In addition to these results, clinical outcomes, concomitant medications, and adverse events that occurred since the previous visit will be recorded on the CRF. Study drug toxicity will also be checked at this visit. Subjects should return the study drug log and remaining study drugs for pill counts and liquid measurements. The final month supply of study drugs and a study drug log will be given to the subject at this visit.

9.7.15 Visit 15

At Visit 15 (month 11), the clinical evaluations will include a routine physical exam, the standard CBC measurement, and a pregnancy test for all female patients of childbearing potential. The same efficacy assessments and *in vitro* measurements that were conducted at Visit 1 will also occur at Visit 15. In addition to these results, clinical outcomes, concomitant medications, and adverse events that occurred since the previous visit will be recorded on the CRF. Study drug toxicity will also be checked at this visit. Subjects should return the study drug log and remaining study drugs for pill counts and liquid measurements. No study drug will be dispensed at Visit 15.

9.7.16 Visit 16 (Study Termination Visit)

At Visit 16 (month 12), the clinical evaluations will include a routine physical exam, the standard CBC and chemistry panel measurements, and a urinalysis (U/A). In addition to these lab results, clinical outcomes, concomitant medications, and adverse events that occurred since the previous visit will be recorded on the CRF. Visit 16 should take place approximately one month after the patient has been taken off of study drug treatment and will serve as the one-month safety follow-up visit.

9.7.17 Early Study Termination/Discontinuation

If a patient discontinues from the study before Visit 16 is completed, or if he/she is discontinued by the Principal Investigator or the Sponsor, the subject is encouraged to come in for one safety follow-up visit. This visit will include all assessments scheduled at Visit 16. If the subject discontinues after Visit 6 and the specimens for the efficacy measurements were not collected at the study visit prior to study discontinuation, the study coordinator must collect them at this visit and send them to the central labs.

10 CLINICAL AND LABORATORY EVALUATIONS

10.1 Efficacy and Safety Laboratory Evaluations

Laboratory evaluations will be performed two times at least 1 week apart prior to randomization. The battery of laboratory tests will be performed over the time of study as appropriate for safety and efficacy. Specific measurement times for each of the tests are outlined below.

Table 10.1 – Summary Table of Studies Done at Local and Central Labs

Visit	Time	CBC, Retic.	Chem. Panel	*Hb F, S, C	**RBC Density, et al.	**Plasma Mg, iMg	#RBC Endothel.	Total Blood Volume
Blood Volume (ml)		3	5	2	8	4	5	
1	Week - 1	x	x	x	x	x	x	27
2	Baseline	x	x	x	x	x	x	27
3	Week 2	x						3
4	Week 4	x						3
5	Week 6	x						3
6	Week 8	x	x	x	x	x	x	27
7	Month 3	x						3
8	Month 4	x	x	x	x	x	x	27
9	Month 5	x						3
10	Month 6	x	x	x	x	x	x	27
11	Month 7	x						3
12	Month 8	x	x					8
13	Month 9	x						3
14	Month 10	x	x					8
15	Month 11	x		x	x	x	x	22
16	Month 12	x	x					8

*Hb F, S, and C will be measured in the laboratory of Dr. Steinberg. For more details refer to section 10.1.2.
**Red blood density, cations, and membrane channel activity, as well as plasma Mg and iMg, will be measured in the laboratory of Dr. Brugnara. For details refer to sections 10.1.3 and 10.1.4.
#Red blood cell endothelial interactions will be measured in the laboratory of Dr. Telen. For details, refer to section 10.1.5.

10.1.1 Standard Laboratory Evaluation (Table 10.2)

CBC: Each site's Hematology Laboratory will measure the standard CBC and differential and reticulocyte count. CBCs will be measured at each visit to monitor for potential toxicities from HU or Mg. Quality Control will be maintained by individual

laboratories' CAP/CLIA certification. Documentation of certification will be required annually.

Chemistry Panel: Each site's Chemistry Laboratory will measure Na, K, Cl, CO_2, Ca, BUN, creatinine, total protein, albumin, total bilirubin, alkaline phosphatase and LDH, using a standard chemistry panel. Chemistries will be measured twice at baseline and subsequently every 2 months to monitor for potential toxicities from HU or Mg. Quality Control will be maintained by individual laboratories' CAP/CLIA certification. Documentation of certification will be required annually.

Urinalysis: Each site's Clinical Laboratory will perform a standard urinalysis at baseline and after 6 and 12 months to monitor safety.

10.1.2 Hemoglobin Content and Alpha Globin Genotype

Hemoglobin S, C, and F: Determination of Hbs S, C, and F will be performed at Visits 1, 2, 6, 8, 10, and 15 in the laboratory of Drs. Steinberg and Chui using the BioRad V Variant II ion exchange high performance liquid chromatography (HPLC) instrument with the β-thalassemia Short Program. This is a well-accepted and reproducible method for the quantitation of Hbs S, C, and F. Further information on quality control can be found in Appendix II.

Alpha Globin Genes: In the laboratory of Drs. Steinberg and Chui, multiplex gap-PCR tests will be performed using methodology designed specifically to detect single α-globin gene deletion of the rightward type ($-\alpha^{3.7}$) and the leftward type ($-\alpha^{4.2}$) at Visit 1.

Genomic DNA will be extracted from peripheral blood leukocytes by standard techniques. Five primers will be used for PCR and subsequent gel electrophoresis to detect normal α-globin genes (control) and ($-\alpha^{3.7}$) and ($-\alpha^{4.2}$) deletions. The protocol is adapted from Tan, et al. (21a).

Table 10.2. Schedule of Standard Hematologic Evaluations, Clinical Outcomes, and Safety Assessments

Visit	Time	Clinical Eval.	CBC*	α genes	U/A	Chemistry Panel **	Adverse Events	Clinical Outcomes ***	Preg. Test
1	Week –1	X	X	X		X	X	X	X
2	Baseline	X	X		X	X	X	X	X
3	Week 2	X	X				X	X	X
4	Week 4	X	X				X	X	X
5	Week 6	X	X				X	X	X
6	Week 8	X	X			X	X	X	X
7	Month 3	X	X				X	X	X
8	Month 4	X	X			X	X	X	X
9	Month 5	X	X				X	X	X
10	Month 6	X	X		X	X	X	X	X
11	Month 7	X	X				X	X	X
12	Month 8	X	X			X	X	X	X
13	Month 9	X	X				X	X	X
14	Month 10	X	X			X	X	X	X
15	Month 11	X	X				X	X	X
16	Month 12	X	X		X	X	X	X	

* Includes Hb, Hct, RBC, WBC, MCV, MCHC, Platelet, Retic or ARC, ANC.

** Includes Na, K, Cl, CO2, Ca, BUN, Cr, total protein, alb, total bilirubin, SGPT/ALT, alk phos, LDH.

*** Includes pain crises, ACS, hospital admissions, transfusion, death, clinical stroke, acute splenic sequestration. Cancer and neuroimaging history collected at Visits 1, 2, 7, 10, 13, and 16.

10.1.3 Biologic Measures of Red Cell Activity (Table 10.3)

Red blood cell hydration status, cation content, K-Cl co-transport activity, Gardos channel activity, and Na/Mg exchanger activity will be determined in the laboratory of Dr. Brugnara (Appendix I). This includes the *primary endpoint* measure of *red cell density*, which will be the proportion of hyperdense RBC (cells with CHCM > 41 g/dL) measured with the ADVIA 120 Hematology System. While several methods for measuring red blood cell density in sickle cell subjects have been described, use of the "hyper" fraction of hyperdense red cells using the ADVIA 120 machine was chosen for the following reasons: pilot data using this technique in HU-treated HbSC subjects have been reported (2); this measurement has been determined to be unaffected by overnight shipping on ice (Appendix IV); and, the same primary endpoint for red cell density is currently being utilized in the CSCC Arginine trial. Use of the same major endpoint

across different CSCC multi-institutional trials will ultimately enhance overall evaluations of different interventions for sickle cell disease.

Studies in both human and mouse red cells have identified Src-family kinase negative modulators of KCl co-transport activity. Preliminary data from human red cells suggest that magnesium supplementation might modify Src-family kinase activity. This will be examined by Dr. DeFranceschi's lab. In addition, reduced cell magnesium has been shown to increase susceptibility to oxidative damage of red cell membrane proteins. Preliminary results have shown significant changes in the profile of red cell membrane oxidation during magnesium supplementation.

Additional laboratory analyses to examine a limited number of samples for red cell membrane kinase activity and oxygen damage to red cell membrane proteins will be conducted in collaboration with Dr. Lucia DeFranceschi in Verona, Italy. These assays will be performed on the first 40 patients enrolled on the study. Dr. DeFranceschi will perform these studies at no additional cost to the study and with no additional requirement of blood from study subjects. (Red blood cells will be obtained from the heparinized blood samples used for total and ionized plasma magnesium assays. These cells would normally be discarded, but instead will be transferred from Dr. Brugnara's lab to Dr. DeFranceschi's lab.) Red blood cells from the same "leftover" samples will be used to evaluate membrane protein oxidation by EMA-labeling of protein-active thiol groups in red cell ghosts before and after magnesium supplementation. One and two-dimensional electrophoresis of red cell membrane proteins will be performed and specific proteins will be identified by mass spectrometry peptide fingerprinting. The 2-DE analysis will allow identification of a number of proteins which may be targets of oxidative damage and may be modulated by magnesium supplementation.

Table 10.3. Schedule of Efficacy Assessments

Visit	Time	Hb F, S, C	Dense cells (%)	RBC Cation	KCl Co-Transp.	Gardos Channel	Na/Mg Exch.	Plasma Mg	Plasma iMg	Kinase Activity/ Oxygen Damage*
1	Week -1	X	X	X	X	X	X	X	X	X
2	Baseline	X	X	X	X	X	X	X	X	X
3	Week 2									
4	Week 4									
5	Week 6									
6	Week 8	X	X	X	X	X	X	X	X	X
7	Month 3									
8	Month 4	X	X	X	X	X	X	X	X	X
9	Month 5									
10	Month 6	X	X	X	X	X	X	X	X	X
11	Month 7									
12	Month 8									
13	Month 9									
14	Month 10									
15	Month 11	X	X	X	X	X	X	X	X	X
16	Month 12									

* Additional laboratory analyses to examine a limited number of samples for red cell membrane kinase activity and oxygen damage to red cell membrane proteins will be conducted in collaboration with Dr. Lucia DeFranceschi in Verona, Italy. These assays will be performed on the first 40 patients enrolled on the study.

ADVIA (Red Cell Density): The ADVIA 120 Hematology System is a fully automated diagnostic instrument. The Sample Shear Valve divides the sample into 5 aliquots for the different types of tests. The reagents and sample aliquots are delivered to their respective reaction chambers for mixing and reaction.

The reported parameters of the measures of red cell metabolism that will be determined by the laboratory of Dr. Brugnara (Appendix I) are:

- Red blood cell hydration status:
  - MCHC (Mean Cell Hemoglobin Concentration) in g/dL
  - % Hypodense cells
  - % Hyperdense cells (% cells with CHCM > 41 g/dL)
  - Hb (Hemoglobin)
  - RBC (Red Blood Cell Count)
  - MCV (Mean Cell Volume)
  - Hct (Hematocrit)
  - MCH (Mean Cell Hemoglobin)
  - CHCM (Cell Hemoglobin Concentration Mean)
  - CH (Cell Hemoglobin Content)
  - RDW (Red Cell Volume Distribution)

  - HDW (Hemoglobin Concentration Distribution Width)
  - Platelet Count, Volume and Distribution Width
  - Reticulocyte % and Reticulocyte Hemoglobin Content (CHr)
  - WBC (White Blood Cell) count and differential
- Cation content:
  - Intracellular Sodium (Na) level in mmol/kg Hb
  - Intracellular Potassium (K) level in mmol/kg Hb
  - Intracellular Magnesium (Mg) level in mmol/kg Hb
- K-Cl co-transport activity:
  - K efflux in mmol/L cell x hour, Na efflux in mmol/L cell x hour
  - K efflux, chlorid-dependent, mmol/L cell x hour
- Gardos channel activity:
  - 86 Rb influx in mmol/L cell x min
- Na/Mg exchanger activity:
  - Mg efflux in mmol/L cell x hour

The primary endpoint measure of red cell density will be the percent of cells with CHCM > 41 g/dL. Further information on quality control can be found in Appendix I.

10.1.4 Circulating Magnesium Levels (Table 10.3)

Results of serum total Mg, ionized Mg (iMg), and Ca^{2+}/Mg^{2+} ratios have been reported to be decreased in both adults and children with sickle cell disease when compared to Caucasians and African American controls (23, 24). Ionized Mg levels have been reported to be more relevant physiologically than total Mg levels (22). A preliminary report suggesting that HU treatment may be associated with further declines in Mg levels in children with sickle cell anemia increases the desirability of monitoring circulating Mg levels in subjects being treated with HU and/or Mg as in this study (24).

Plasma total Mg (mmol/L) and iMg (mmol/L) levels will be performed and quality controls will be measured in the laboratory of Dr. Carlo Brugnara (Appendix I). Assays will be performed using the Nova Biomedical CCX Analyzer. Further information on quality control can be found in Appendix II. (Intra-erythrocyte Mg will be measured as indicated in Section 10.1.3).

10.1.5 Red Cell-Endothelial Interactions (Table 10.4)

While red cell adhesion is thought to contribute to vaso-occlusion in SCD, most studies of adhesion have concentrated on cells from patients with homozygous Hb S (SS RBC), while little interest has been paid to adhesion of red cells from other sickling disorders. In Hb SS, HU treatment has been shown to reduce adhesion to endothelial cells as well as to laminin and TSP (25, 26), although no similar studies of HbSC RBC have been reported, with the exception of one study, which at least implicates similar red cell changes during vaso-occlusion for both Hb SS and HbSC RBC (27). HU also decreased the expression of adhesion molecules expressed on reticulocytes (28), although no careful study of other RBC adhesion molecules before and after HU treatment has been made.

Vaso-occlusion in HbSC disease may also involve adhesion of other cells, including platelets and neutrophils, as well as alterations of the endothelium. Several markers of non-RBC adhesion and endothelial damage have been studied in HbSC patients, although once again less attention has been paid to this disorder in the literature than to Hb SS. For example, TSP levels, possibly related to platelet activation, have been reported to be normal in steady state Hb SS but are elevated during vaso-occlusion (29). HbSC patients (n=3) in steady state had normal or low TSP levels in this report, but they were not studied during vaso-occlusive episodes. SVCAM, a marker of endothelial damage, has also been reported to be elevated in patients with SCD, including at least one patient with HbSC (30). Furthermore, HU may improve the endotheliopathy of SCD, as well as reduce the increased expression of adhesion molecules by neutrophils (31). In addition, HU changes the morphology of human endothelial cells and reduces their ability to sustain adhesion of both normal and SS RBC (32).

While less attention has been paid to SC RBC adhesion because SC RBC generally adhere less well than do SS RBC in vitro, HbSC red cells are known to bind moderately more avidly to laminin than normal RBC, although not as well as SS RBC (Udani, Telen, unpublished data). Adhesion of SC RBC to endothelial cells also has been reported to be increased relative to normal (AA) RBC, but again their adhesion is less marked than that of SS RBC. In general, SS and SC RBC bind more avidly than AA RBC to several

substrates, including the extracellular matrix proteins thrombospondin and laminin, and to endothelial cells.

Because adhesion of SC RBC has not been well or systematically studied, and because highly sensitive assays for adhesion to two of the three best characterized substrates for sickle cell adhesion [laminin and endothelial cells (ECRFs)] are available, the effects of hydroxyurea and magnesium on HbSC RBC adhesion before and after treatment will be examined. In addition, since SS RBC have recently been shown to exhibit remarkably increased adhesion to both laminin and endothelial cells when exposed to the stress hormone epinephrine, this responsiveness in SC RBC will be measured before and after treatment (33, 34).

In order to assess the effects of hydroxyurea and magnesium on HbSC RBC adhesion, the following will be measured in vitro before and during therapy:

1. Adhesion to immobilized laminin during continuous flow (cells/mm²)
2. Adhesion to ECRF endothelial cells during intermittent flow ((% adherent cells) at 1, 2 and 5 dynes/cm^2
3. Expression of receptors for laminin, TSP, and endothelial cells (B-CAM/LU, CD47, VLA-4, and LW) by quantitative Western Blot chemiluminescence
4. Red cell surface phosphatidylserine exposure (annexin V binding) by flow cytometry
5. Adhesive response to epinephrine as measured by adhesion to ECRF (% adherent cells)) at 1, 2 and 5 dynes/cm^2

These assays will provide a qualitative picture of how the SC red cell surface is altered in response to HU and Mg, and how these alterations affect adhesion. Detailed methodology and quality control measures for these assessments are found in Appendix III.

Table 10.4. Schedule of *in Vitro* Red Cell-Endothelial Interaction Assessments

Visit	Time	Laminin	Endothelial Cells	Receptors*	Response to Epinephrine
1	Week -1	X	X	X	X
2	Baseline	X	X	X	X
3	Week 2				
4	Week 4				
5	Week 6				
6	Week 8	X	X	X	X
7	Month 3				
8	Month 4	X	X	X	X
9	Month 5				
10	Month 6	X	X	X	X
11	Month 7				
12	Month 8				
13	Month 9				
14	Month 10				
15	Month 11	X	X	X	X
16	Month 12				

* Includes receptors for laminin, TSP, and endothelial cells (B-CAM/LU, CD47, VLA-4, LW), and red cell surface phosphatidylserine (annexin V binding).

This protocol is sponsored by
the National Heart, Lung, and Blood Institute

11 SAFETY EVALUATIONS AND REPORTING PLAN

11.1 Safety Assessments Overview

Subjects will be evaluated every two weeks for the first eight weeks after randomization. Subsequently, subjects will have routine evaluations every four weeks. At each visit, subjects and/or their parents will be queried regarding recent medical events or procedures. Specific events will be documented at specific visits to ascertain the nature and treatment of the event, including pain crises, episodes of acute chest syndrome, cancer, transfusions, neuroimaging, and hospital admissions. These reportable events and diagnoses will be followed up by the nurse coordinator, who will review hospital charts, medical records, and office visit records for documentation in the follow-up visit forms in the CRF. Subjects will continue receiving the same regimen until they have been followed for one year and had their one-year evaluation.

11.2 Adverse Events

An adverse event (AE) is defined for this study as any untoward medical occurrence in a subject or clinical investigation subject who is administered clinical trial material that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to HU or Mg pidolate. AE data are recorded on the CRF form.

Examples of an AE include:

- Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency or intensity of the condition.
- Significant or unexpected worsening or exacerbation of the condition/indication under study.
- A new condition detected or diagnosed after study drug administration even though it may have been present prior to the start of the study.

- Signs, symptoms, or clinical sequelae of a suspected overdose of either study drug or a concurrent medication ("overdose" per se, should not be reported as an AE/SAE).
- Pre- or post-treatment events that occur as a result of protocol-mandated procedures (e.g., invasive protocol-defined procedures, modification of a subject's previous drug treatment regimen).

An AE does **not** include

- Medical or surgical procedures (e.g., colonoscopy, biopsy). The medical condition that leads to the procedure is an AE.
- Social or convenience hospital admissions where an untoward medical occurrence did not occur.
- Day to day fluctuations of pre-existing disease or conditions present or detected at the start of the study that do not worsen.
- The disease/disorder being studied, or expected progression, signs, or symptoms of the disease/disorder being studied unless more severe than expected for the subject's condition.

11.3 Serious Adverse Events

A serious adverse event (SAE) is defined as any untoward medical occurrence that at any dose:

- Results in death.
- Is life-threatening (i.e., an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe).
- Requires hospitalization or prolongation of existing hospitalization.
- Results in persistent or significant disability/incapacity.
- Results in a congenital anomaly birth defect.
- In the opinion of the investigator, important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above may be considered serious. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

11.4 Assessment of Adverse Event Severity, Relationship to Treatment, and Expectedness

The investigator who identifies the adverse event will also grade the severity and determine the relationship of the event to the treatment. The following scale will be used to "grade" the severity of all adverse events.

1. Mild. Awareness of sign, symptom, or event, but easily tolerated; does not interfere with usual daily activities or tasks.
2. Moderate. Discomfort enough to cause interference with usual daily activity; may warrant therapeutic intervention.
3. Severe. Incapacitating; inability to perform usual activities and daily tasks; significantly affects clinical status; requires therapeutic intervention.
4. Life-threatening. Adverse event is life-threatening.
5. Death. Adverse event causes death.

The standard nomenclature for defining the causal relationship between an AE and the study drug used by CSCC is listed in the following table. The category that overall best "fits" the relationship between the adverse event and the study drug should be chosen and recorded on the CRF and SAE form, if necessary.

Table 11.2. Relationship Between Treatment and AE

Unrelated	• No temporal association to study product. • An alternate etiology has been established or event is consistent with sequelae of sickle cell disease (see below). • The event does not follow the known pattern of response to study product. • The event does not reappear or worsen with re-challenge.
Probably not related / remote	• No temporal association to study product. • Event could readily be produced by clinical state, environmental or other interventions. • The event does not follow the known pattern of response to study product. • The event does not reappear or worsen with re-challenge.
Possibly related	• Reasonable temporal relationship to study product. • The event is not readily produced by clinical state, environmental, or other interventions. • The event follows a known pattern of response to the study product or as yet unknown pattern of response.

Probably related	• There is a reasonable temporal association with the study product. • The event is not readily produced by clinical state, environmental, or other interventions. • The event follows a known pattern of response to the study product. • The event decreases with de-challenge.
Definitely related	• There is a reasonable temporal relationship to the study product. • The event is not readily produced by clinical state, environmental, or other interventions. • The event follows a known pattern of response to the study product. • The event decreases with de-challenge and recurs with re-challenge.

Since all subjects enrolled in this study have sickle cell disease, it is anticipated that certain adverse events will occur related to the progression of the disease and this should be taken into account when determining whether or not an event is related to the therapy. Table 11.1 lists common sickle cell related events. Events on this list can still be related to therapy if the investigator determines that study drug may have triggered the event or made it more severe.

Table 11.1. List of Adverse Events Related to Sickle Cell Disease

Acute chest syndrome Anemia Aplastic crisis Aplastic crisis/anemia Arthralgia Avascular necrosis of hip/shoulder Avascular necrosis of the femoral head Bone infarction Cardiomegaly Cerebrovascular accident Cholecystitis, hepatic sequestration Cranial nerve palsy Decreased kidney function Decreased lung function Delayed growth/puberty Depressed ESR	Elevated urinary urobilinogen Pyelonephritis Hyperplastic bone marrow Hyposthenuria Hypoxemia (PO2 < 65mm Hg) Infection, pneumococcal Jaundice Leukocytosis Meningitis Pain, joint Pain, long bone	Pain, severe abdominal Priapism Pulmonary embolism Pulmonary hypertension Pulmonary parenchymal infiltrates on chest x-ray Pyelonephritis Renal failure Renal insufficiency/albuminuria Renal papillary necrosis Retinculocytosis (10%-20%) Retinal Disease Retinal hemorrhage Rhabdomyolysis Sepsis Skin ulcers Splenic sequestration

An unexpected AE is an adverse reaction, the nature or severity of which is not consistent with current prescribing information for HU or Mg pidolate.

11.5 Monitoring of Adverse Events

Every AE must be followed to a satisfactory outcome or stabilization of the event, even when this requires a time period beyond the scope of the study (this is particularly applicable to SAEs). Outcome includes information on recovery and any sequelae, as well as specific tests and/or treatments that may have been required and their results. For a fatal outcome, cause of death and a comment on its possible relationship to the suspected reaction should be provided. All AEs will be collected after the subject signs informed consent and will continue for 30 days after the last dose of study drug.

The terms used to define outcome are as follows (outcome of reaction/event at the time of last observation):

- Ongoing
- Resolved without sequelae

- Resolved with sequelae
- Death

Actions taken in response to an AE and follow-up results must be recorded in the subject's medical record (this includes follow-up laboratory results). Any treatment administered for the adverse event must be recorded in the subject's CRF. When subjects are discontinued from the study due to an AE, relevant clinical assessments and laboratory tests will be repeated as necessary until final resolution or stabilization occurs.

11.6 Reporting of Serious Safety Issues; Suspension Guidelines

11.6.1 Reporting of Serious Adverse Events

Serious adverse event reporting will begin with events that arise after the Informed Consent is signed until 30 days after the last dose of study drug. Within 1 business day of the realization that an unexpected SAE has occurred to a study subject, a study investigator must make an initial report tothe CSCC Statistics and Data Management Center SAE Regulatory Specialist.

The report should describe the event as fully as possible. The initial SAE reports received from the site should include the following minimum information: an identifiable subject, study product, an identifiable reporting source, and an event or outcome that can be identified as serious. Supporting documentation (CRF pages, lab reports, summary notes, autopsy reports) should accompany the report.

All participating sites will be expected to report fatal or life threatening SAES to their local IRBs according to standard institute guidelines and procedures.

The site investigator, the study coordinator, the Medical Monitor, and the SDMC SAE Regulatory Specialist will collaborate to prepare a report of the unexpected SAE using the current version of FDA's SAE reporting forms. A fatal or life-threatening, drug-related unexpected SAE will be reported to the DSMB and the IND holder's IRB within 7 calendar days of the receipt of the initial report by the SDMC SAE Regulatory Specialist. A non-fatal, non-life-threatening, drug-related unexpected SAE will be

reported to the DSMB and the IND holder's IRB within 15 calendar days of the receipt of the initial report by the SDMC SAE Regulatory Specialist.

The PI will submit the DSMB report to the Chair of the DSMB Subcommittee appointed to monitor this study and to the NHLBI Project Officer. The PI will submit the SAE report to all study investigators. A non-fatal, non-life-threatening expected SAE will be reported to the DSMB in the semi-annual report. Each study investigator will submit SAE reports to the local Institutional Review Board (IRB) and other local authorities in accordance with the institution's regulations.

All serious adverse events, regardless of expected status, are also recorded in the Adverse Events section of the subject's CRF.

The site investigator will follow the progress of a subject who experiences an unexpected SAE until the SAE is resolved or considered stable. When the unexpected SAE has not been resolved by the report deadline, the site investigator will make follow-up reports in accordance with directions from the DSMB, the FDA, and/or the site's IRB.

11.6.2 Reporting of All Serious Safety Concerns

Serious safety issues that arise in this study will be brought to the attention of the CSCC Data and Safety Monitoring Board (DSMB), which will make recommendations to the National Heart, Lung, and Blood Institute (NHLBI) regarding possible suspension or termination of the study. The NHLBI will consider the DSMB's recommendations, determine an appropriate action and notify the Principal Investigator (PI) and the CSCC Statistics and Data Management Center (SDMC). The PI will notify all participating investigators, who will implement the actions directed by NHLBI. This section defines "serious safety issues" and describes procedures for bringing them to the attention of the DSMB.

The SDMC or PI will make the following types of reports that can alert the DSMB to a potential safety issue:

- Ad hoc reports of fatal, life-threatening, or unexpected serious adverse events that are made within 7 or 15 calendar days, as specified in Section 11.7.1.

- Reports of quarterly statistical analyses of all serious adverse events. The SDMC makes such analyses quarterly, but files a report to the DSMB only when analyses indicate that a safety issue has arisen, as defined by the "alert" criteria.
- Reports of semi-annual SDMC analyses of all adverse events and of adverse clinical laboratory trends. These reports will highlight any safety issues revealed by the analyses that meet the "alert" criteria.

Safety AlertsThree months after the first subject is enrolled in the study, and at the end of each 3 month period thereafter, if any SAEs (expected or unexpected) have been reported in the study during the preceding 3 months, the SDMC will:

- Use the current version of the MedDRA dictionary to code all AEs (serious or not) that have been recorded on study AE forms.
- Make a "snapshot" copy of the adverse events data, including MedDRA codes.
- Create frequency tables of treatment x occurrence (yes or no, since inception of the study) of all subjects. The counting units are subjects, not events.
- Compute Fisher's Exact Test (FET) statistic to test the alternative hypothesis that occurrence of SAEs is not independent of treatment group. The FET p-value is not adjusted for multiplicity.
- If the FET p-value is less than the critical value shown in Table 11.3 and the active treatment group has a higher AE rate, the SDMC will conduct further statistical analyses as indicated by the circumstances and report the results to the Chair of the DSMB subcommittee monitoring this study, the PI, and the NHLBI Project Officer.

The SDMC will not file a report of all SAEs if none of the FET p-values is less than the critical value shown in Table 11.3 or if the relative risk is less than 1.

11.6.3 Reporting of All Adverse Events (Serious and Non-serious)

Six months after the first subject is enrolled in the study, and at the end of each 6 month period thereafter, the SDMC will:

- Use the current version of the Medical Dictionary for Regulatory Activities (MedDRA) to code all AEs that have been recorded on study AE forms.
- Make a "snapshot" copy of the adverse events data, including MedDRA codes.
- Calculate safety alerts based on clinical laboratory trends as specified in Section 11.7.5.

- Collate all new SAE reports and those that have been updated since the last report, using MedWatch forms or narratives.
- Including all of this information, prepare the study's semi-annual report and present it to the DSMB and the NHLBI Project Officer.

11.6.4 Reporting of Adverse Clinical Laboratory Trends

An *adverse clinical laboratory trend* is a shift, in an adverse direction, in the mean (or median) change-from-baseline of a clinical laboratory parameter that is more adverse in an active treatment group than in a control group (e.g., mean ALT change-from-baseline increases significantly more in the active treatment group than in the control group).

Six months after the first subject is enrolled in the study, and at the end of each 6 month period thereafter, the SDMC will:

- Make a "snapshot" copy of the study's clinical laboratory data.
- Perform an appropriate statistical analysis of clinical laboratory change-from-baseline data for each clinical laboratory evaluation obtained in this study.
- Perform an appropriate statistical test of H_o: (The mean [or median, or proportion, as appropriate] change-from-baseline of the clinical laboratory values for the active treatment group is the same as for the control group), vs. H_a: (The mean [or median, or proportion] change-from-baseline in the active treatment group is "worse" than for the control group). The meaning of "worse" depends upon the specific clinical lab measurement. The test statistic p-value is not adjusted for multiplicity.
- If the hypothesis test p-value is less than the critical value shown in Table 11.3, the SDMC will conduct further statistical analyses as indicated by the circumstances and highlight this finding in the semi-annual DSMB report.
- Collaborate with the PI to incorporate the results into the study's semi-annual report to the DSMB and the NHLBI Project Officer.

Table 11.3. Summary of Procedures and Timing for Alerting the DSMB and NHLBI Project Officer of Possible Serious Safety Issues

Situation or Event	Summary of Procedure (See text for details.)	Critical Value for DSMB "Alert"
Study Drug Related, Unexpected SAEs	1. Site investigator notifies SDMC SAE Regulatory Specialist within 1 business day . 2. SDMC SAE Regulatory Specialist prepares report using FDA forms and submit report to DSMB, NHLBI Project Officer, IRBs, study investigators. Report: a. Fatal or life-threatening: within 7 calendar days. b. Otherwise: within 15 calendar days.	Alert all cases.
All SAEs	SDMC performs quarterly analyses of MedDRA-coded SAEs, tabulates subjects with SAEs classified by highest-level MedDRA term. Report only when p < critical value and active treatment group has higher AE rate.	$p < 0.01$ p not adjusted for multiplicity
Adverse Events (all)	SDMC performs semi-annual analyses of MedDRA-coded AEs, tabulates subjects with AEs classified by highest-level MedDRA term. Report every 6 months. Alert only when FET p < critical value and active treatment group has higher AE rate.	$p < 0.01$ p not adjusted for multiplicity
Adverse Clinical Lab Trends	SDMC performs semi-annual analyses of clinical lab change-from-baseline using analyses appropriate for the data type. Report every 6 months. Alert only when p < critical value and change is in "adverse" direction.	$p < 0.005$ p not adjusted for multiplicity

11.7 Subject Discontinuation Due to Adverse Event(s)

The following criteria will be used to determine whether subjects exhibit toxicities of the study drug(s) sufficient to require discontinuation of the study.

While HU and Mg have been shown to have little toxicity when given in the doses outlined in this study, we will closely monitor signs, symptoms, and laboratory findings

to assess for unexpected toxicities. Drugs will be discontinued in any subject who experiences the following:

1. Decline in Hb level to < 5 g/dL.
2. Increase in Hb level to > 13.5 g/dL (because of viscosity concerns).
3. Initiation of chronic transfusion.
4. Hepatic dysfunction (SGPT > 2x upper limit of normal).
5. Renal toxicity (creatinine ≥ 1.2 < 18.0 years of age; ≥ 1.4 mg/dL, ≥ 18.0 years of age).
6. Pregnancy.
7. Stroke.
8. Pulmonary failure requiring intubation.
9. Grade 3 or 4 toxicity lasting longer than 2 weeks.
10. Decision of the PI (e.g., subject is non-compliant).

Subjects will be discontinued for hepatic dysfunction or renal toxicity only if either one is not resolved with dose reduction(s) in the study drug (HU) that is associated with the toxicities. If the site PI has concerns with the level of either the SGPT or the creatinine of the subject, the Operations Committee for the CHAMPS study will be contacted and each situation will be evaluated on a case-by-case basis.

A subject will be considered to be "off study" if:

1. Consent is withdrawn by the subject or the subject's parent/guardian.
2. Subject is lost to follow-up.
3. Subject is deceased.

In addition, subjects will also have treatments discontinued if they become unable to orally ingest the study drug or at the request of the subject for any reason. In the event that drugs are stopped, subjects will continue to be followed by study personnel to assess for potential side effects of their administration. Subjects who receive a single transfusion can continue receiving therapy, but laboratory efficacy measurements will not be assessed until their HbA decreases to less than ten percent. All other efficacy measures and all safety measures will continue to be gathered on these subjects.

11.8 Pregnancy Reporting

HU and Mg have not been found to have toxicity in pregnant subjects, although HU in high doses had teratogenic effects in animal studies. Therefore, pregnant subjects and

female subjects who are nursing will be excluded from the study. Female subjects of childbearing potential will be regularly assessed for pregnancy throughout the study at every visit (excluding Visit 16 – Study Termination Visit). In the event of a test indicating the study subject is pregnant, she will be informed of this result and will immediately have treatment discontinued. All study participants, including males and females, will be asked to abstain from having sex or to use an appropriate form of contraception.

11.9 Clinical and Safety Monitoring

11.9.1 Subject Safety Monitoring

The Data and Safety Monitoring Board (DSMB) has been appointed by and is responsible to NHLBI. The DSMB has adopted a written charter that has been approved by NHLBI and will govern its activities. The DSMB will decide how often they need to review the safety data for each protocol. A month prior to the scheduled meeting time, the SDMC will take all available data and generate a report of all relevant safety information (see above). Upon review of the safety data, the DSMB will make a recommendation to continue the trial or stop it due to safety concerns. DSMB surveillance of this multi-center study will be linked to surveillance of an ongoing single-center CSCC study at St. Jude Children's Research Hospital. The latter is a Phase I trial to estimate the MTD of Mg pidolate in children with Hb SS who are already receiving HU. Data regarding Mg toxicity in the Phase I trial will be reviewed by the DSMB and will be utilized when indicated to assess toxicity in the multi-center Hb SC disease study.

11.9.2 Clinical Monitoring Plan

A clinical research associate (CRA) appointed by the Statistical and Data Management Center (SDMC) will assess overall compliance with the clinical protocol and data quality at all participating centers at least once per year for active studies.

11.9.3 Plan for Reporting Protocol Violations

All protocol violations and deviations will be reported as soon as possible using the SDMC Deviation/Violation Form. This form will ask for a description of the event and what corrective action is planned.

12 DATA COLLECTION AND DATA MONITORING

12.1 CRF and Source Documentation

The site study coordinator will complete a Case Report Form (CRF) for each subject. A CRF manual will be provided to each site to assist in correct CRF completion. On all other study documents, subjects will be identified by a CSCC subject number assigned at enrollment, and will not be identified by name.

The SDMC will provide web-based software to provide data entry screens for data capture. The screens will be accessible via the CSCC website and require Center-specific user ID/password privileges. Weekly reports will be posted to the CSCC secure website to monitor enrollment and completeness of data.

12.2 Data Management

The SDMC will provide statistical and data management support for the study. Validation rules will be applied at several points in the data management process. An error correction procedure will be applied to correct data values that fail validation rules.

12.3 Staff Training and Data Monitoring

Prior to the onset of enrollment, clinical study coordinators and data coordinators will be centrally trained to ensure adherence to the protocol and assure the highest possible data quality. Training will be led by a combination of investigators and other staff from the clinical centers, the SDMC, and NHLBI. Training presentations will address informed consent procedures, study operations and protocol requirements, data collection procedures, maintenance of source documentation, CRF completion and review, routine reporting requirements, data entry and management, and CSCC and NHLBI policies and procedures. As needed and as time allows, face-to-face training will be provided by SDMC staff as part of periodic site visits.

After training, site coordinators will have the responsibility of monitoring CRFs and source documents for accuracy, protocol compliance, subject safety, and adherence to guidelines outlined in the CSCC Manual of Procedures.

As referenced above, centers will be site-visited by the appointed CRA. At each site visit, recruitment guidelines and study eligibility criteria will be reviewed. As the study progresses, informed consents and completed data forms may be reviewed during site visits and compared to source documentation (medical or center records) to confirm accuracy.

13 STATISTICAL ANALYSIS

13.1 Analysis Populations

Randomized subjects who are not dosed will be replaced. Data collected on subjects who do not complete the study will be used whenever possible. The exact criteria used to establish each analysis population and any predefined reasons for eliminating subjects will be identified and documented before the study is unblinded.

Intent-to-Treat (ITT) / Safety population: All randomized subjects who receive any clinical trial material (i.e., any dose of HU, Mg pidolate, or placebo) will be included in the ITT/Safety population. Subjects in the ITT population will be classified according to the treatment group to which they were randomized, regardless of what study drug they received.

Per-Protocol population: The per-protocol population consists of all subjects from the ITT/Safety population who have no major protocol violations and have efficacy information for at least 2 months of the study.

All analyses will be performed on the ITT population. Efficacy analyses will be repeated on the per-protocol population.

13.2 Statistical Methods

13.2.1 Study Population

Subject disposition, populations, demographic and baseline information will be summarized by age strata and treatment arm.

13.2.2 Efficacy Analyses

All efficacy analyses will be performed on the ITT population while the primary and some secondary analyses will be repeated on the per-protocol population.

The Safety Pilot phase will be used to evaluate the safety of magnesium after 10 subjects per treatment arm have been enrolled and had a follow-up time of at least 2 months. This will not be a formal interim analysis, as safety results will be evaluated and no hypothesis

will be tested. Safety endpoints will be summarized by age and treatment groups and descriptively analyzed for differences across treatment groups using a Fisher's Exact Test as the data allow.

In keeping with the 2x2 factorial design of the study, the effect of treatment on the primary outcome of change from baseline percent of RBCs > 41 g/dL to the number at two months will be analyzed using analysis of covariance, with all four treatment groups and controlling for baseline number of RBCs > 41 g/dL. In the primary analysis, all four treatment arms will remain in the model and the combination therapy will be considered a separate treatment group from the single exposure groups. Each active treatment group will be compared to the placebo group.

As a secondary analysis, the additive effect of HU and Mg will be examined by testing whether the treatment interaction term (based on subjects receiving both therapies) is significantly different than zero. This would be the case if the combination arm had much more or much less than the sum of the treatment effects in the single therapy arms. Because the study is not powered to detect an interaction, it is unlikely that it will be found. If no evidence of an interaction is found, a two-way ANOVA model adjusting for baseline number of RBCs > 41 g/dL will be fit, and the effects of each therapy will be estimated.

Also, as a secondary analysis, the change from baseline percent of RBCs > 41 g/dL measured at two, four, six and eleven months will be analyzed using a generalized linear mixed model. This model will allow comparisons of the RBC density over the four treatment groups, controlling for time and baseline percent of RBCs > 41 g/dL as fixed effects and subject as a random effect.

Secondary endpoints will be analyzed using a generalized linear mixed model over the longitudinal measurements (2, 4, 6, and 11 months post-randomization). This includes the following secondary endpoints:

1. Standard hematologic parameters [hemoglobin level, mean cell volume (MCV), reticulocyte count, white blood cell count, platelet count, absolute neutrophil count (ANC)].
2. Hemoglobin S, C, and F levels.

3. Red Cell Metabolic Studies: K-Cl co-transport activity, Gardos channel activity, Na-Mg exchanger activity, red cell cation content, intracellular Mg.
4. Plasma total Mg and ionized Mg (iMg) levels.
5. Adhesion Studies: Adhesion of red cells to laminin, thrombospondin, and endothelial cells; expression of receptors for laminin, thrombospondin, and endothelial cells; red cell surface phosphatidylserine; adhesive response to epinephrine.

This model will allow comparisons of each secondary outcome over the four treatment groups, controlling for time and baseline measurement as fixed effects and subject as a random effect.

Differences across treatment groups for the clinical endpoints of death, clinical stroke, cancer, acute splenic sequestration, pain crises, episodes of acute chest syndrome, episodes of acute splenic sequestration, hospitalizations, neuroimages, and transfusions will be analyzed using Fisher's exact test, if the number of events is sufficient. Otherwise, these data will be summarized by treatment group.

All primary and secondary efficacy measures will be summarized by treatment group and measurement time (if appropriate), using relevant descriptive measures (e.g., mean and standard deviation for continuous outcomes and percents for categorical outcomes). Additionally, graphs of the laboratory measures over time may be used to illustrate longitudinal relationships.

13.2.3 Safety Analyses

Treatment emergent AEs, drug-related AEs, SAEs, and drug-related SAEs (as determined by the investigator) will be categorized by body system and MedDRA (Version 6.0 or later) preferred term. A treatment emergent AE is one that started or worsened in severity during or after the administration of study drug.

The assessment of safety data will be based mainly on the tabulation of adverse events (AE), by age group and treatment arm. Additionally, AEs will be tabulated by severity and relationship to drug. Serious AEs will be described in detail. Comparisons across treatment groups, stratifying on age group will be made using a Cochran-Mantel-Haenszel chi-square test on any AEs that occur in more than 5% of the subjects.

Additionally, blood chemistry and hematology laboratory measurements will be tabulated by age group, treatment group, and measurement time.

13.2.4 Interim Analyses

No formal interim analyses will be performed in this study. Only the safety data from the Safety Pilot phase of the study will be reviewed by the DSMB.

13.3 Statistical Considerations

13.3.1 Covariates

Baseline laboratory measurements will be controlled when analyzing laboratory outcomes. Other relevant clinical covariates such as age may be considered in the analysis of clinical, laboratory, pharmacokinetic, and safety outcomes as needed.

13.3.2 Multi-center Studies

Randomization will be stratified by center to preserve a balance across treatment groups within each center, thus ensuring center effects are even between the treatment groups.

13.3.3 Multiple Comparisons and Multiplicity

The primary hypothesis of no difference between the four treatment groups consists of three tests (each active treatment arm compared to placebo). Using a Bonferroni adjustment, we would divide our 0.05 alpha by three, implying that we would test each hypothesis using a 0.017 alpha. All secondary analyses should be considered descriptive and no adjustments for multiple comparisons will be made.

13.3.4 Examination of Subgroups

Subgroup analyses by age group (<16 years old vs. ≥ 16 years old) may be performed to examine differences in treatment effect. Any subgroup analyses will be performed as exploratory analyses and represented as such.

13.3.5 Missing Data

Data from subjects who are lost to follow-up will be used as much as possible since the analyses allow for missing data. If deemed necessary, a comparison of completers versus non-completers will be performed.

13.4 Sample Size

In order to ascertain the number of subjects appropriate to use in this Phase II, exploratory study, we need to make certain educated assumptions. The pilot study examining the effect of shipping on RBC density found a mean of 7.5% (SD = 3.00) to be a reasonable estimate of the number of percent RBCs > 41 g/dL for subjects with HbSC disease (See Appendix IV). Based on the literature (2), we anticipate that HU will decrease this number by at least 20% down to 6.0%. There is no current literature to base an assumption of the effect Mg will have on erythrocyte density, but we wish to power our tests to detect a similar difference of 20%. If we then conservatively assume that taking both therapies is equivalent to taking one therapy, we would see the following post-treatment mean percent of RBCs >41 g/dL. These assumptions imply we will be analyzing a treatment difference of 1.5 from the mean placebo change from baseline of 0.

Table 13.1. Expected Post-Treatment Mean Percent of RBCs > 41 g/dL by Treatment Group - Assuming a Negative Interaction (i.e., Taking Both Therapies Is No Better than Taking One).

	Placebo	Magnesium
Placebo	7.5	6.0
Hydroxyurea	6.0	6.0

Since the analysis will focus on change from baseline, another important factor to consider is the variance and covariance of the difference scores (post minus pre) for an individual. The variance of the change from baseline for an individual is equal to the sum of the pre and post measurement variances, minus twice the covariance of pre and post measurements. Using our pilot study-supplied standard deviation of 3.00 and assuming equal variances across the pre and post measurements, we can consider a range of correlations between the pre and post measurements. We expect these measurements

to be highly correlated since our literature-cited means reflect a steady state measurement of several HbSC subjects. If we consider a correlation of 0.75, we would have a standard deviation of the change from baseline of 2.12. If the true correlation between subjects is 0.8, we would have a standard deviation of the change from baseline of 1.90. Although the analysis will adjust for baseline measurements, the sample size calculations will assume independence between baseline measurements and change from baseline measurements as well as no site effects; therefore, the sample size calculations will be based on multiple comparisons within a one-way ANCOVA analysis. The post minus pre difference scores will be the response variable.

We will be performing three comparison tests (each active treatment arm compared to placebo) so we wish to adjust our alpha for multiple comparisons. Using a Bonferroni adjustment, we would divide our 0.05 alpha by three, giving us a 0.0167 alpha for each hypothesis.

Putting this all together, we assume an equal standard deviation of 2.12 (corresponding to a correlation of 0.75), a two-sided alpha of 0.0167, and using a one-way ANCOVA to generate our test statistics, we would need 47 evaluable subjects per treatment group to achieve 85% power to detect the 20% difference between each of the treatment groups compared to placebo.

If we wish to vary our assumptions, we would see differences in the number of subjects needed. A higher correlation between pre and post therapy measurements would increase our power to detect the difference by decreasing the variability of our endpoint. For example, 38 evaluable subjects ensure 80% power to detect the 20% difference with a higher correlation of 0.8. Additionally, Bonferroni adjustments are known to be conservative. Our assumptions are based on the best information we have at this point and certain adjustments towards a more conservative approach (implying larger sample size) have been used.

14 HUMAN SUBJECTS PROTECTION

14.1 Discontinuation of Study

Although there are few known risks associated with taking HU and Mg, the safety of the subjects will be monitored by an independent Data and Safety Monitoring Board (DSMB). Subjects can withdraw from the study at any time. Discontinuation of any part of the protocol does not interfere with the subject receiving their routine medical care.

The NHLBI reserves the right to discontinue the study at any time for administrative reasons. The DSMB will monitor the trial by periodically examining the unmasked safety data. The DSMB can recommend discontinuation of the study due to safety concerns at any time. Investigators will be reimbursed for reasonable expenses incurred to the date of discontinuation on the basis of completed subjects. The Federal Drug Administration also has the right to discontinue the study at any time.

The benefits of improving our understanding of the mechanisms and impact of taking HU and/or Mg in subjects with SCD strongly outweigh the minimal risks associated with this therapy. If efficacious, this therapy could be used at little cost to improve the disease progression in other SCD subjects.

All data collected for the purposes of this study will be kept as confidential as other medical records. Data will be stored in one location in a locked filing cabinet to be accessed only by study personnel with the direct permission of the PI. No names will appear on the data; however, subject materials will be identifiable through a unique code number assigned to the subject at enrollment. This master list with the subject's name and his/her identification number will be locked in a separate filing cabinet to further ensure confidentiality. Any published findings as a result of the study will not identify participants by name.

14.2 Disclosure of Data

The investigator, his or her staff and associates, and the appropriate regulatory agencies may use the information included in this protocol as necessary for the conduct of the trial and the safety of subjects. Data from the trial are confidential and may not be disclosed without the written permission of the NHLBI.

14.3 Publication of Research Findings

Manuscript(s) and abstract(s) prepared from the data collected during this trial will be prepared by the study investigators and the SDMC.

15 OBTAINING INFORMED CONSENT

The informed consent will be obtained by one of the study investigators or his designee in the presence of at least one witness. Patients identified as eligible for the study will be invited to participate. Patients will be consented at their local institutions as per local IRB guidelines in accordance with the Code of Federal Regulations (21 CFR 50 and 21 CFR 50.27 Documentation of Informed Consent). The patient and a legal guardian will be introduced to the study. Objectives and procedures required during the study will be discussed with the family and patient, while reading the informed consent. Patients older than 18 years-old will sign their own informed consent statement. The legal guardian will sign the informed consent if the patient is a minor. Verbal and/or written assent will be obtained as mandated by each site's local IRB.

See Appendix VII for the IRB Approval Process for Informed Consent Statements at each participating site.

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the National Heart, Lung, and Blood Institute

16 SUBJECT COMPENSATION

Subjects will be reimbursed $35 for each visit to help defray their costs of participation.

17 PROTOCOL REVISION AND AMENDMENT PROCESS

If the study protocol is revised or amended during the enrollment period of the study, the protocol will go through an approval process by the NHLBI and/or the Data Safety Monitoring Board (DSMB) depending on the nature of the changes. For administrative changes, such as typographical errors or clarifications to the existing document, only the approval by NHLBI is necessary before the protocol (a clean and marked copy), a numbered memorandum outlining the changes to the protocol along with justifications for the changes, and a new protocol signature page updated with the new protocol version number on it is distributed to the sites. For a protocol amendment in which a revision is made that impacts one or more of the study procedures (such as a change to the inclusion/exclusion criteria), NHLBI and DSMB approval must be received. Once the approval is received, the revised protocol (a clean and marked copy), a signed letter from NHLBI documenting DSMB approval for the protocol amendment, a numbered memorandum outlining the changes to the protocol along with justifications for the changes, and a protocol amendment signature page updated with the new protocol version number for the Principal Investigator to sign will be distributed to each of the participating sites.

A study document tracker will be maintained by the SDMC to track the participating sites' progress in resubmitting all protocol revisions and amendments to their local IRB to ensure that all sites are using the same protocol when enrolling subjects into the study.

18 PROTOCOL SIGNATURE PAGE

I, _____, MD agree to conduct:

Effectiveness of Hydroxyurea and Magnesium Pidolate Alone and in Combination in Hemoglobin SC Disease: A Phase II Trial

I understand that no deviations from this protocol (Version 6.1, dated December 13, 2007) may be made without the written permission of the NHLBI CSCC Protocol Chair, except where necessary to eliminate immediate hazard(s) to trial subjects, or when the change(s) involve only logistical or administrative aspects of the trial.

Name: _____

Signature: _____

Site: _____

Date: _____

19 LIST OF INVESTIGATOR(S) AND CLINICAL LABORATORY(S)

This section lists the name(s) and title of the investigator(s) who is/are responsible for conducting the trial, and the address and telephone number(s) of the trial center(s).

Investigator	Address and Telephone Of Center
George Buchanan, MD	University of Texas Southwestern Medical Center @ Dallas Pediatrics Department 5323 Harry Hines Boulevard Dallas, TX 75390-9063
Clinton Joiner, MD	Cincinnati Children's Medical Center Division of Hematology/Oncology 3333 Burnet Avenue Cincinnati, OH 45229
Ronald L. Nagel, MD	Albert Einstein College of Medicine Division of Hematology Ullman Building, Room 921 1300 Morris Park Avenue Bronx, NY 10461
Kwaku Ohene-Frempong, MD	Children's Hospital of Philadelphia 34th Street & Civic Center Blvd. Philadelphia, PA 19104
Martin Steinberg, MD	Boston Medical Center One Boston Medical Center Place, FGH-2 Boston, MA
Marie Stuart, MD	Thomas Jefferson University Hematology Division/Pediatrics Dept. College Building, Suite 727 1025 Walnut Street Philadelphia, PA 19107
Marilyn Telen, MD	Duke University Medical Center Division of Hematology Box 2615 Durham, NC 27710
Elliott Vichinksy, MD	Children's Hospital & Research Center at Oakland Department of Hematology/Oncology 747-52nd Street Oakland, CA 94609
Winfred Wang, MD	St. Jude Children's Research Hospital Department of Hematology/Oncology 332 North Lauderdale Bldg. R-6010 Mail Stop Code 763 Memphis, TN 38105

Name and address of the clinical laboratory (if central laboratory is used), or the names and addresses of all laboratories and technical institutions to be used for processing samples, etc.

CLINICAL LABORATORY	ADDRESS AND TELEPHONE
Dr. Carlo Brugnara Children's Hospital Department of Laboratory Medicine	300 Longwood Avenue, Bader 766 Boston, MA 02115 Phone: (617) 355-6610 Fax: (617) 730-0383 Email: carlo.brugnara@childrens.harvard.edu
Dr. Martin Steinberg/Dr. David Chui Boston University - Sickle Cell	88 East Newton Street, E-211 Boston, MA 02118 Phone: (614) 414-1020 Fax: (617) 414-1021 Email: msteinberg@medicine.bu.edu
Dr. Marilyn Telen Duke University Medical Center	Box 2615 Rm 333 Medical Science Building Durham, NC 27710 Phone: (919) 684-5378 Fax: (919) 681-7688 Email: telen002@mc.duke.edu
Dr. Lucia de Franceschi University of Verona	Policlinico GB Rossi 37134 Verona Italy ldefrances@mail.univr.it Phone:045-8074918 Fax: +39-045-580111

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21 APPENDICES

APPENDIX I – BLOOD SPECIMENS FOR RED CELL DENSITY AND OTHER METABOLIC ASSAYS (LABORATORY OF DR. BRUGNARA)

Subject samples will be identified with a unique subject identifier and the date of specimen collection. Subject names will not be used.

Measures of Cellular Effects of Therapy:

1. Cell volume and hemoglobin concentration (MCHC) with percent of dense cells (Bayer ADVIA 120).
2. Cell Na, K, and Mg content.
3. Activity of the erythrocyte K/Cl co-transporter, Gardos channel, Na/Mg exchange.

Quality Control

To control the quality and reproducibility of the assays, Dr. Brugnara's laboratory will run tests on normal subjects concurrently with this protocol's study samples. Before the study begins, the assays will be performed on 3 subjects' samples; the assays will be repeated 5 times in each normal subject to determine reproducibility and precision. During the study, normal samples will be tested in parallel with the study samples every 2 weeks.

The normal blood is obtained from discarded samples and from collection of blood with informed consent per Dr. Brugnara's protocol entitled " Cellular Determinants of Red Cell Sickling." This protocol has been approved by the Children's Hospital, Boston.

Based on the initial assessments, Dr. Brugnara's laboratory will establish limits of acceptability for this repeated assay that will be used to validate and accept the results obtained in the study samples.

All QC data will be collected and organized in specific QC tables.

Specific ion transport assays:

- Cation content:
  - Intracellular Na level in mmol/kg Hb
  - Intracellular K level in mmol/kg Hb

  - Intracellular Mg level in mmol/kg Hb
- K-Cl co-transport activity:
  - K efflux in mmol/L cell x hour,
- Gardos channel activity:
  - 86 Rb influx in mmol/L cell x min
- Na/Magnesium exchanger activity:
  - Mg efflux in mmol/L cell x hour

Quality Control

To control the quality and reproducibility of the assays, we will study at the beginning of the study, 3 normal subjects, and repeat each assay 5 times in each of these normal subjects, to determine reproducibility and precision. During the study period, we will run one of these normal subjects in parallel with the study samples every two weeks.

Based on the initial assessments, we will establish limits of acceptability for this repeated assay that will be used to validate and accept the results obtained in the study samples.

All QC data will be collected and organized in specific QC tables.

Requirements: Two pediatric EDTA tubes, 10 ml total blood volume. An aliquot from one tube will be transferred to the lab of Dr. Steinberg for HbF determination.

Measures of Plasma Total and Ionized Magnesium Levels:

Quality control will be maintained using the following measures:

Ionized magnesium: 5 controls, provided by Nova Biomedical will be run every day on the Nova Biomedical CCX Analyzer, before starting any samples. This will be performed with two instruments, therefore, everything will be done in duplicate. The instruments calibrate themselves every two hours. The magnesium ion-selective electrodes are replaced if the controls are not within the value required by the lab's standards. In addition, each ion-selective electrode is replaced after 300 samples.

Total magnesium: one control will be run every day, before any samples are run. The instrument checks itself before starting a run. The instrument will be recalibrated every month or if new reagents are needed.

Requirements: One small green top (sodium heparin) tube, 2 ml, kept at 4°C

Samples will be shipped priority overnight in wet ice to:

Dr. Carlo Brugnara
Children's Hospital, Boston
Department of Laboratory Medicine
300 Longwood Avenue, Bader 766
Boston, MA 02115
Phone: (617) 355-6610
Fax: (617) 730-0383
Email: brugnara@tch.harvard.edu

Prior to study activation, we will ship 3 control samples to Dr. Brugnara to test for specimen stability. If indicated, we will modify the shipping procedures.

Measures of Red Cell Membrane Src-Family Kinase Activity and Oxidative Damage:

1) Src-family kinase activity in red cell membranes
2) Red cell membrane protein oxidation by 1 and 2-DE and mass spectrometry peptide finger-printing

These studies will be performed in the laboratory of Dr. Lucia DeFranceschi in Verona, Italy on samples processed and shipped to her from the laboratory of Dr. Carlo Brugnara in Boston, MA. The studies will be done on the first 40 patients enrolled in the study (the pilot group), after which it will be determined if the studies are feasible and worthwhile. The studies will be run at no additional cost to the CHAMPS study and without additional blood being required from study subjects. Packed red blood cells from the heparinized samples used for measuring total and ionized plasma magnesium levels will be utilized; these cells would otherwise be discarded.

Samples will be shipped priority overnight in wet ice to:

Dr. Lucia de Franceschi
University of Verona
Policlinico GB Rossi
37134 Verona Italy
ldefrances@mail.univr.it
Phone:045-8074918
Fax: +39-045-580111

APPENDIX II – BLOOD SPECIMENS FOR ALPHA-GLOBIN GENE SEQUENCING AND HB S, C, AND F (LABORATORY OF DR. STEINBERG)

Subject specimen will be identified with a unique subject identifier and the date of specimen collection. Subject names will not be used.

Hemoglobin S, C, and F levels will be measured in the laboratory of Dr. Martin Steinberg using approximately 2 ml aliquots of EDTA anticoagulated blood. This specimen will be taken from the specimen shipped to the laboratory of Dr. Brugnara. Measurements will be performed using HPLC.

Single α-globin gene deletion of the rightward type ($-\alpha^{3.7}$) is common among African Americans. Single α-globin gene deletion of the leftward type ($-\alpha^{4.2}$) is rare in this population. Diagnosis of these α-globin gene deletions requires DNA-based diagnostics. In the laboratory of Drs. Steinberg and Chui, multiplex gap-PCR tests will be performed using methodology designed specifically to detect these two deletions at visit 1.

Genomic DNA will be extracted from peripheral blood leukocytes by standard techniques. Five primers will be used for PCR and subsequent gel electrophoresis to detect normal α-globin genes (control) and ($-\alpha^{3.7}$) and ($-\alpha^{4.2}$) deletions. The protocol is adapted from Tan, et al. (21a).

Quality Control

The quality control process will include testing at four-month intervals of quality control samples along with subject samples. The quality control (QC) material will be prepared with known Hb S, C, and F concentrations and will be tested blinded, in triplicate, in the same manner as subject samples. The concentration of Hb S, C, and F in the QC samples will be prepared according to published range of Hb S, C, and F levels. We will evaluate a high Hb S, C, and F value (abnormally high), normal and a low level. The acceptable values for each point will be evaluated by analyzing the mean value and standard deviation of the determination. An assay is considered within control range when all the QC values are within ± 2 standard deviations. Sample QC values outside ± 2 SD will be considered in error and the sample results will be rejected. A table with the QC data will be prepared each time QC is evaluated.

Requirements: Two ml of EDTA anticoagulated blood will be obtained from specimens sent to the lab of Dr. Brugnara, and shipped to:

Dr. David Chui
Boston University - Sickle Cell
88 East Newton Street, E-248
Boston, MA 02118
Phone: 617-414-1018
Fax: 617-414-1021
Email: david.chui@bmc.org

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APPENDIX III – BLOOD SPECIMENS FOR ADHESION ASSAYS (LABORATORY OF DR. TELEN)

Subject samples will be identified with a unique subject identifier and the date of specimen collection. Subject names will not be used.

Flow Chamber Methodology

In vitro studies of cell adhesion during flowing conditions best represent in vivo events, as compared to adhesion assays in which cells are simply allowed to incubate overlaid on a chemical or cellular substrate (e.g., laminin or endothelial cells) and then washed off by either noncontrolled forces (e.g., pipette washes) or controlled forces (e.g., rotary motion devices). Flow chambers can produce either a constant shear stress throughout the chamber or a variable shear stress, produced by creating a variable height for the chamber. We have used this latter type of chamber extensively, so that we can then choose to study adhesion at several different shear stresses in the same experiment. We plan to use this latter method for studies of SC RBC, because we believe it is potentially more sensitive to weak adhesive interactions than the fixed height chamber, as both detachment curves over a range of shear stresses as well as absolute adhesion at given shear stresses can be easily quantitated. The variable height flow chamber (Figure 1) consists of a narrow gasket of thickness H_o with the upper surface milled into a Plexiglas plate and inclined at an angle α; h(x) is measured with a micrometer. The design of this chamber allows shear stress along the walls to be accurately measured from fluid viscosity, position along the length of the chamber, and flow rate (35, 36). An infusion pump from a reservoir maintained at 37º C will generate flow rates. For a Newtonian fluid, the wall shear stress ${}^{t}w$ (dynes cm^{-2}) is:

$$t_w = \frac{6\mu Q}{wh(x)^2}$$

where, μ is the fluid viscosity at 37°C, and Q is the volumetric flow rate. Wall shear stresses range from 0.6 to 150 dyne/cm^2. Viscosities are measured with a couette viscometer (Rheometric Scientific, Piscataway, NJ). For shear stresses below 100 dyne/cm2, the medium will consist of DMEM, which is Newtonian with a viscosity

of 0.0085 g/cm-s at 37° C.

For experiments examining red cell adhesion to laminin, glass slides will be coated by overnight incubation with 10 µg/ml laminin, rather than with cells, as illustrated in Figure 1. For assays of adhesion to endothelial cells, confluent ECRF endothelial cells will be grown and prepared on glass slides coated with gelatin. In both instances, the slide is mounted in the flow chamber and the unit assembled. 20 ml of SC

RBC (2 x10^7/ml) suspended in Hank's PBS (with Ca^{++} and Mg^{++})) are infused into the chamber at a constant flow rate of 3 ml/min, followed by 10 min of washing with the same buffer at the same flow rate. Adhesion is then quantitated in seven fields exposed to shear stresses ranging from 0.3 to 2.7 dynes/cm^2. The local height at each field photographed is measured using the calibrated vernier scale of the fine focus, and shear stress is calculated using the equation given above.

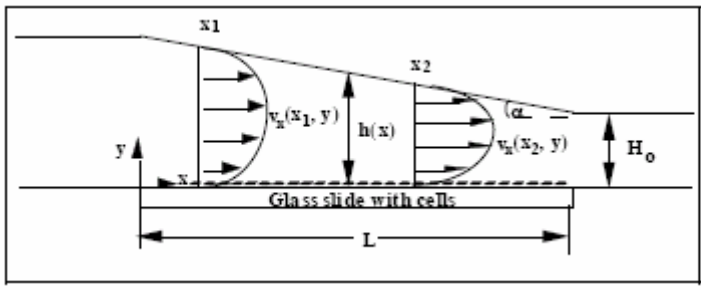

Figure 1. Schematic diagram of the variable height laminar flow chamber. Cells attach to a glass slide on the lower surface. Fluid flows though a gap of height h which varies linearly with position. The angle of inclination (a = 0.3235°) is sufficiently small such that inertial effects in the vertical direction are small and the velocity profile is parabolic.

Results from preliminary studies employing a graduated height flow chamber, using SC and SS RBC and laminin as the adhesive substrate, are shown in Figure 2.

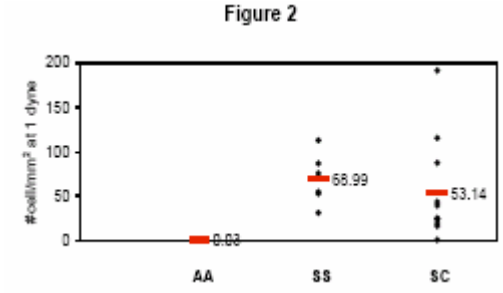

Figure 2 compares the adhesion of SS RBC and SC RBC to immobilized laminin in the flow chamber, at a shear stress of 1 dyne/cm^2. As shown, although the mean adhesion of SC RBC samples is lower than for SS RBC samples, it is still considerably higher than normal (AA) RBC. Mean adhesion of all samples tested are shown by the bars and adjacent values.

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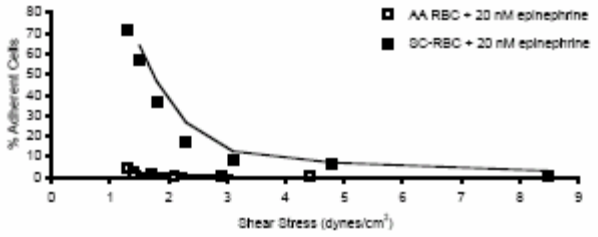
Figure 3



In Figure 3, the ability of epinephrine-treated SC RBC to adhere to endothelial cells is compared to adhesion by similarly treated normal RBC. Adhesion is easily visualized with shear stresses ranging from 1-5 dynes/cm^2. Moreover, this figure represents a relatively weakly adherent SC RBC sample, as many samples exhibit much higher numbers of adhesive cells/mm^2. Therefore, we feel that this type of flow chamber, rather than a constant height flow chamber, will be most sensitive to the degree of adhesion exhibited by SC RBC.

Schedule of Adhesion Assays and Sample Needs:

In the current protocol we will obtain two baseline blood samples during the randomization phase, and then samples at 2 months, 4 months, 6 months, and 12 months during the treatment phase. We plan to use the same schedule for the adhesion assays. Moreover, we can usually perform the needed assays using the blood left over in a routine 5 ml purple top tube (such as that used first to perform a routine automated blood count), or we can simply obtain a separate 5 ml purple top tube. In other words, we routinely perform this assay with small quantities of cells, and therefore will not add greatly to the amount of blood drawn for protocol-related studies.

Stimulated Adhesion Assays:

Measurement of the ability of SC RBC to adhere to endothelial cells will be measured after a one minute exposure of cells to epinephrine. We have shown that adrenergic receptor signaling, which involves cAMP and protein kinase A, upregulates the ability of SS RBC to adhere to both laminin and endothelial cells (33, 34). Finally, we have recently shown that pretreatment of SS RBC with either forskolin or physiological concentrations of epinephrine, followed by transfusion of these cells *in vivo*, leads to increased SS RBC adhesion and vaso-occlusion (37), suggesting that responsiveness of SS RBC to epinephrine can be pathophysiologically important in SCD. Finally, neither SS RBC nor SC RBC adhere to endothelial cells unless either the endothelial cells are activated (e.g. with TNFα or platelet activating factor) or the RBC are activated by epinephrine or another agent that increase intracellular camp. Therefore, we will address measurement of the responsiveness of SC RBC to epinephrine as part of our assays for adhesion to endothelial cells. By measuring how SC RBC adhesion is affected by

stimulation with forskolin and epinephrine. These assays will be performed as previously described, using the sensitive graduated height flow chamber (33).

We expect to find that at baseline SC RBC are intermediate compared to normal and SS RBC in their ability to respond to epinephrine with increased adhesion to endothelial cells. Since thus far we have found that SS RBC responses are similar vis-à-vis both laminin and endothelial cells, we will examine only adhesion to laminin by unstimulated cells and adhesion to endothelial cells in the context of epinephrine-stimulated adhesion of SC RBC, as a cost-saving approach to these studies.

Assays of Adhesion Receptor and Surface Phosphatidylserine Expression:

The laminin and endothelial cell receptors (B-CAM/LU and LW, respectively) are both over-expressed by SS RBC as compared to normal African-American controls, while the VLA-4 receptor for TSP is over-expressed by SS reticulocytes (38). In addition, SS and SC RBC have increased exposure of phosphatidylserine, which can be measured by annexin V binding. And phosphatidylserine has also been proposed to be involved in adhesion to thrombospondin (39). AnnexinV binding assays will be performed by routine flow cytometric methods in order to assess how drug therapy affects exposure of phosphatidylserine. Adhesion molecule expression will be assayed by Western Blot and quantitated by chemiluminescence.

Quality control will be maintained using the following mechanisms:

A. Surface markers (B-CAM/LU, CD47, VLA-4, LW, red cell surface phosphatidylserine [annexin V binding]):

1. Immunological and Other Reagents for Flow Cytometry and Western Blot:
    - Antibodies are either obtained commercially and used within their expiration dates or are well-described and published.
    - All antibodies are tested monthly for their reactivity with both Hb AA and HbSS control cells, to ensure that antibody strength (titer) is maintained.
    - Reagents for annexin V binding (phosphatidylserine expression) are obtained commercially and calibrated using normal and calcium-ionophore treated cells (negative and positive controls, respectively).
    - Ghost membranes are prepared from erythrocytes and frozen at -80^{o} C. Patient ghosts samples will be analyzed following SDS-PAGE and

Western blot. Quantification of Western Blots will be performed by chemiluminescence.

2. Erythrocytes: All assays will be performed promptly, as specified in the protocol
3. Flow cytometry:
    - All assays will be performed by the Duke Comprehensive Cancer Center Flow Cytometry facility, and instrumentation is calibrated daily.
    - Routine daily, weekly and monthly maintenance will be performed.
    - Raw Data will be stored in secure files on the hard drive of the flow cytometry instrument and backed-up daily to the Cancer Center Server and to Flow Cytometry facility media.
    - Hard copies will be printed daily.
    - Subjects samples will be run according to routine protocols for human red blood cells by a technician familiar with the requirements for gating and assaying erythrocytes.
    - All instruments will be maintained via standing service contracts.
    - Subject sample controls: In addition, we will submit blinded samples from three subjects not enrolled in the study once every four months.
    - When not in use, all antibodies and other reagents will be refrigerated. Certain stock reagents may be frozen, according to the manufacturer's recommendations.

B. Adhesion assays:

1. Erythrocytes: All assays will be performed promptly and cells will be used the day they are received or the day after, as specified in the protocol.
2. Adhesion reagents:
    - Laminins 10/11 will be bought commercially and then tested to ensure that they produce the same degree of adhesion as previous batches. When necessary, various lots will be screened and titrated to maintain constant assay conditions.
    - Reagents will also be tested monthly for maintenance of ability to support adhesion.
3. Endothelial cells:
    - We are using ECRF cells. We have demonstrated that ECRF are equivalent to HUVECs for their adhesion properties and are less expensive and less laborious to maintain.
4. Adhesion assays:
    - All assays will be performed at 37°C as previously described using a microscope stage containing a thermal plate.
    - All buffers will be prewarmed to 37°C.
    - Epinephrine will be pharmaceutical grade and freshly prepared for each day's assays.
    - Infusion pumps will be calibrated daily.
    - All assays will be performed in a standardized timed manner, to ensure

that all measurements are made at standardized times as stipulated in the published method and in the protocol.

- All adhesion will be documented through a digital camera and data stored on a Compaq 7500 computer. Daily backups are made to hard disc and to the departmental server.

Requirements: One pediatric EDTA tube, 5 ml total blood volume. Sample should be put on ice within two hours of being drawn, in order to ensure similar handling of all samples. Sample may be used for automated cell counting (complete blood count) before being shipped).

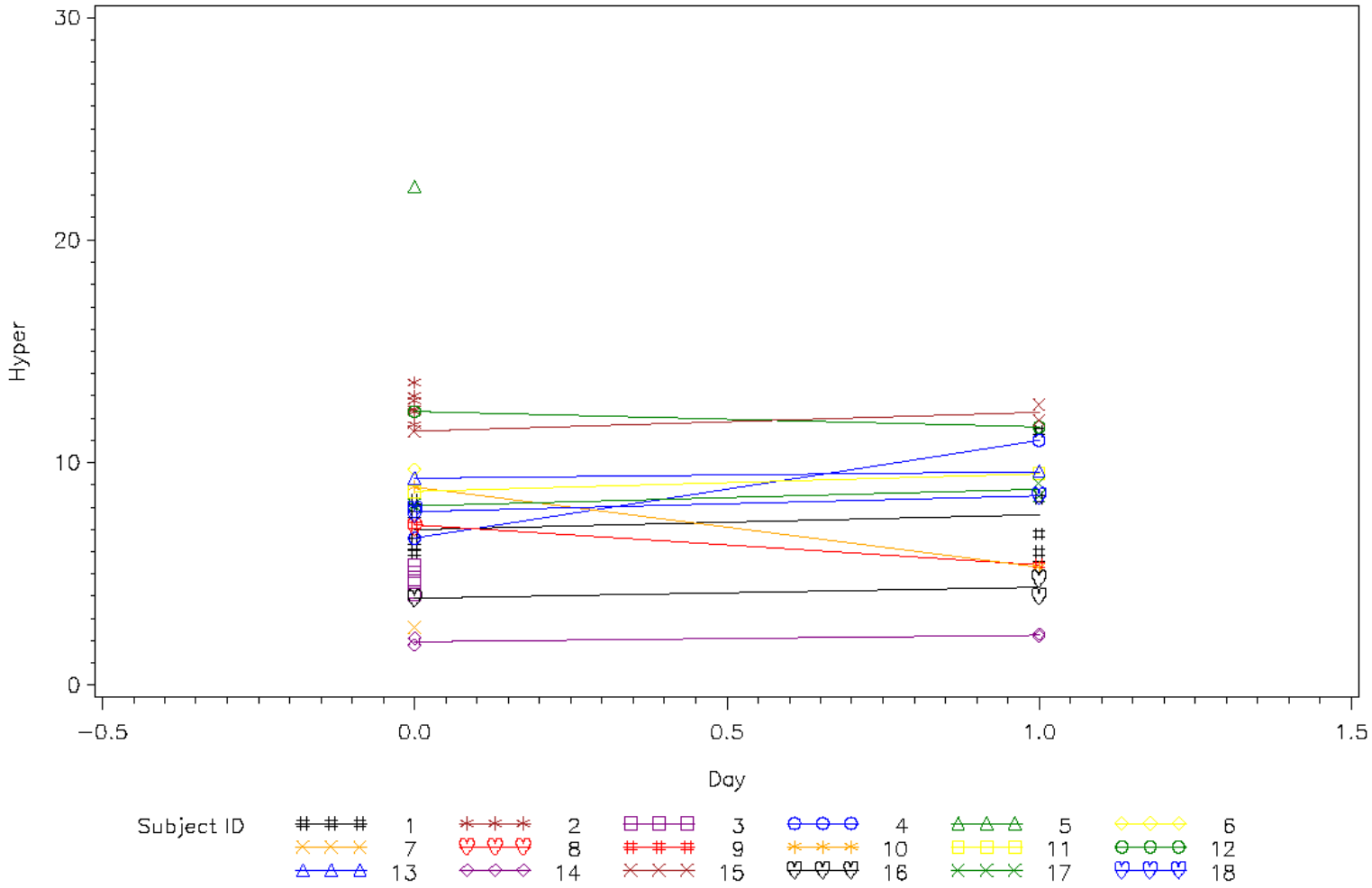
Samples will be shipped overnight on wet ice to:

Dr. Marilyn J. Telen
Duke University Medical Center
Box 2615
Medical Science Building, Room 333
Durham, NC 27710
Phone: (919) 684-5378
Fax: (919) 681-7688
Email: telen0002@mc.duke.edu

APPENDIX IV – PILOT STUDY TO ASSESS THE IMPACT OF SHIPPING ON RBC MEASUREMENTS

To determine whether an artifact in red cell density measurement (the primary endpoint) resulted from shipping of specimens from local Centers to the Central Lab, the following procedure was performed. Blood specimen from HbSC subjects were collected in a standard manner in either Boston or Memphis. At least two tubes were collected and standard blood counts on an ADVIA instrument were determined at each of the two sites. The second tube was packed on ice in a standard shipping container identical to the one used in the CSCC Multi-center Arginine trial. This specimen was then shipped by Federal Express to another address in the same city, making sure that the specimen arrived within 24 hours. The specimen was then taken to the original ADVIA machine and the same blood counts obtained. The results of this informal study involving 18 sickle cell subjects are shown in Figure I of Appendix IV. Two specimens (subjects 9 and 10) were shipped in smaller containers and therefore did not have enough ice to keep the tubes cold for 24 hours. As can be seen in the figure, the hyperdense fraction of cells decreased significantly in these two cases. One additional case (subject 14) was found to have HbS-β+ thalassemia rather than HbSC disease; this subject had the lowest percentage of hyperdense cells (~ 2%) before and after shipping. As can be seen in the figure, the average percent hyper cells at both pre- and post-shipping time points was approximately 8% with a range of 4-12%. Except for one case (subject 4, increase in percent hyper cells), all cases in which the ice was adequate showed stable fractions of hyperdense cells.

Figure 1: Proportion of hyperdense ("hyper") cells in individual HbSC disease subjects measured by the ADVIA 120 instrument before and after overnight (24 hr.) shipping



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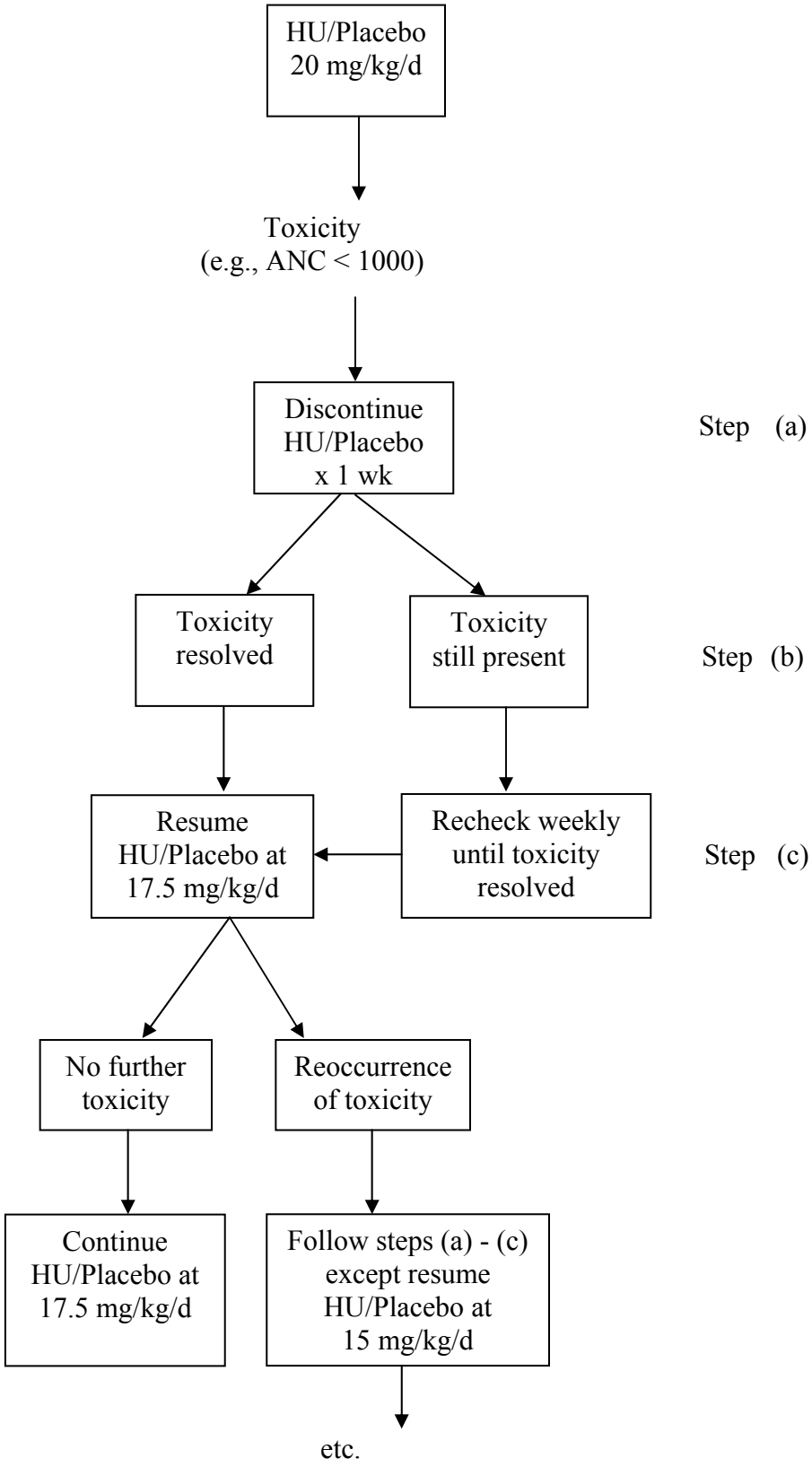
APPENDIX V – HYDROXYUREA-PLACEBO DOSING TABLE AND DOSE ADJUSTMENT

caps = number of capsules per day. If more than one number, the subject should take the first number on the first day, the second on the 2nd day, etc, and then repeat. E.g. if dose is 2/1/1: 2 pills on Day One, 1 pill on Day Two, 1 pill on Day Three, then repeat (2 pills on Day Four, 1 pill on Day Five, 1 pill on Day Six, repeat). Actual doses below are close approximations of the prescribed dose. Equivalent dosing may be used if necessary. E.g., a child with a dose of 500 mg who cannot swallow the large capsules may be given alternate a dose of 400 mg with a dose of 600 mg.

		20/mg/kg/d			17.5 mg/kg/d (1st Toxicity)			15 mg/kg/d (2nd Toxicity)			12.5 mg/kg/d (3rd Toxicity)		
Wt. (kg)	Wt. Range (kg)	HU/ Placebo Dose (mg)	500 mg (# caps)	200 mg (# caps)	Dose (mg)	500 mg (# caps)	200 mg (# caps)	Dose (mg)	500 mg (# caps)	200 mg (# caps)	Dose (mg)	500 mg (# caps)	200 mg (# caps)
15	12.6 - 17.5	300		2/1	262.5		2/1/1	225		2/1/1/1/1/1/1	187.5		1
20	17.6 - 22.5	400		2	350		2/2/2/1	300		2/1	250	1/0	
25	22.6 - 27.5	500	1		437.5		3/2/2/2	375		2	312.5		2/1
30	27.6 - 32.5	600		3	525	1		450		2	375		2
35	32.6 - 37.5	700	1	1	612.5		3	525	1		437.5		2
40	37.6 - 42.5	800		4	700	1	1	600		3	500	1	
45	42.6 - 47.5	900	1	2	787.5		4	675	1	1	562.5		3
50	47.6 - 52.5	1000	2		875		4	750	1	1	625		3
55	52.6 - 57.5	1100	1	3	962.5	1	2	825		4	687.5	1	1
60	57.6 - 62.5	1200	2	1	1050	2		900	1	2	750	1	1
65	62.6 - 67.5	1300	1	4	1137.5	1	3	975	2		812.5		4
70	67.6 - 72.5	1400	2	2	1225	2	1	1050	2		875	1	2
75	72.6 - 77.5	1500	3		1312.5	1	4	1125	1	3	937.5	1	2
80	77.6 - 82.5	1600	2	3	1400	2	2	1200	2	1	1000	2	
85	82.6 - 87.5	1700	3	1	1487.5	2	2	1275	1	4	1062	1	3
90	87.6 - 92.5	1800	2	4	1575	3		1350	2	2	1125	1	3
95	92.6 - 97.5	1900	3	2	1662.5	2	3	1425	2	2	1187.5	2	1
100	97.6 - 102.5	2000	4		1750	3	1	1500	3		1250	3/2	
105	102.6 - 107.5	2100	3	3	1837.5	2	4	1575	2	3	1312.5	1	4
110	107.6 - 112.5	2200	4	1	1925	3	2	1650	3	1	1375	2	2
115	112.6 - 117.5	2300	3	4	2012.5	4		1725	3	1	1437.5	2	2
120	117.6 - 122.5	2400	4	2	2100	3	3	1800	2	4	1500	3	
125	122.6 - 127.5	2500	5		2187.5	3	3	1875	3	2	1562.5	2	3


HU/Placebo
20 mg/kg/d
Toxicity
(e.g., ANC < 1000)
Discontinue
HU/Placebo
x 1 wk
Step (a)
Toxicity
resolved
Toxicity
still present
Step (b)
Resume
HU/Placebo at
17.5 mg/kg/d
Recheck weekly
until toxicity
resolved
Step (c)
No further
toxicity
Reoccurrence
of toxicity
Continue
HU/Placebo at
17.5 mg/kg/d
Follow steps (a) - (c)
except resume
HU/Placebo at
15 mg/kg/d
etc.

APPENDIX VI – MAGNESIUM PIDOLATE-PLACEBO DOSING TABLE & DOSE ADJUSTMENT

		Initial Dose		Dose after 1st Toxicity		Dose after 2nd Toxicity	
Wt. (kg)	Wt. Range (kg)	Mg Pidolate 0.3 mEq/kg BID (mEq)	(ml)	Mg Pidolate 0.225 mEq/kg BID (mEq)	(ml)	Mg Pidolate 0.15 mEq/kg BID (mEq)	(ml)
15	12.6 - 17.5	4.5	2.2	3.4	1.7	2.2	1.1
20	17.6 - 22.5	6.0	3.0	4.5	2.2	3.0	1.5
25	22.6 - 27.5	7.5	3.7	5.6	2.8	3.7	1.9
30	27.6 - 32.5	9.0	4.5	6.8	3.4	4.5	2.2
35	32.6 - 37.5	10.5	5.2	7.9	3.9	5.2	2.6
40	37.6 - 42.5	12.0	6.0	9.0	4.5	6.0	3.0
45	42.6 - 47.5	13.5	6.7	10.1	5.0	6.7	3.4
50	47.6 - 52.5	15.0	7.5	11.2	5.6	7.5	3.8
55	52.6 - 57.5	16.5	8.2	12.4	6.2	8.2	4.1
60	57.6 - 62.5	18.0	9.0	13.5	6.7	9.0	4.5
65	62.6 - 67.5	19.5	9.7	14.6	7.3	9.7	4.9
70	67.6 - 72.5	21.0	10.5	15.8	7.9	10.5	5.2
75	72.6 - 77.5	22.5	11.2	16.9	8.4	11.2	5.6
80	77.6 - 82.5	24.0	12.0	18.0	9.0	12.0	6.0
85	82.6 - 87.5	25.5	12.7	19.1	9.5	12.7	6.4
90	87.6 - 92.5	27.0	13.5	20.2	10.1	13.5	6.8
95	92.6 - 97.5	28.5	14.2	21.4	10.7	14.2	7.1
100	97.6 - 102.5	30.0	15.0	22.5	11.2	15.0	7.5
105	102.6 - 107.5	31.5	15.7	23.6	11.8	15.7	7.9
110	107.6 - 112.5	33.0	16.5	24.7	12.3	16.5	8.2
115	112.6 - 117.5	34.5	17.2	25.9	12.9	17.2	8.6
120	117.6 - 122.5	36.0	18.0	27.0	13.5	18.0	9.0
125	122.6 - 127.5	37.5	18.7	28.1	14.0	18.7	9.4

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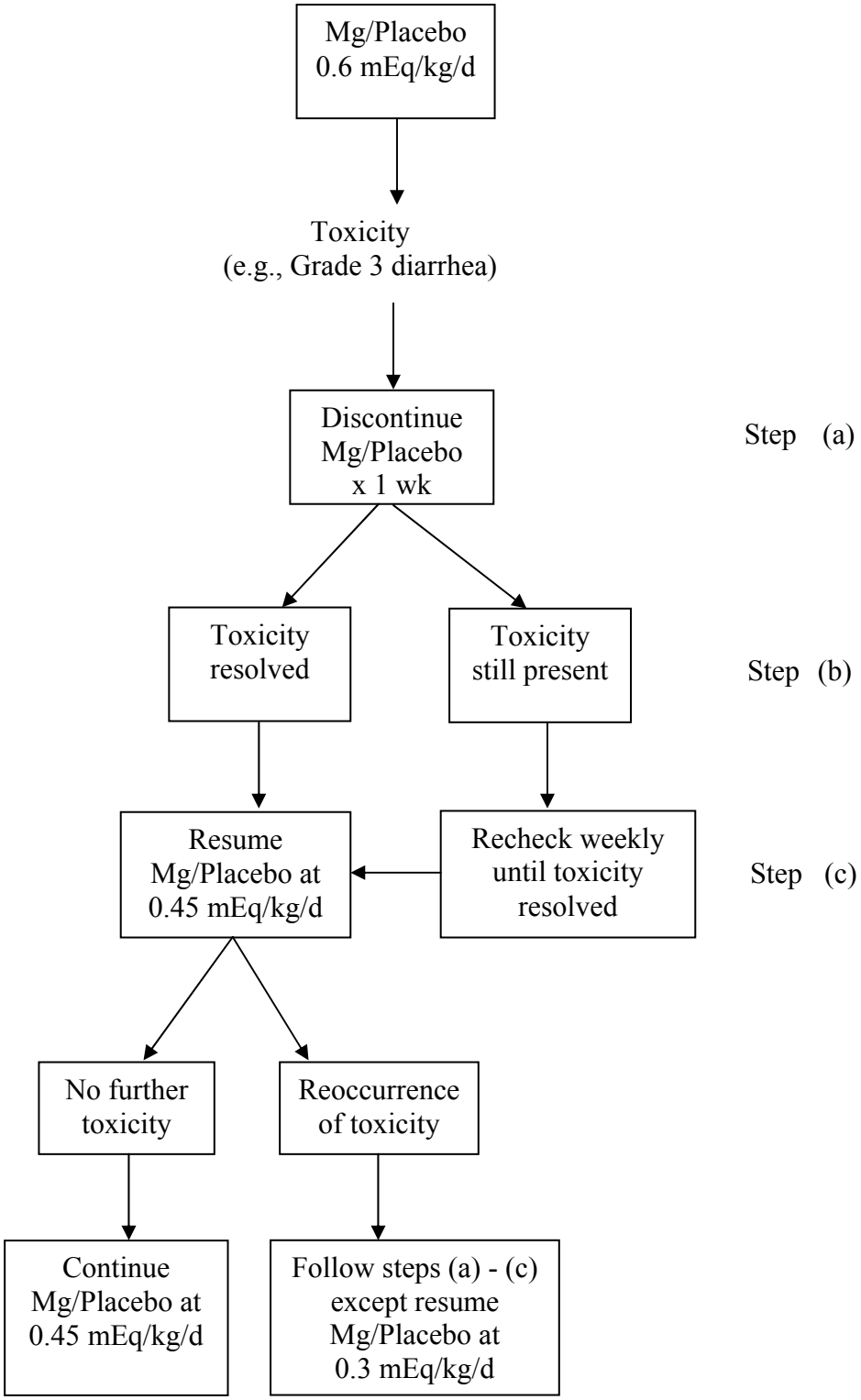
Mg/Placebo
0.6 mEq/kg/d
Toxicity
(e.g., Grade 3 diarrhea)
Discontinue
Mg/Placebo
x 1 wk
Step (a)
Toxicity
resolved
Toxicity
still present
Step (b)
Resume
Mg/Placebo at
0.45 mEq/kg/d
Recheck weekly
until toxicity
resolved
Step (c)
No further
toxicity
Reoccurrence
of toxicity
Continue
Mg/Placebo at
0.45 mEq/kg/d
Follow steps (a) - (c)
except resume
Mg/Placebo at
0.3 mEq/kg/d

APPENDIX VII – IRB APPROVAL PROCESS FOR INFORMED CONSENT STATEMENTS FOR THE HYDROXYUREA AND MAGNESIUM PIDOLATE STUDY FOR SC PATIENTS

The informed consent statement template is written in compliance with the Code of Federal Regulations (21 CFR 50.30), and is adapted to meet the needs of each participating site's local IRB. The informed consent describes in non-technical language the purpose of the study, the activities and procedures involved, the expected duration, the potential risks, benefits, and discomforts of participation, and alternatives to study participation. Each patient must be informed that participation in the study is voluntary, that he/she may withdraw from the study at any time, and that withdrawal of consent will not affect his/her subsequent medical treatment or relationship with the treating physician.

The informed consent forms of each participating site must be approved by the NHLBI, which is serving as both the study's sponsor and IND holder. The local Principal Investigator is responsible for ensuring that the informed consent is obtained from each research subject before that subject participates in the research study, for providing the subject with a copy of the signed consent, and ensuring proper documentation of the process. All recruiting materials (such as a study brochure) will be approved by NHLBI.

The CSCC Statistics and Data Management Center (SDMC) will track forms through this process; sites will not be allowed to enroll study subjects until the process is completed.

The table below outlines the informed consent statement approval process each site must complete before enrolling subjects into the study.

Step	Action	Status
1	The HU-Mg Protocol Team develops an informed consent template	Completed
2	The template is reviewed and approved by the SDMC and Dr. Greg Evans at the NHLBI.	Completed
3	A subcommittee of the Study PI, SDMC staff, and Dr. Evans develops a checklist that is used to review each site's consent forms to assure that the forms meet all regulatory requirements	Completed
4	Each site prepares a template-based consent form(s) for submission to its own IRB.	
5	Using the checklist, the SDMC and NHLBI staff reviews the consent forms: • Forms will be returned to the site investigator for revision if they: o do not satisfy the requirements on the checklist o misstate a point (e.g., underplay a potential adverse event) • After forms are revised by the site, they are re-reviewed by SDMC staff.	
6	Following approval from the SDMC, each site submits its consent forms to its own IRB.	
7	If the site IRB requests changes, the modified consent forms are sent to the SDMC and reviewed as in Step 5, to assure that none of the key elements in the consents have been removed during the IRB review process: • Forms will be returned to the investigator for revision for the same reasons listed in Step 5 • When forms are resubmitted to the IRB, changes must be discussed with the IRB • If forms are acceptable, go to Step 8	
8	A copy of the IRB approval and approval-stamped consents are sent to the SDMC.	
9	The IRB approval and approval-stamped consents are forwarded from the SDMC to the NHLBI.	