CLINICAL PROTOCOL

PROTOCOL TITLE: **Randomized Trial of Oral Dexamethasone for Acute Chest Syndrome**

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1 SYNOPSIS

Title of the Protocol: Randomized Trial of Oral Dexamethasone for Acute Chest Syndrome
Overview: This study tests the effectiveness of oral dexamethasone for the acute chest syndrome (ACS) of sickle cell disease (SCD) in children and adults in the context of standard supportive care. This study primarily aims to determine whether dexamethasone shortens the duration of signs and symptoms of and medical intervention for ACS.
CSCC Protocol Chair: Charles T. Quinn, M.D.
Intervention: Individuals meeting entry criteria will be randomized to receive either dexamethasone 0.3 mg/kg (12 mg maximum single dose) or an equivalent amount of placebo. The study drug will be given by mouth every 12 hours until discharge from the hospital or for a maximum of 4 doses (2 days), whichever occurs first. Thereafter, study drug will be tapered over 6 days for a total duration of therapy not to exceed 8 days.
IND Holder: Charles T. Quinn #74,481
Objectives: **Primary Objective:** To determine whether dexamethasone decreases the duration of signs and symptoms of ACS. **Secondary Objectives:** To determine (1) whether dexamethasone reduces morbidity associated with ACS; (2) whether a tapered regimen of dexamethasone prevents rebound VOC; (3) whether dexamethasone modulates the endothelial phenotype to a less proadhesive, and procoagulant state, and to determine if the discontinuation of dexamethasone will be associated with a rebound in the expression of endothelial activation markers of adhesion and coagulation; (4) whether dexamethasone prevents subacute adverse effects of ACS on pulmonary function; and (5) whether dexamethasone modulates white cell activation and if the discontinuation of dexamethasone will be associated with a rebound in white cell activation.
Hypotheses/Estimates: We will be testing the null hypothesis of no difference in duration of signs and symptoms of ACS among individuals with SCD. The alternative hypothesis tested is that oral dexamethasone is a safe and effective adjuvant treatment that can shorten the duration of signs and symptoms for individuals with SCD who are hospitalized for the treatment of ACS. This is a hypothesis-testing superiority trial.
Criteria for Evaluation: **Efficacy:** **Primary Endpoint**: The duration of signs and symptoms of ACS or duration of hospitalization, whichever is less. The duration of signs and symptoms of ACS is defined as the interval between study entry and the time at which tachypnea, hypoxemia, increased work of breathing, and thoracic pain have resolved and supplemental oxygen and ventilatory support are no longer utilized. **Secondary Endpoints**: Clinical endpoints include: rating of pain, duration of hospitalization, supplemental oxygen, hypoxemia, and fever, number of transfusions, quantification of opioid use, rebound hospitalizations, pulmonary radiographic findings, and pulmonary function testing results. Laboratory endpoints include: $VCAM_1$, $ICAM_1$, P-selectin, L-selectin, vWF multimers, endothelial and monocyte microparticles, WBTF, NO, and $sPLA_2$. **Safety:** Adverse events (AEs), vital signs, physical examination, concomitant medications, complete blood count (CBC), urine output and daily fluid balance, blood culture, pulse oximetry, reticulocyte count, and baseline and interim history.
Study Design: Phase III randomized, double-blind, controlled clinical trial with two arms.
Study Population: Individuals of both genders 5 years of age and older with Hgb SS and Hgb $S\beta^0$are eligible. **Major Inclusion Criteria**: New episode of ACS; diagnosis of ACS within preceding 24 hours; and provision of informed consent. **Major Exclusion Criteria**: Prior participation in this study; corticosteroids contraindicated for comorbid conditions; corticosteroid treatment in preceding 2 weeks.
Clinical and Laboratory Evaluations: The primary endpoint is the duration of signs and symptoms ACS (resolution of tachypnea, hypoxemia, increased work of breathing, and thoracic pain as well as the cessation of medical interventions to support respiration). Other clinical evaluations include subject pain, requirement for opioid analgesics, number of transfusions, use of supplemental oxygen, duration of hypoxemia, duration of hospitalization, and duration of fever. A chest radiograph and spirometry will be performed at a follow-up visit. A panel of biomarkers related to endothelial activation, coagulation, and nitric oxide (NO) bioavailability will be obtained at several time points.
Sample Size: At least 56 subjects with SCD who are hospitalized for ACS.
Randomization: Subjects in each of the two age groups (5-17 and ≥ 18 years of age) will be randomized to either the dexamethasone or placebo group (0.3 mg/kg every 12 hours x 4 doses, then tapered). Randomization will be stratified by site, age group, and severity of ACS to preserve balance across treatment groups.

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Title of the Protocol: Randomized Trial of Oral Dexamethasone for Acute Chest Syndrome
Data Analyses: We will evaluate the difference in duration of symptoms for ACS between the two treatment groups using a generalized linear mixed model (GLMM). Here, the natural log of the duration of symptoms will act as the dependent variable of interest and the treatment status as the grouping factor. As a secondary analysis, the duration of hospitalization, supplemental oxygen, and fever will be analyzed in a similar manner. Laboratory parameters will be analyzed using a GLMM model, with treatment group, baseline laboratory value, age group, severity, and site as fixed effects and subject as a random effect. Clinical endpoints and AEs will be summarized by treatment and analyzed for differences across treatment group. Two formal interim analyses will be performed on the primary efficacy variable. The first interim analysis will take place when 56 subjects have resolved signs and symptoms of ACS OR have been discharged from the hospital. In this interim analysis, the primary efficacy hypothesis will be tested at the $\alpha = 0.001$ level. The study will be stopped if significant efficacy is identified. The second interim analysis will be performed when 112 subjects have resolved signs and symptoms of ACS OR have been discharged from the hospital. The primary efficacy hypothesis will be tested at the $\alpha = 0.01$ level. If significant efficacy is identified for total subjects and for children, the study will be stopped. Otherwise, the study will continue enrollment to 112 children, after which the primary efficacy hypothesis will be tested on the $\alpha = 0.039$ level.
Human Subjects: Potential risks include avascular necrosis, vaso-occlusive crisis, mood changes, hypertension, nausea, vomiting, dyspepsia, gastric ulceration, delayed wound healing, acne, and infection.

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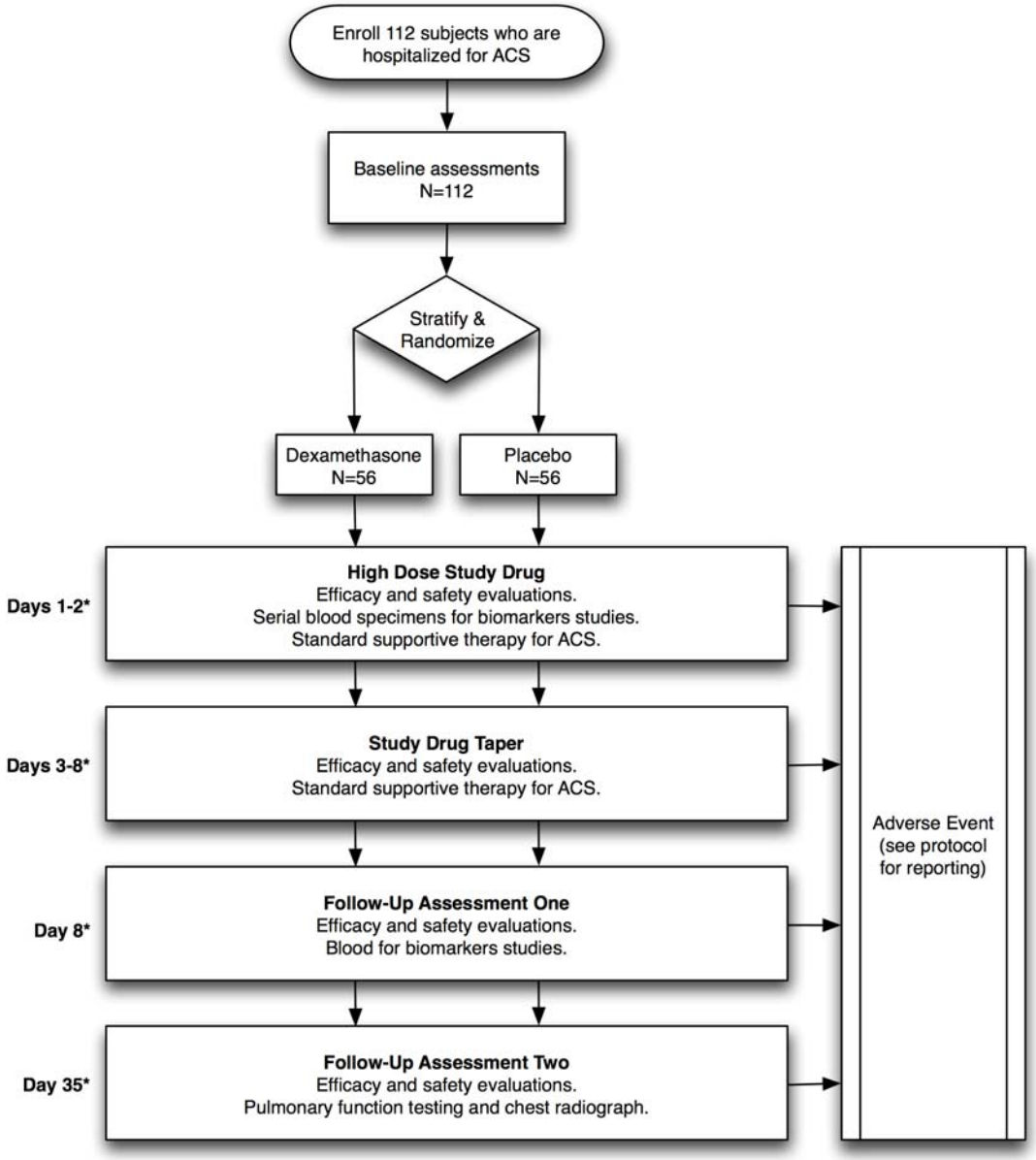
2 SUBJECT FLOW DIAGRAM



*Exact timing may vary—see protocol.

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4 ABBREVIATIONS

ACS	Acute Chest Syndrome
ACTH	Adrenocorticotropic hormone
AE	Adverse Event
CBC	Complete Blood Count
CPT	Chest physiotherapy
CRF	Case Report Form
CSCC	Comprehensive Sickle Cell Centers
CSCC-CTN	Comprehensive Sickle Cell Centers- Clinical Trials Network
CSSCD	Cooperative Study of Sickle Cell Disease
CV	Curriculum Vita
D5 0.45 NS	One-quarter normal saline with 5% dextrose
DSMB	Data and Safety Monitoring Board
FDA	Food and Drug Administration
FEF25-75%	Forced expiratory flow between 25 and 75% of expired vital capacity
FET	Fisher's Exact Test
FEV_1	Forced expiratory volume in 1 second
FVC	Forced Vital Capacity
g/dL	Gram per Deciliter
GCP	Good Clinical Practice
GLMM	Generalized linear mixed model
Hgb	Hemoglobin
Hgb S	Sickle hemoglobin
Hgb SS	Homozygous Sickle Cell Anemia
ICAM-1	Intercellular Adhesion Molecule-1
ICH	International Conference on Harmonisation
IND	Investigational New Drug
IRB	Institutional Review Board
ISM	Independent Safety Monitor
ITT	Intent-to-treat
IV	Intravenous
LOS	Length of Stay
MedDRA	Medical Dictionary for Regulatory Activities, Version 6.0 or later
MEq/L	Milliequivalents per liter
mg	Milligram
mg/dL	Milligram per deciliter
mg/kg	Milligram/kilogram
mL	Milliliter
mm3	Cubic millimeters
NF_KB	Nuclear factor kappa B
NHLBI	National Heart, Lung, and Blood Institute
NO	Nitric Oxide
NPO	Nil per os (nothing by mouth)
O_2	Oxygen saturation
p.o.	per os (by mouth)
PA	Posteroanterior
PEFR	Peak expiratory flow rate
PFE	Pulmonary fat embolism
PFT	Pulmonary Function Testing
PI	Principal Investigator
PRBCs	Packed red blood cells
PRC	Protocol Review Committee
PRN	pro re nata (as needed)
RBC	Red Blood Cell
RhoFED	Rho Federal Systems Division, Chapel Hill, NC

SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SCD	Sickle Cell Disease
SDMC	Statistics and Data Management Center, located at RhoFED
SMB	Safety Monitoring Board
$sPLA_2$	Secretory Phospholipase A2
TF	Tissue Factor
TSP	Thrombospondin
VCAM-1	Vascular Cell Adhesion Molecule-1
VOC	Vaso-occlusive Complications
WBC	White Blood Cell
WBTF	Whole Blood Tissue Factor

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5 BACKGROUND AND RATIONALE

Introduction: SCD is a chronic disorder caused by a mutant hemoglobin. The substitution of the amino acid valine by glutamic acid in the sixth position of the beta subunit of hemoglobin produces sickle hemoglobin (Hgb S). This mutation promotes the hydrophobic interaction and polymerization of deoxygenated Hgb S molecules, producing rigid, elongated red blood cells (RBCs), or sickle cells, that obstruct the microcirculation. The gene for Hgb S arose in historically malarious regions of the world because the heterozygous state offered protection against the malaria parasite, and the gene is now found in individuals of African, Arabic, Hispanic, Greek, and Italian descent. The gene frequency of Hgb S is particularly high in individuals of African ancestry in the United States, among whom 9% are heterozygotes. SCD occurs in individuals who are homozygous for Hgb S or are compound heterozygotes for Hgb S and certain other abnormal hemoglobins. Approximately 1 in 600 African-Americans has SCD and at least 60,000 individuals are affected in the United States.[1] SCD is characterized by chronic anemia and episodic "vaso-occlusive" crises. Acute pulmonary disease, descriptively termed "acute chest syndrome" (ACS),[2] is one of these events.

Acute Chest Syndrome: ACS is defined clinically as a new pulmonary radiographic infiltrate and concurrent signs and symptoms of acute pulmonary disease.[3, 4] Fever and cough are common clinical findings in young children with ACS, but chest pain, shortness of breath, chills, a productive cough, and hemoptysis are more common in older individuals. About two-thirds of patients with ACS are hypoxemic, and most have an acute 1 to 2 g/dL decrease in hemoglobin concentration. The signs and symptoms of ACS may precede the appearance of an infiltrate on a chest radiograph. Lower and middle lobes are affected more often than upper lobes in adults, although upper lobe disease is more common in children. Bilateral infiltrates or involvement of multiple lobes predicts severe disease.

Significance: ACS is a common cause of hospitalization for patients with SCD, second only to painful crisis.[5,6] This complication is not always apparent at the time of admission to the hospital, however. Patients may have fever or acute pain, especially in

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the chest or back, but no respiratory symptoms or pulmonary infiltrates, and develop ACS two or three days later. The Cooperative Study of Sickle Cell Disease (CSSCD) showed that ACS occurs more frequently in young children and among individuals with homozygous sickle cell anemia (Hgb SS).[5] The highest incidence of ACS, 25.8 episodes per 100 patient-years, occurs in children, ages 2 to 4 years with Hgb SS, whereas the incidence is 8.8 episodes per 100 patient-years in adults with Hgb SS. The mean length of hospitalization for all patients with ACS is about 7 days, but children stay in the hospital about 3 fewer days than adults.[7] ACS is the most common cause of death due to SCD. 25% of SCD deaths are related to ACS,[7] and the death rate of ACS is 1.8 % in children and 4.3 % in adults.[6] Despite the gravity of this complication, the treatment of ACS is only supportive and often inadequate. Better treatments are clearly needed for this common and serious complication.

Pathophysiology: There are several presumed causes or antecedents of ACS, including infection, pulmonary fat embolism, hypoventilation and atelectasis, pulmonary edema, pulmonary infarction, and bronchospasm.[7] Any of these may act concurrently, and the distinction of ultimate cause is difficult. Vichinsky and colleagues analyzed 671 episodes of ACS in 538 patients who underwent extensive evaluation for infection and fat embolism by bronchoscopy, serologic assays, microbiologic cultures, molecular techniques, and histologic analysis.[3] A specific etiology was identified in only 38 % of episodes, although a cause was imputed in 70 % of episodes for which "complete" data were available. Infections with bacterial, atypical bacterial, and viral agents were the most commonly identified cause, followed by fat embolism, either alone or in combination with infection. Over half of the episodes had no identifiable cause or were attributed to infarction by exclusion.

The final common pathway in the pathogenesis of ACS is probably pulmonary vascular occlusion and pulmonary endothelial injury. Rigid, irreversibly sickled erythrocytes may occlude the pulmonary vasculature during ACS,[8,9] but the genesis of this occlusion is likely far more complex.[10] For example, studies have shown that sickle erythrocytes bind to pulmonary endothelium via vascular cell adhesion molecule-1 (VCAM-1), whose expression is increased by hypoxia and cytokines, and this binding is not counter-

balanced by cytoprotective mediators like NO.[11] Such excessive and uncontrolled adhesive interactions between erythrocytes and the pulmonary endothelium likely initiate and propagate ACS.

Pulmonary fat embolism (PFE) is one recently recognized cause of ACS.[12] Fatty bone marrow may be released into the blood as a result of bone marrow necrosis caused by a vaso-occlusive bony crisis. Embolic fat activates secretory phospholipase A_2 ($sPLA_2$), an enzyme that cleaves phospholipids and liberates free fatty acids. These fatty acids injure the pulmonary endothelium.[13] Fatty acids also increase the expression of VCAM-1 and promote the adhesion of erythrocytes to endothelium in vitro—evidence for pathologic adhesive interactions in PFE.[11] Inflammatory leukotrienes and prostaglandins are also generated when $sPLA_2$ releases arachidonic acid.[14,15] Indeed, the serum concentration of $sPLA_2$ is a laboratory marker of ACS.[16,17] Patients with ACS have an increased serum concentration of $sPLA_2$, and the concentration correlates with the severity of ACS. There is also a temporal correlation between $sPLA_2$ concentration and the time-course of ACS: $sPLA_2$ increases before ACS is clinically apparent, peaks at the onset, and declines during resolution. [16,17]

Chronic Pulmonary Disease: The pulmonary histopathology of SCD is characterized by intimal hyperplasia and pulmonary fibrosis.[18,19] Recurrent episodes of ACS may cause this injury.[19] Some patients consequently develop a debilitating pulmonary disease characterized by chest pain, dyspnea, chronic pulmonary infiltrates, and hypoxemia.[18, 19, 20, 21] This condition can progress to pulmonary hypertension, cor pulmonale, and even death.[21] Several investigators have used pulmonary function testing to characterize and define the frequency of pulmonary disease in SCD.[20, 22 23] These studies show that obstructive lung disease occurs frequently in this population, that it may precede restrictive lung disease,[22] and that it may be associated with prior episodes of ACS.[23] Therefore, treatments that decrease the severity of ACS might also prevent or decrease late sequelae.

Treatment: ACS is the clinical manifestation of some complex combination of toxic pulmonary endothelial injury, abnormal cellular adhesive interactions, vascular occlusion, the elaboration of inflammatory cytokines, infection, and regional alveolar

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hypoxia. However, our limited understanding of ACS has hindered the development of specific or targeted therapies. The treatment of patients with ACS is primarily supportive, involving watchful waiting and attentive management of pain, hydration, oxygenation, and ventilation. Antibiotics are used empirically, although infectious agents are not identified in most patients.[2, 3, 24, 25, 26] Simple or exchange transfusion of packed RBCs may be beneficial for many reasons, but this therapy has not been tested in rigorous clinical trials.[27, 28, 29] Likewise, there are only anecdotal reports of the benefit of inhaled NO.[30] More effective therapy is needed.

Steroids: Glucocorticoids have a potential therapeutic role because they inhibit several ostensible steps in the pathogenesis of ACS. Steroids attenuate the inflammatory process, inhibit the enzyme $sPLA_2$, and prevent cytokine-induced expression of adhesion molecules by endothelial cells.[11, 31, 32, 33] Thus, steroids could impede the process of vascular occlusion, endothelial injury, and inflammation. Further justification for the study of steroids for ACS can be inferred from related conditions. For example, pulmonary fat embolism due to trauma can be prevented, and possibly treated, with steroids.[13, 34, 35] Likewise, if there is a component of reactive airway disease to ACS, like asthma, steroids might also be beneficial.[36] Glucocorticoid therapy of ACS, therefore, deserves further investigation.

Preliminary Studies: Steroid hormones have been studied for the treatment and prevention of acute SCD crises for many years. Androgenic and progestational steroids reverse in vitro sickling and can alleviate pain, decrease crisis frequency, increase erythrocyte mass, and decrease transfusion requirements.[37,38,39,40,41] Nevertheless, these agents are rarely used for SCD. Glucocorticoid hormones are less well studied, and little is known about their therapeutic potential.

In 1951, Dr. Roland Scott, a legendary figure in SCD clinical research, described his use of cortisone and adrenocorticotropic hormone (ACTH) to treat acute painful episodes in two children with SCD.[42] He showed a temporal correlation between the use of these agents and the abatement of pain. He concluded that this therapy "…seem[ed] to be of definite value in relieving the acute pain and distress associated with the crisis…" Another physician, who was also a father of three children with sickle cell anemia,

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reported a similar observation in 1979. He administered oral dexamethasone at the onset of crisis and noted consistent subsidence of symptoms within 48 hours.[43] He then tapered the dexamethasone over 3 to 7 days. He observed that dexamethasone had "unequivocal and impressive" benefit, and that dexamethasone "…may not only rapidly reverse symptoms but also decrease the number of crises." Although anecdotal, later in 1979, these observations prompted further study by Franklin and Linch. They studied the in vitro effect of dexamethasone on sickle erythrocytes by measurements of oxygen affinity and enumeration of sickled forms.[44] They found no effect of dexamethasone in these assays, but concluded that "corticosteroids might still produce symptomatic improvement [of painful crises] by reducing local inflammation."

In 1994, Griffin and coworkers at U.T. Southwestern Medical Center at Dallas (UT Southwestern) noted the similarity of several clinical features of painful crises to inflammatory reactions and thought that anti-inflammatory glucocorticoids might therefore be beneficial.[45] They conducted a randomized, double-blind, placebo-controlled trial of high-dose intravenous methylprednisolone for children with SCD who were hospitalized for acute, severe pain. They found that methylprednisolone (15 mg/kg given 24 hourly until discharge without tapering) decreased the duration of pain and the length of hospitalization, by about 24 hours, when compared to placebo. There was a reduction of opioid use as well. They reported no complications of this therapy but noted that patients who were treated with methylprednisolone tended to have recurrence of pain (i.e., rebound attacks) after discontinuation of steroids. Also in 1994, de Araújo and colleagues reported a beneficial effect of intravenous hydrocortisone, dipyrone, or both agents for patients with painful crises, as measured by subjective reports of pain.[46]

Based on these initial observations, Bernini et al. at UT Southwestern were motivated to study the use of steroids for ACS. They conducted a prospective, randomized, double-blind, placebo-controlled clinical trial of dexamethasone for children with mild or moderately severe ACS and reported their findings in 1998.[47] Dexamethasone was tested instead of methylprednisolone because of its longer half-life and better tissue penetration—hypothetically improving efficacy and decreasing the likelihood of rebound attacks. Dexamethasone was given as 0.3 mg/kg intravenously (IV) every 12 hours for 4

doses without tapering. Compared to placebo, dexamethasone reduced the length of hospitalization by 33 hours—a 40 % reduction, which was statistically and clinically significant. Moreover, there were significant decreases in the duration of supplemental oxygen, duration of opioid analgesia, need for transfusion, occurrence of clinical deterioration, and persistence of fever (see Table 5.1). No direct adverse effects were attributable to steroids. However, in the 72 hours following discharge, 6 patients were re-admitted to the hospital (4 with pain, 1 with ACS, 1 with stroke) from the dexamethasone group compared to 1 with aplastic crisis in the placebo group. Although the difference in number of re-admissions was not statistically significant, one can infer a "rebound" effect after discontinuation of steroids.

Table 5.1 Bernini et al. (1998): Effect of dexamethasone on ACS in children

	Dexamethasone (n=22)	Placebo (n=21)	P-value
Length of hospitalization (Hr.)	47	80	0.005
Duration of oxygen therapy (Hr.)	30	60	0.004
Duration of opioid therapy (Hr.)	19	76	<0.001
Blood transfusions (No.)	2	10	0.013
Clinical deterioration (No.)	1	14	<0.001
Readmission within 72 hours after discharge (No.)	6	1	0.095
Readmission within 72 hours after discharge with ACS (No.)	1	0	1.000

A follow-up study by Huh et al. at UT Southwestern further tested the efficacy of dexamethasone for ACS, published to date in abstract form only.[48] This prospective, randomized, double-blind, placebo-controlled clinical trial used the same dose and frequency as the Bernini study, but it specified 6 doses (3 days) instead of 4 doses (2 days). This longer duration of therapy was chosen in an attempt to prevent the rebound effect. The trial was terminated early due to slower-than-anticipated accrual, thus the power of the study to detect the specified difference in the primary outcome variable (duration of hospitalization) was decreased to 45% from the calculated 80%. The outcomes are summarized in Table 5.2. In summary, dexamethasone decreased the duration of hypoxemia and possibly decreased the duration of hospitalization, duration of opioid treatment, and the use of transfusions. However, the longer course of

dexamethasone without a taper did not prevent rebound readmissions for vaso-occlusive complications (VOC).

Table 5.2 Huh et al. (2004): Effect of dexamethasone on ACS in children

	Dexamethasone (n=32)	Placebo (n=32)	P-value
Length of hospitalization (Hr.)	60.7	87.0	0.065
Duration of oxygen therapy (Hr.)	17.9	45.5	0.01
Duration of opioid therapy (Hr.)	28.2	54.1	0.09
Blood transfusions (Mean No./episode)	0.47	0.53	0.09
Readmission within 7 days after discharge (No.)	10	2	0.02
Readmission within 7 days after discharge with ACS (No.)	1	0	1.00

It is possible that steroid-induced leukocytosis could contribute to the pathophysiology of ACS or possibly mediate rebound vaso-occlusive episodes. Indeed, there are case reports of VOC associated with leukocytosis induced by colony stimulating factors in patients with SCD. However, ACS itself is often accompanied by leukocytosis, so any specific additional effects of steroids might be difficult to discern. Moreover, corticosteroids may be beneficial in the setting of leukocytosis because of anti-inflammatory and anti-adhesive effects. It is plausible that individuals who have the highest white blood cell (WBC) counts might benefit most from steroids, and that leukocytosis in the absence of steroids might be harmful (such as after steroid therapy is stopped). We examined the effects of dexamethasone on WBC count to determine whether leukocytosis predicted rebound crises in the study of Huh et al. (unpublished data; Figure 5.1). We found that baseline and peak total leukocyte counts did not differ between the dexamethasone and placebo groups, and peak leukocyte count did not predict rebound.

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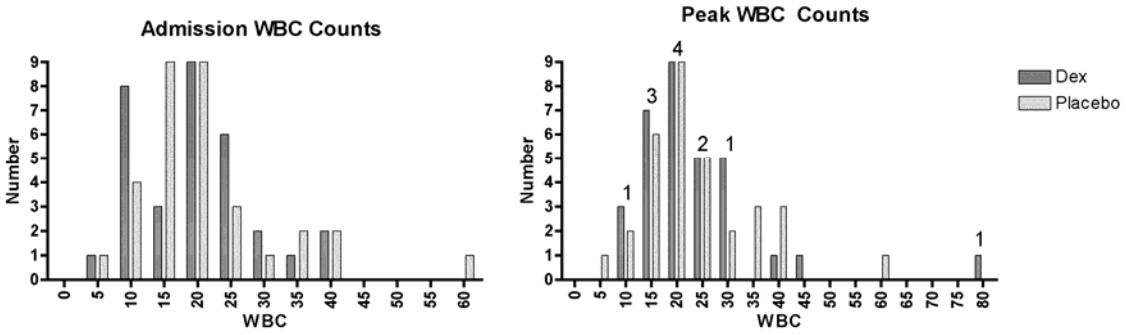
Figure 5.1 Histograms of total WBC counts by study arm.



Numbers above bars indicate the number of subjects who were admitted for "rebound" crisis according to peak WBC count during treatment for ACS. For example, 3 subjects among the 13 (7 dexamethasone + 6 placebo) who had a peak white count in the 15 - 20,000 /mm^3 range were readmitted.

The entire literature of glucocorticoid therapy of SCD is summarized above. However intriguing and promising, clinicians should remain hesitant to use steroids because of real or potential toxicities. Repetitive or prolonged use of steroids can cause avascular necrosis, a condition to which patients with SCD are predisposed, although not all individuals may be at risk. [49,50] Whether a brief course of dexamethasone might contribute to avascular necrosis is not known.[51, 52] There are case reports of steroids precipitating severe vaso-occlusive crises, perhaps by causing leukocytosis, and fat embolism.[53,54] Paradoxically, steroids are also used to prevent and for treatment of fat embolism.[34, 35] Some are also concerned that steroids simply "mask" the signs of symptoms of a crisis and have no direct therapeutic benefit.

Nevertheless, dexamethasone is the first treatment shown to benefit patients with ACS in randomized, placebo-controlled trials. [47, 48] But the generalizability of these trials is limited, given that sample sizes were small, only children were studied, and individuals with severe ACS were few. The rebound effect is also worrisome. In addition, the statistical analysis and interpretation of the results was clouded because the observations were not all independent—some children with multiple episodes of ACS were included more than once in the trial.

Thus, further study of this promising treatment in a broad population is needed to clearly define its benefits and potential toxicities. This study aims to determine whether a tapered regimen of dexamethasone can both be effective and eliminate rebound VOC. The taper will follow 4 doses of the study drug as specified in the Bernini study,[47]

because simply extending the duration of (un-tapered) dexamethasone to 6 doses, as in the Huh study,[48] did not prevent rebound attacks. Both adults and children who have ACS of any severity will be studied, dose modifications for marked leukocytosis will be incorporated, and individuals will participate only once. This study will also measure differences in pulmonary function at a 1-month follow-up visit, and a panel of biomarkers will be obtained at several time points during the course of study drug treatment.

Rationale for Biomarker Assessment in ACS:

Endothelial activation and phenotype. Previous studies have shown that ACS is associated with endothelial activation characterized by a pathologic over-expression of proadhesive molecules, such as VCAM-1, that is not counterbalanced by cytoprotective and anti-adhesive mediators such as nitric oxide (NO).[11] Hypoxia plays a major role in endothelial cell activation and circulating blood cell-endothelial adhesion,[55,56] tissue factor expression enhancing thrombin formation (with concomitant further endothelial activation, increased permeability and heterotypic cell-endothelial interactions[57,58,59]), and the exocytosis of endothelial Weibel-Palade Bodies.[60] Weibel-Palade release of endothelial P-selectin is a crucial modulator of the early phases of polymorphonuclear adhesion to the vascular wall and leukostasis-induced pulmonary injury.[61] P-selectin also facilitates the production of monocyte-derived procoagulant and proadhesive tissue factor-positive microparticles,[62, 63] the binding of sickle red cells to the vascular endothelium [64] and the genesis of a pro-inflammatory phenotype.[65, 66] The endothelial Weibel-Palade bodies under hypoxic conditions also release high molecular weight multimers of von Willebrand factor (vWF), further recruiting platelets to areas of hypoxia-induced vascular damage. Thus, these putative metabolites or soluble plasma biomarkers that we plan to measure during ACS are mainly endothelial derived (VCAM-1, ICAM-1, P-selectin, vWF multimers and NO metabolites) or are functional cell microparticles formed as a result of endothelial activation (endothelial and monocyte microparticles), with whole blood tissue factor an index of both endothelial and monocyte activation. Since ACS is a syndrome of acute pulmonary endothelial injury related to the final common pathway of hypoxia on a background of chronic endothelial activation, the evaluation of these biomarkers that have potent effects on pulmonary

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pathophysiology is appropriate, with precedence for their use in both SCD and other states of endothelial activation and lung injury.[67, 70]

Leukocyte activation. During the early rolling phase of the inflammatory cascade, shedding of L-selectin occurs from the activated polymorphonuclear surface, with elevated levels of plasma sL-selectin noted in some reports of patients with SCD. [75, 76] Since dexamethasone modulates white cell activation, it is also possible that an additional effect of glucocorticoids may be mediated via modulation of polymorphonuclear leucocyte and monocyte activation, with concomitant effects on sL-selectin and whole blood tissue factor expression respectively.

Rebound vaso-occlusion. Glucocorticoids not only attenuate the inflammatory response,[71] but also inhibit the cytokine-induced surface expression of proadhesive endothelial molecules and activate NO synthase.[31,72,73] These may be the mechanisms of the beneficial therapeutic effects of glucocorticoids for ACS, and these effects will be identified in our study. However, the rebound phenomenon is troublesome. The occurrence of rebound VOC suggests a perturbation in homeostatic mechanisms related to adhesion and coagulation. Evidence for such a possibility has been recently provided by Belcher et al. who have demonstrated that in sickle cell transgenic mice expressing human α, β^S and $\beta^{S\text{-Antilles}}$ globins, dexamethasone pretreatment for 3 days inhibited inflammation, leukocyte rolling and vaso-occlusion in a reperfusion injury model (hypoxia-reoxygenation) concomitant with a decrease in endothelial cell nuclear factor kappa B (NF_KB), intercellular adhesion molecule-1 (ICAM-1), and VCAM-1 levels in lungs and skin.[74] VCAM-1 or ICAM-1 blockade with monoclonal antibodies mimicked dexamethasone by inhibiting vaso-occlusion and leukocyte adhesion in the sickle mice, demonstrating that endothelial activation with VCAM-1 and ICAM-1 expression are necessary for hypoxia-induced vaso-occlusion. However, following steroid discontinuation, reperfusion injury in the steroid-treated mice resulted in a dramatic increase in NF_KB activation with increased levels of ICAM-1 and VCAM-1 concomitant with vascular stasis and venular occlusion. These data strongly argue that proadhesive and also perhaps procoagulant molecules (such as tissue factor, which is partially under NF_KB regulation), may increase markedly after steroid withdrawal and possibly mediate

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rebound vaso-occlusion. As such, a tapered regimen is included in this trial of dexamethasone for ACS.

Summary. In this study, serial biomarker assays will delineate whether steroid therapy modulates the endothelial phenotype and whether discontinuation of steroids will be associated with a rebound in markers of endothelial activation. The current study provides a unique opportunity to test this hypothesis in patients with SCD. We will also serially measure $sPLA_2$ activity to determine if therapeutic benefit of dexamethasone may also be, at least in part, related to the inhibition of $sPLA_2$ by dexamethasone. If we are to use this powerful anti-inflammatory agent for ACS or painful crisis, it is crucial that we begin to understand the biomarker profile induced both by steroid administration and its discontinuation. That is, we need to understand the biologic basis of both its beneficial effects and deleterious rebound complications, such that our therapy can be tailored appropriately.

This clinical trial will be conducted in compliance with the protocol, International Conference on Harmonisation (ICH), Good Clinical Practice (GCP) guidelines, and other applicable regulatory requirements.

6 STUDY OBJECTIVES AND PURPOSE

6.1 Primary Objective:

To determine whether dexamethasone decreases the duration of signs and symptoms of ACS. The duration of ACS will be assessed by ACS Assessment (Appendix 3) that incorporate the resolution of tachypnea, hypoxemia, increased work of breathing, and thoracic pain as well as the cessation of medical interventions to support respiration. We predict that dexamethasone will decrease the time to pulmonary recovery.

6.2 Secondary Objectives:

To determine whether dexamethasone reduces morbidity associated with ACS. We predict that dexamethasone will decrease subjects' pain, the requirement for opioid analgesics, the number of transfusions, the use of supplemental oxygen, the duration of hypoxemia, the duration of hospitalization, and the duration of fever.

To determine whether a tapered regimen of dexamethasone prevents rebound VOC. We will study whether a tapered regimen of oral dexamethasone prevents the recurrence of ACS or the development of other VOC soon after ACS. Abrupt discontinuation of a short course of IV corticosteroid may precipitate an early relapse of VOC. As such, we wish to determine whether a longer, tapered course of oral dexamethasone can prevent the relapse of ACS or VOC and readmission to the hospital. We will also document any treatment-related toxicities.

To determine whether dexamethasone modulates the endothelial phenotype to a less proadhesive and procoagulant state, and to determine whether the discontinuation of dexamethasone will be associated with a rebound in the expression of endothelial activation markers of adhesion and coagulation. We predict that the dexamethasone-treated group will demonstrate a decrease (compared to admission values) in plasma levels of $VCAM_1$, $ICAM_1$, P-selectin, whole blood tissue factor (WBTF), tissue factor- (TF-) and $VCAM_1$-positive endothelial microparticles, and ultra-large vWF multimers (vWF/ULVF) at the 48 hour and discharge blood samplings. Inter-group comparisons (placebo vs dexamethasone) will also demonstrate a decrease following dexamethasone when compared to placebo-treated controls. We also predict that the dexamethasone-treated subject group may demonstrate a rebound in plasma levels of $VCAM_1$, $ICAM_1$, P-selectin, vWF, WBTF, and TF and $VCAM_1$-positive endothelial microparticles concomitant with steroid discontinuation.

To determine whether dexamethasone prevents subacute adverse effects of ACS on pulmonary function. Recurrent ACS may cause pulmonary injury and contribute to the development of chronic restrictive lung disease. Decreasing the severity of ACS with dexamethasone might also decrease the severity of late sequelae. We predict that subjects treated with dexamethasone will have better measurements of pulmonary function and more rapid resolution of radiographic pulmonary disease than those treated with placebo.

To determine whether dexamethasone modulates white cell activation and whether the discontinuation of dexamethasone will be associated with a rebound in white cell activation. We predict that plasma L-selectin levels, and monocyte-derived circulating

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TF-positive microparticles will decrease during dexamethasone treatment and possibly rebound following steroid discontinuation.

7 STUDY DESIGN

7.1 Description of Study Design

7.1.1 Description Of Primary And Secondary Endpoints

The *primary endpoint* is the duration of signs and symptoms of ACS or the duration of hospitalization, whichever is less. The duration of signs and symptoms of ACS is defined as the interval between first dose of study drug and the time at which the elements of the ACS assessment are satisfied. The ACS assessment incorporate assessments of tachypnea, hypoxemia, work of breathing, thoracic pain, and medical interventions to support respiration (Appendix3). The elements of the ACS assessment will be formally assessed and recorded every 4 hours until discharge from the hospital.

The *secondary endpoints* include the following:

- Amount and type of opioid used;
- Number and type (simple or exchange) of transfusions received;
- Duration of Supplemental oxygen;
- Duration of hospitalization;
- Duration of fever;
- Duration of hypoxemia;
- Rebound hospitalizations;
- Results of pulmonary function testing (FVC, FEV_1, $FEF_{25-75\%}$, PEFR, and flow-volume loop);
- Pulmonary radiographic findings (resolved, improved, unchanged, and worse); and,

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- Rating of pain [Oucher or numeric rating scale (both are 0-10 scales)].

The *safety endpoints* include the following:

- Vital signs, including blood pressure;
- Physical examination;
- Urine output and daily fluid balance;
- Blood culture (for fever);
- CBC;
- Reticulocyte count;
- Peripheral oxygen saturation by pulse oximetry;
- AE monitoring;
- Baseline history and interim history (including pain history, pain treatment, respiratory symptoms, inpatient or outpatient evaluations, and management of SCD-related complications); and,
- Concomitant medications monitoring.

The *laboratory endpoints* include the following:

- Plasma soluble biomarkers (VCAM-1, ICAM-1, P-selectin, L-selectin and vWF multimers);
- Flow Cytometry for endothelial microparticles ($VCAM_1$ and TF) and monocyte microparticles (TF);
- Nitric Oxide metabolites;
- Whole blood tissue factor (WBTF)
- $SPLA_2$

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7.1.2 Description of Type of Study

This is a multi-center, randomized, double-blind, placebo-controlled Phase III clinical trial that will include at least 56 subjects with SCD who are hospitalized for ACS. Individuals who meet entry criteria will be randomized to receive either dexamethasone or placebo. Dexamethasone will be administered initially as 0.3 mg/kg by mouth every 12 hours for a maximum of 4 doses (2 days) or until discharge from the hospital, whichever occurs first. Afterward, the study drug will be tapered over 6 days for a total duration of therapy not to exceed 8 days. Due to possible age-related and ACS severity-related differences in the duration of signs and symptoms of ACS, randomization will be stratified by age and severity of ACS (see Section 8.3). We are enrolling a maximum of 112 pediatric subjects. We will enroll adult subjects at the rate site participation will allow, which we anticipate will be no more than 56 subjects. Interim analyses will be performed when a total of 56 subjects and 112 subjects have completed their hospital stay. If we do not stop enrollment after these interim analyses are completed, we will continue to enroll up to a maximum total of 112 pediatric subjects. The efficacy and safety of dexamethasone will be compared to placebo in the context of standard supportive care for ACS.

8 SELECTION AND WITHDRAWAL OF SUBJECTS

Subjects will be enrolled by study personnel or the physicians who care for them in the emergency department or the inpatient ward after providing informed consent for participation in the study. **Enrollment and Randomization must occur within 24 hours of the diagnosis of ACS, which is timed from the diagnostic chest radiograph. Enrollment is defined as the date and time of signing the informed consent form.**

8.1 Inclusion Criteria

Subjects who meet all of the following criteria are eligible for enrollment into the study:

1. Age ≥ 5 years;
2. Diagnosis of sickle cell anemia (Hgb SS) or sickle-β^0-thalassemia (Hgb Sβ^0);

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3. Current episode of ACS defined as a new lobar or segmental pulmonary infiltrate seen on a chest radiograph and two or more of the following findings in the 24 hours preceding enrollment (signing consent):
    - temperature $\geq 38.5^{o}C$,
    - tachypnea,
    - dyspnea or increased work of breathing,
    - chest wall pain, and
    - oxygen saturation of < 90% in room air by pulse oximetry;

4. Subjects must have **one** or more of the following findings at the time enrollment:
    - tachypnea,
    - dyspnea or increased work of breathing,
    - chest wall pain, and
    - oxygen saturation of < 90% in room air by pulse oximetry;

5. Current episode of ACS diagnosed within the preceding 24 hours;
6. Ability to take medication in capsule form;
7. Written, informed consent provided by the subject and/or parent(s) or guardian(s) before study entry.

8.2 Exclusion Criteria

Subjects who meet any of the following criteria are disqualified from enrollment in the study:

1. Prior participation in this study;
2. A condition that will likely be exacerbated by corticosteroid therapy, including:
    - diabetes mellitus,
    - hypertension,
    - esophageal or gastrointestinal ulceration or bleeding, or
    - known avascular necrosis;
3. Diagnosis of ACS in the 3 months preceding enrollment;

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4. Treatment with oral or parenteral corticosteroid therapy for any reason within the preceding 14 days;
5. Use for respiratory illness in the preceding 3 months of:
    - oral corticosteroids, or
    - parenteral corticosteroids;
6. A chronic pulmonary condition that requires treatment with corticosteroids;
7. Current participation in a program of chronic transfusions.
    - "Current participation" denotes that the subject's last transfusion was given in the 4 months preceding study entry
    - "Program of chronic transfusions" is defined as a regimen of serial simple or exchange transfusions given at least every 6 weeks for at least 3 consecutive transfusions for the prevention of SCD-related complications.
8. Pregnancy;
9. Treatment with any investigational drug in preceding 90 days.
10. A history of either tuberculosis or a positive skin test for tuberculosis;
11. Known infection with HIV or a current systemic fungal infection.

8.3 Stratification

Before randomization, subjects will be stratified by site, age (< 18 years or ≥ 18 years) and by severity of ACS (defined below). Stratification by age and severity of ACS will be done to ensure balance across treatment groups. Strata will not be adequately sized (i.e., powered) to analyze the primary endpoint in each stratum.

Mild to moderately severe ACS is defined by all the diagnostic criteria for ACS listed in the subject eligibility section in addition to the following:

- transcutaneous oxygen saturation of ≥ 85% in room air, and
- segmental or lobar infiltrates that involve no more than two lobes by chest radiography.

Severe ACS is defined by all the diagnostic criteria for ACS listed in the subject eligibility section and one or more of the following:

- impending respiratory failure or need for invasive or non-invasive ventilatory support,

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- transcutaneous oxygen saturation of < 85% in room air or ≤ 90% despite maximal supplemental oxygen, or
- segmental or lobar infiltrates that involve three or more lobes by chest radiography.

8.4 Subject Discontinuation

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 subjects:

1. New hypertension (not pre-existing) that requires treatment with antihypertensive medications;
2. Stroke;
3. Gastrointestinal hemorrhage; and
4. Pregnancy.

AEs caused by participation in the study may necessitate modifications to a subject's level of participation or discontinuation of subjects from participation in the study. Subjects who discontinue early from the study will be replaced.

Subjects who discontinue prematurely from the study for any reason will be encouraged to maintain the follow-up clinic visits that are scheduled after discharge. Study personnel will contact subjects by phone to encourage attendance.

9 TREATMENT OF SUBJECTS

9.1 Treatments

9.1.1 Description of Study Drug and Dosing Regimen

Dexamethasone is a commercially available synthetic corticosteroid hormone that is used primarily as an anti-inflammatory agent. It is available in tablets (several manufacturers), and as a solution for IV injection (several manufacturers).

Potential AEs, especially when given chronically, include hypertension, hyperphagia, weight gain, hyperglycemia, striae, acne, osteopenia, personality changes, gastrointestinal ulceration and hemorrhage, and growth retardation. Dexamethasone, like other steroids, may cause avascular necrosis of bone.

The dosing of dexamethasone, or an equivalent amount of placebo, is specified in Table 9.1. The initial dose (0.3 mg/kg every 12H) is given for a total of 2 days (4 doses) or until hospital discharge, whichever occurs first. Note: Time Zero is defined as the initial dose of study drug and the first 24 hour period begins with the first dose. All subsequent doses and study assessments are scheduled from this time point. Afterward, all subjects will receive the taper as specified in Table 9.1. All study drug should be given by mouth. If medications temporarily cannot be given by mouth (e.g. NPO status for a diagnostic test or altered mental status) then the temporary administration of the study drug by vein during this time period is acceptable. The dose of IV study drug is the same as the oral formulation. The study drug should be given by mouth as soon as oral medications can be tolerated.

Table 9.1 Dose and Frequency of Study Drug

Dose (mg/kg)	Max single Dose (mg)	Frequency	24hr dose (mg/kg)	Duration (days)	
0.3	12	Every 12H	0.6	2 (or hospital discharge)	"High Dose"
0.3	12	Every 24H	0.3	2	"Taper"
0.2	8	Every 24H	0.2	2	
0.1	4	Every 24H	0.1	2	

9.1.2 Dose Modification or Interruption of Study Drug

If the total leukocyte count exceeds 50,000/mm^3 during the course of "high dose" study drug (0.3 mg/kg every 12 hours), then the taper will be started immediately. That is, if the leukocyte count exceeds 50,000/mm^3, the next dose of study drug (0.3 mg/kg) will be given 24 hours (instead of 12 hours) after the preceding dose of study drug, and the taper should be continued as specified (Table 9.1). If the total leukocyte count exceeds 50,000/mm^3 during the "taper", then the taper will be continued as specified (Table 9.1).

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The doses of study drug must be given no more than 60 minutes early or late from the scheduled time (this does not apply to the first dose of study drug which is given within 2 hours of randomization). A protocol deviation form must be completed in the event a dose is outside this time interval or completely missed, however the subject may continue in the study. Missed doses of study drug will not be made up.

Dose Scheduling Modification during the Taper or home administration of study drug will be as follows:

- The Taper dosing may be modified by +/- 4 hours at Hospital Discharge to accommodate the subject's out-patient schedule. A time for each dose should be ascribed each day using the +/- 4 hour window. Then, based on that day's dose time, if it is more than 8 hours late, omit the dose. If a dose is omitted, still use the ascribed time schedule for the next dose.
- Subjects will be instructed to return all unused study drug at the Follow-up clinic visit. Unused study drug will be returned to the site pharmacist for drug accountability according to each institutions policy.

The doses of the study drug will not otherwise be modified or temporarily interrupted. Study drugs may be stopped according to subject discontinuation guidelines (Section 8.4) or because of a severe AE (Section 11.8).

9.1.3 Packaging, Labeling, and Blinding of Study Drug

All study drug (active and placebo) will be prepared by the central study pharmacist at Children's Medical Center Dallas, Dallas TX, and shipped to participating centers' pharmacies for dispensing. Oral study drug will be available only in capsule formulation (over-encapsulated tablets). Placebo and active study drug capsules will appear identical. Oral study drug will not be available in a liquid preparation because of the inability to produce an identically flavored placebo formulation. IV placebo will be normal saline. The active and placebo IV study drugs will be clear solutions delivered in pre-filled syringes.

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Study drug will be delivered to the inpatient ward in single-dose units for each individual subject and labeled in accordance with guidelines for all inpatient medications. The label will indicate the name of the drug to be either "ACS oral study drug" or, if needed, "ACS IV study drug".

9.1.4 Return and Destruction of Study Drug(s)

Unused inpatient study drug will be returned to the participating centers' hospital pharmacies for disposal according to their standard operating procedures.

9.1.5 Guidelines for Supportive Care

Antimicrobial therapy: All subjects will receive antimicrobial therapy directed against the most commonly isolated organisms in ACS: *Chlamydia pneumoniae*, *Mycoplasma pneumoniae*, *Staphylococcus aureus* (coagulase positive), *Streptococcus pneumoniae*, and *Mycoplasma hominis*. Cefuroxime (50 mg/kg/dose [maximum 1500 mg] IV every 8 hours) and azithromycin (10 mg/kg/dose [maximum 500 mg] by mouth (p.o.) on day 1, then 5 mg/kg/dose [maximum 250 mg] p.o. on days 2 through 5) are recommended antibiotics after a blood culture is obtained. The choice of the antibiotic is at the discretion of the local physician/investigator.

Antimicrobial therapy may be altered appropriately if a specific organism is isolated.

Intravenous fluids: IV fluids will be infused to correct any dehydration and to maintain adequate hydration. D5 0.45 NS with 10 mEq/L potassium chloride will initially be infused at 1 to 1.5 times the calculated maintenance rate (a higher rate may be needed to correct any dehydration) and continued until adequate hydration can be maintained by drinking. The daily maintenance fluid volume is calculated by adding 100 ml/kg/day for the first 10 kg of body weight, 50 ml/kg/day for the second 10 kg of body weight, and 20 ml/kg/day for weight greater than 20 kg. This total daily volume is divided by 24 (hours) to arrive at the hourly infusion rate in ml/hour (the suggested maximum infusion rate is 125 ml/hr). *The rate and composition of the fluid may be modified at the discretion of the attending physician, with the caveat that over-hydration is to be avoided*.[59]

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Respiratory therapy: Supplemental oxygen will be administered to maintain a peripheral oxygen saturation of at least 90% by pulse oximetry. Oxygen will be discontinued when the subject maintains a peripheral oxygen saturation of at least 92%, or a documented baseline value, in room air. Arterial blood gas measurements may be performed at the discretion of the attending physician. All subjects should perform incentive spirometry with 10 breaths every two hours from 8 am to 10 pm and every two hours while awake at night.[77] Young children who are unable to perform incentive spirometry will perform a similar age-appropriate activity, such as blowing bubbles. Chest physiotherapy (CPT) may be prescribed at the discretion of the attending physician. Nebulized bronchodilators may be prescribed at the discretion of the attending physician, and they should be strongly considered for subjects with wheezing or clinical evidence of lower airway obstruction.

Gastrointestinal prophylaxis: All subjects should receive empiric prophylactic treatment with an H_2- (histamine$_2$-) receptor antagonist, such as ranitidine (2-4 mg/kg/day divided BID with a maximum daily dose of 300 mg), for at least the 8 days of study drug treatment. A different H_2-receptor antagonist or a proton pump inhibitor may be used instead of ranitidine at the treating physician's discretion.

Analgesia: Subjects who are in pain will be treated with ibuprofen (10 mg/kg/dose [maximum 600 mg] p.o. every 6h) and if needed acetaminophen/codeine (1 mg/kg/dose of codeine p.o. every 4-6h). The ibuprofen and acetaminophen/codeine may be alternated every 2 hours. If pain is severe, or oral analgesia is not sufficient, morphine (0.1 mg/kg/dose IV every 2h prn) can be given. The use of continuous infusion morphine or other parenteral opioid analgesics requires caution. Dosing and selection of analgesics can be modified at the discretion of the treating physician to safely achieve optimal pain control.

Transfusion: Attending physicians may prescribe transfusion outside of these guidelines if deemed clinically appropriate. All transfused packed red blood cells (PRBCs) will have extended antigen matching for C, D, E, and Kell, except in the case of emergency.

- **Simple transfusion** of PRBCs will be given when the hemoglobin (Hgb) concentration is ≤ 5 g/dL or it has decreased 2 g/dL or more from the subject's baseline Hgb (recorded in the individual's database or medical record). Simple transfusion will also be given when the Hgb concentration has decreased 1 g/dL or more from the baseline Hgb if the subject has hypoxemia requiring supplemental oxygen. The expected post-transfusion Hgb should not exceed 11 g/dL to avoid hyperviscosity and the possibility of worsening the ACS or causing a stroke.
- **Exchange transfusion** is indicated for any of the following: extensive bilateral pulmonary disease, hypoxemia not corrected by supplemental oxygen, or rapid clinical deterioration. Automated red cell exchange (erythrocytapheresis) will be performed unless technical difficulties necessitate a manual exchange. The goal of exchange transfusion is a Hgb of 9 – 10 g/dL and a % Hgb S of 30% or less.

Other: An irritant or osmotic laxative is recommended for all subjects who receive opioids because of the expected constipation. Diphenhydramine or hydroxyzine may be given as needed for pruritus.

9.1.6 Guidelines for Discharge and Follow-up

Discharge: Subjects may be discharged from the hospital at the discretion of the treating attending physician, but the suggested discharge criteria are delineated in Appendix 3.

Medications: Any remaining doses of study medication, as outlined above, will be completed by mouth at home. Subjects will also complete any remaining doses of the 5-day course of azithromycin. Any additional antibiotic therapy can be chosen by the attending physician as deemed necessary for good subject care.

Follow-up: A follow-up appointment in the sickle cell clinic will be made for approximately 1 week after study enrollment and 1 month after discharge. At each visit a standardized interval history (between discharge and the follow-up visit) will be obtained for pain intensity, pain treatment, respiratory symptoms, and any inpatient or outpatient evaluation and management of SCD-related complications.

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9.2 Randomization and Masking

After enrollment, subjects will be randomized to one of two treatment arms. Subjects in Arm 1 will receive oral dexamethasone. Subjects in Arm 2 will receive oral placebo. A computer-generated, adaptive randomization schema will be used to allocate subjects 1:1 between the two treatment arms. Randomization will be stratified by site, age and the severity of ACS (see Section 8.3). In order to increase the likelihood of balance in treatment allocations, subjects will be randomized using the standardized range variation (79) of the sequential allocation algorithm of Pocock and Simon (80), a minimization method. This method attempts to achieve treatment balance on several subject characteristics (i.e. age group, site, and severity of ACS 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 (81). 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, a continuous updating of the information related to previous treatment allocations is required, thus a centralized randomization system will be used.

Pharmacists at each center will be unblinded and will utilize information from the interactive voice/web randomization system (IVRS) at the SDMC to obtain a new treatment assignment for a subject and provide each subject with appropriate dosing of both therapies. The IVRS 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.

The time interval between Randomization and the first dose of study drug administration will be no longer than 2 hours. In the event a subject's first dose occurs outside the 2-hour time limit, the subject will still be included in the study and the analysis. This is in the spirit of the intention-to-treat analysis. The amount of time in excess of the 2-hour limit until the first dose of study drug will also be recorded. This event will be documented as a protocol deviation.

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The subject's physicians, nurses, and ancillary support staff will not be aware of the treatment assignment. The placebo formulations will be identical in appearance and taste to the active compounds (see Section 9.1.3).

9.3 Prior and Concomitant Therapy

No oral or parenteral corticosteroids should have been used in the 14 days preceding enrollment; oral corticosteroids must not have been used in the preceding 3 months; parenteral corticosteroids for respiratory illness must not have been used in the preceding 3 months; and any investigational drug should not have been administered in the preceding 90 days (see exclusion criteria, Section 8.2). There are no other restrictions of prior or concomitant therapy.

9.4 Subject Compliance

Study center personnel or hospital staff nurse caring for the subject will dispense and witness all study drug doses during the hospitalization. Follow-up phone calls to encourage the ingestion of any remaining doses of study drug will be made by the study nurse until the completion of the regimen. The study nurse will also remind and encourage attendance of the follow-up visits during the phone contacts.

10 CLINICAL AND LABORATORY EVALUATIONS

10.1 Efficacy and Safety Assessments

The following efficacy assessments and evaluations will be performed during the study at times outlined in Table 10.2:

- Duration of ACS signs and symptoms (Appendix 3);
- Amount and type of opioid used;
- Number and type (simple or exchange) of transfusions received;
- Duration of hypoxemia;
- Duration of hospitalization;

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- Duration of fever;
- Rebound hospitalizations;
- Results of pulmonary function testing (FVC, FEV_1, $FEF_{25-75\%}$, PEFR, and flow-volume loop);
- Pulmonary radiographic findings (resolved, improved, unchanged, and worse); and,
- Rating of pain [Oucher or numeric rating scale (both are 0-10 scales)].

10.2 Safety Assessments

The following safety assessments and evaluations will be performed during the study, at times outlined in Table 10.2:

- Vital signs, including blood pressure;
- Physical examination;
- Urine output and daily fluid balance;
- Blood culture (for fever);
- CBC;
- Reticulocyte count;
- Peripheral oxygen saturation by pulse oximetry;
- AE monitoring;
- Baseline history and interval history (including pain history, pain treatment, respiratory symptoms, inpatient or outpatient evaluations, and management of SCD-related complications); and,
- Concomitant medications monitoring.

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10.2.1 Investigational Laboratory Studies (Biomarkers)

All subjects will provide blood for the biomarkers studies on the 4 different days specified in Table 10.1. Approximately 8.5 mL of blood will be needed for the set of assays on a given day; thus a total of approximately 34 mL will be needed for the five sets of assays over the 8-day course of study drug. The baseline sample (Day 0) will be drawn at study entry *before study drug is administered*. Subsequent samples will be timed according to study-drug treatment. The Day 1 sample will be drawn 6-12 hours after the 2nd dose of study drug (this must be before the 3rd dose of study drug is administered). The Day 2 sample will be drawn 6-12 hours after 4 doses of study drug (this must be before the 5th dose is administered), which marks the beginning of the steroid taper. If a subject is discharged from the hospital before receiving 4 doses of high-dose (0.3 mg/kg every 12 hours) dexamethasone therapy, then the Day 2 sample will instead be drawn at the time of discharge, which also marks the beginning of the steroid taper for that individual. In this event, the Day 2 sample should be drawn prior to beginning the steroid taper. The Day 8 sample will be obtained after the completion of the steroid taper. For individuals who received fewer than 4 doses of high-dose (0.3 mg/kg every 12 hours) dexamethasone, the Day 8 sample will be obtained after the completion of the steroid taper instead between Days 7-10. The Day 1, 2, and 8 samples should be drawn 6-12 hours after the preceding dose of study drug and prior to the next dose if applicable. Study personnel will record the date and hour the samples are drawn. If the Biomarker samples cannot be obtained within the indicated intervals, the samples should be collected, nevertheless, as close to the time interval as possible. Study personnel will record the date, time, and reason the samples were collected outside the designated interval.

Samples will be processed and shipped from participating centers to the laboratory of Drs. Stuart and Setty (See Appendix 1) or Dr. Kuypers (See Appendix 4).

Table 10.1 Investigational Laboratory Studies (Biomarkers)

Biomarker Assessed	Baseline (Day 0)	Day 1 (6-12 hrs after 2nd dose)	Day 2 (6-12 hrs after 4th dose or discharge)	Day 8 (Day 7-10 or taper end)
Plasma soluble biomarkers (VCAM-1, ICAM-1, P-selectin, L-selectin and vWF multimers).*	X	X	X	X
Flow Cytometry for endothelial microparticles ($VCAM_1$ and TF) and monocyte microparticles (TF).*	X	X	X	X
Nitric Oxide metabolites.*	X	X	X	X
Whole blood tissue factor (WBTF).*	X	X	X	X
$SPLA_2$**	X	X	X	X

*Stuart and Setty Lab

**Kuypers lab

10.3 Evaluations by Study Visit

10.3.1 Baseline Evaluations

A chest radiograph is necessary for the diagnosis of ACS, the medical care of the subject, and study enrollment. Immediately after enrolling a subject, the elements of the ACS assessment will be assessed and the following additional assessments performed or measurements taken: history (which includes the collection of any transfusions within 24 hours of enrollment), physical exam, vitals signs (includes blood pressure monitoring), a CBC, blood culture (for those subjects with fever), a pregnancy test if applicable, pain intensity (by Oucher or numeric rating scale), and peripheral oxygen saturation (by pulse oximetry) [See Table 10.2.] Additionally, a blood sample will be drawn for the central laboratory measurements (see Table 10.1).

10.3.2 Evaluations During Study

The elements of the ACS assessment will be assessed and recorded every 4 hours (Appendix 3). Note: Time Zero is defined as the initial dose of study drug and the first 24 hour period begins with the first dose. All subsequent doses and study assessments

are scheduled from this time point. The first every 4 hour ACS assessment begins as close as possible to 4 hours after the first dose of study drug and the next routine nursing assessment. The studies required on a daily basis include a CBC and a reticulocyte count. Follow-up blood cultures are obtained at the discretion of the attending physician but should be considered every 24 hours if the subject is persistently febrile (temperature ≥38.5ºC). A daily weight, physical exam, and vital signs (every 4 hours which includes blood pressure) will be recorded on the subject's medical record. Subjects will be monitored by continuous pulse oximetry until there has been no requirement for supplemental oxygen for 24 hours and there is clinical improvement. Oxygen saturation (O_2) will be recorded at least every 4 hours with the vital signs until pulse oximetry is stopped. A semi-quantitative, age-appropriate rating of pain intensity [the 0-10 Oucher Scale (age < 10 years) or the 0-10 numeric rating scale (age ≥ 10 years)] will also be obtained by the nurse and recorded with each set of vital signs. A follow-up chest radiograph is not required, but it can be obtained if clinically appropriate. The daily amount and type of opioid analgesia as well as the number and types of transfusions will be recorded. Accurate, cumulative fluid balance measurements (ins and outs) will be recorded every 8 hours on the subject's medical record. AEs will be recorded as they occur throughout hospitalization. The duration of fever, hypoxemia, and hospitalization will also be recorded. Table 10.2 summarizes these evaluations. Furthermore, blood samples will be drawn for the central laboratory measurements at Baseline and Days 1, 2, and 8 (See Table 10.1.).

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Table 10.2 Clinical and Clinical Laboratory Evaluations and Procedures

	Inpatient				Inpatient or Outpatient	
	Baseline	Daily	Inter-mittent	Contin-uous	Follow-up #1(7-10 d) [6]	Follow-up #2 [6]
ACS assessment[1]	X		Every 4h		X	X
History	X				Interval[2]	Interval[2]
Physical exam	X	X				X
Weight	X	X				X
Vital signs	X		Every 4h[5]		X	X
CBC, reticulocyte count	X	X			X	X
Blood culture	X	X[3]				
Chest radiograph	X					X
Pain scale[4]	X		Every 4h		X	X
Concomitant Medications	X	X	X	X	X	X
Opioids used (quantified)		X				
Pulse oximetry (record every 4 hrs)	X			X (q4h)	spot	spot
Fluid balance (ins and outs)			Every 8h			
Pregnancy test (if applicable)	X					
Study drug administration			Every 12-24h			
Occurrence of AEs or SAEs				X	X	X
Number of transfusions	X	X			X	X
Pulmonary function testing						X
Compliance					X	X

[1]Appendix 3.
[2]Includes pain history, pain treatment, respiratory symptoms, inpatient or outpatient evaluations and management of SCD-related complications (rebound VOC).
[3]Only if temperature ≥38.5ºC or at discretion of attending physician
[4]Oucher Scale (age <10 years) or numeric rating scale (age ≥10 years)
[5] Vital signs recorded every 4 h and I/Os every 8 h on subject's medical record. Study personnel will collect the maximum values of the subject's vital signs every 24h.
[6]Follow-up #1 is after completion of study drug between days 7-10. Follow-up #2 is thirty days (+/- 3 days) after discharge from hospital and no more than 60 days after study enrollment

10.3.3 Follow-up Studies

The required measurements and studies at the Follow-up clinic visits are an ACS assessment, CBC, reticulocyte count, oxygen saturation by pulse oximetry, vital signs (includes blood pressure monitoring), and a pain assessment. At each visit, a standardized interval history for pain intensity, pain treatment, and respiratory symptoms will be obtained, as well as any inpatient or outpatient evaluation and management of SCD-related complications which includes the collection of any transfusions the subject received, and an assessment of compliance with the home medications will be completed. AEs occurring after discharge will be captured, especially events requiring hospitalization. **Any additional hospitalizations in the 1 week after discharge for**

VOC will be counted as "rebound VOC" hospitalizations. Additionally, the following will be obtained at the 1-month follow-up only: physical examination, a chest radiograph (PA and lateral views) and pulmonary function testing (PFT) without methacholine challenge or bronchodilators. PFTs will include measurement of forced vital capacity (FVC), forced expiratory volume in 1 second (FEV_1), average forced expiratory flow between 25 and 75% of expired vital capacity ($FEF_{25-75\%}$), and peak expiratory flow rate (PEFR). Also, the flow-volume loop will be recorded and the FEV_1/FVC will be calculated.

11 ADVERSE EVENTS

11.1 Safety Assessment Overview

Subjects will be evaluated every day while hospitalized. Subsequently, subjects will have 2 scheduled evaluations approximately 1 week after study enrollment and 1 month after discharge. At these follow-up visits, subjects and their parent(s) will be queried regarding recent medical events and/or procedures. Specific events will be documented to ascertain the nature and treatment of the event, including pain crises, episodes of acute chest syndrome, transfusions, 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 case report form (CRF).

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 administered a pharmaceutical product 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 the medicinal (investigational) product. AE data are recorded on the CRF.

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.

11.2.1 Adverse Events related to Increased Blood Pressure.

All site Principal Investigators are required to review blood pressure results at study enrollment, once daily during hospitalization, and at both follow-up clinic visits. The following blood pressure results must be reported as Adverse Events:

- A single blood pressure in which the systolic pressure is ≥140 mmHg ***and*** the diastolic pressure is ≥90 mmHg;
- a systolic pressure ≥140 mmHg on 2 more occasions in a rolling 24 hour period regardless of diastolic pressure;
- a diastolic pressure ≥90 mmHg on 2 more occasions in a rolling 24 hour period regardless of systolic pressure.

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The AE form will allow commentary about suspected causes of the hypertension (e.g., crying or severe pain). Additionally, the Serious Adverse Event criteria will be reviewed to determine if any of the AE's related to increased blood pressure meet these definitions and will be reported accordingly.

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 inpatient 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.

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11.4 Assessment of Adverse Event Severity and Relationship to Treatment

The following scale will be used to "grade" the severity of all AEs.

Table 11.1 Severity Scale for AEs

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 Table 11.2. The category that overall best "fits" the relationship between the AE 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. • 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.

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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 11.3 List of Adverse Events Related to Sickle Cell Disease

Acne	Fluid Retention	Pain, long bone
Acute chest syndrome	Gastric Ulceration	Pain, severe abdominal
Anemia	Gastritis	Pancreatitis
Aplastic crisis	Glucosuria	Priapism
Aplastic crisis/anemia	Hand-foot syndrome	Psychoses
Arthralgia	Hematuria	Pulmonary thromboembolism (age ≥18 yr only)
Avascular Necrosis	Hemiplegia	Pulmonary hypertension
Avascular necrosis of hip/shoulder	Hemolysis	Pulmonary parenchymal infiltrates on chest x-ray
Avascular necrosis of the femoral head	Hepatosplenomegaly	Pyelonephritis
Bone infarction	Hyperglycemia	Renal failure
Cardiomegaly	Hyperplastic bone marrow	Renal insufficiency/albuminuria
Cerebrovascular accident	Hypertension	Renal papillary necrosis
Cholecystitis, hepatic sequestration	Hyposthenuria	Reticulocytosis (∃10%–20%)
Cranial nerve palsy	Hypoxemia (PO2 < 65mm Hg)	Retinal disease
Decreased kidney function	Increased appetite	Retinal hemorrhage
Decreased lung function	Infection, pneumococcal	Rash
Delayed growth/puberty	Insomnia	Rhabdomyolysis
Delayed wound healing	Jaundice	Sepsis
Depressed ESR	Leukocytosis	Skin ulcers
Depression	Meningitis	Splenic sequestration
Dyspepsia	Mood Changes	Striae
Elevated urinary urobilinogen	Nausea and vomiting	Vaso-occlusive crisis
Fever	Pain, joint	Weight Gain
		Pulmonary parenchymal infiltrates on chest x-ray

An unexpected AE is an adverse reaction, the nature or severity of which is not consistent with the Dexamethasone label. Cases in which the AE is more severe than currently described in the Investigator's Brochure are also considered "unexpected."

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

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a fatal outcome, cause of death and a comment on its possible relationship to the suspected reaction should be provided.

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, and
- 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 AE 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

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 unexpected SAEs that are made within 7 or 15 calendar days, as specified in subsequent paragraphs.

- Reports of quarterly statistical analyses of all SAEs. 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 AEs and of adverse clinical laboratory trends. These reports will highlight any safety issues revealed by the analyses that meet the "alert" criteria.

11.6.1 Reporting of Serious Adverse Events

SAE 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 day of the realization that an SAE has occurred to a study subject, a study investigator must make an initial report 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 (e.g., 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 Medical Monitor, and the SDMC SAE Regulatory Specialist will collaborate to prepare a report of the SAE using the current version of the FDA's SAE reporting forms. A fatal or life-threatening unexpected SAE will be reported to the DSMB within 7 calendar days of the receipt of the initial report by the SDMC SAE Regulatory Specialist. A non-fatal, non-life-threatening, unexpected SAE will be reported to the DSMB 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. Each study investigator will submit the SAE report to

the local institutional review board (IRB) and other local authorities in accordance with the institution's regulations.

All SAEs, regardless of expected status, are also recorded in the AE section of the study's CRF.

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

11.6.3 Safety Alerts

Three 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 AE data, including MedDRA codes.
- Create frequency tables of treatment x occurrence (yes or no, since inception of the study) of all subjects. One table will be created for each highest level MedDRA term for which SAEs have been reported. 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 11.4 or if the relative risk is less than 1.

11.6.4 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 MedDRA dictionary to code all AEs that have been recorded on study AE forms.
- Make a "snapshot" copy of the AE data, including MedDRA codes.

- Create frequency tables of treatment x occurrence (yes or no, since inception of the study) of all subjects. One table will be created for each highest-level MedDRA term for which AEs have been reported. The counting units are subjects, not events.
- Compute Fisher's Exact Test (FET) statistic to test the alternative hypothesis that occurrence of AEs 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 alert the DSMB to 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.

11.6.5 Reporting of Adverse Clinical Laboratory Trends

An *adverse clinical laboratory trend* is a shift, in an adverse direction, 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 or a higher proportion of subjects with an abnormal reading 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 data for each clinical laboratory evaluation obtained in this study.
- Perform an appropriate statistical test. If the hypothesis test p-value is less than the critical value shown in Table 11.4, 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.

11.6.6 Reporting of Vaso-Occlusive Crisis Rebounds

Three months after the first subject is enrolled in the study, and at the end of each 3-month period thereafter, the SDMC will:

- Make a "snapshot" copy of the rebound data.
- Create frequency tables of treatment x occurrence of rebound (yes or no, since inception of the study) of all subjects. The counting units are subjects, not rebound events.
- Compute Fisher's Exact Test (FET) statistic to test the alternative hypothesis that occurrence of rebound is not independent of treatment group. The FET p-value is not adjusted for multiplicity.
- If the FET p-value is less than 0.025 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 rebounds if the FET p-value is less than 0.025 or if the relative risk is less than 1. A more detailed description characterizing the probability of triggering an alert under different scenarios of rebound rates is found in Appendix 2.

Table 11.4 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"
Unexpected SAEs	Site investigator notifies SDMC SAE Regulatory Specialist and PI within 8 hours. Site investigator, SDMC SAE Regulatory Specialist and PI prepare report using FDA forms and submit report to DSMB, NHLBI Project Officer, IRBs, study investigators. Report: Fatal or life-threatening: within 7 calendar days. 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 AE(s)

The following criteria will be used to determine whether or not subjects exhibit toxicities of the study drug(s) sufficient to require discontinuation from the study.

We will closely monitor signs, symptoms, and laboratory findings to assess for unexpected toxicities. Oral dexamethasone will be discontinued in any subject who experiences the following:

1. New hypertension (not pre-existing) that requires treatment with antihypertensive medications;
2. Stroke;

3. Gastrointestinal hemorrhage; or
4. Pregnancy.

In addition, subjects will also have dexamethasone discontinued if they become unable to orally ingest dexamethasone or, at the request of the subject, for any reason. In the event that dexamethasone is stopped, subjects will continue to be followed by study personnel to assess for potential side effects of dexamethasone administration.

11.8 Pregnancy Reporting

Because of potential adverse effects on the fetus, pregnant subjects will be excluded from the study. Female subjects who are pubertal will be regularly assessed throughout the study. In the event of a test indicating the study subject is pregnant the subject will be informed of this result and will immediately have dexamethasone discontinued.

11.9 Clinical and Safety Monitoring

11.9.1 Subject Safety Monitoring

The DSMB (SMB, ISM) will be appointed by, and responsible to the NHLBI.

The DSMB will review a summary of the safety data for the study 6 months after the first subject is enrolled and every 6 months after that. 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.

11.9.2 Clinical Monitoring Plan

A study coordinator will be appointed to assess the overall enrollment and compliance with the clinical protocol at all participating centers. Sites will be visited by a trained clinical monitor once every year for the duration of the trial. The monitoring will focus on data quality and provide feedback to the sites on how well they are capturing and entering data.

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 CRF for each subject. A CRF manual will be provided to each site to assist in correct CRF completion. CRF data must be currently maintained and up-to-date.

Study participants must not be identified by name on any study documents. Subjects will be assigned and identified by CSCC subject numbers assigned at enrollment.

The specifics of source documentation maintenance will be detailed in the protocol manual of procedures.

12.2 Data Management

RhoFED's internet-based remote data entry system will be used to capture the data for this study. Using this system, clinical site personnel use an internet browser (Internet Explorer or similar) to key data into electronic CRF. The screens will be accessible via the CSCC website and require center-specific user ID/password privileges. Univariate data validation tests are performed as the data are keyed, and most implausible data values are resolved immediately. Data are not stored on the site's computer. At the end of each "page," data are submitted to RhoFED's secure web server using SSL (128 byte public key encryption methodology) and stored in the study's "operational database." (The database used for capturing, validating, updating, and storing the data is called an "operational database.") The database is backed up nightly; backup tapes are saved in a secure, off-site location. Authorized site personnel may log in to the system, review and correct previously entered data, key additional data, or lock records to prevent further inadvertent modifications at any time.

AEs will be coded at RhoFED using the MedDRA dictionary. AE codes will be entered into the operational database.

Approximately 1 month prior to each DSMB meeting RhoFED will take a "snapshot" of the operational database, create analysis datasets, perform statistical analyses, and prepare a DSMB report.

Upon completion of processing, the operational database is subjected to database closure procedures and subsequently locked.

After the operational database is locked, RhoFED's programming team will create and validate an analysis database. The analysis database will be the basis for statistical analyses of the data.

12.3 Staff Training

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.

12.4 Data Monitoring

After central training, individual sites will monitor CRFs and source documents for accuracy, protocol compliance, subject safety, and adherence to guidelines.

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

13 STATISTICAL ANALYSIS

Analyses will be prospectively described in a Statistical Analysis Plan (SAP). Additional statistical procedures may be used if necessary (e.g., data transformations). In general, data will be summarized by treatment and age group using univariate statistics (e.g., N, mean, standard deviation, median, minimum, and maximum) or frequency (e.g., N, percentage), as appropriate.

13.1 Analysis Populations

The general population will consist of hospitalized subjects 5 years of age and older with a diagnosis of SCD, who have experienced a current episode of ACS and who have provided a written informed consent. 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 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 had no major protocol violations and who were followed until discharge from 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

Descriptive summaries will be generated to describe the disposition of all enrolled subjects. Descriptive summaries will also be generated for all relevant baseline variables

by age strata, severity strata, and treatment group. These variables include, but are not limited to, demographic data, medical history, and study populations.

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. Center will be included in the models as permitted by sample size. Center will be eliminated from the models if it appears to be an insignificant variable in the model.

The primary analysis will compare the duration of ACS signs and symptoms (as assessed by the ACS assessment, Appendix3) or the duration of hospitalization for ACS, whichever happens first, of subjects in the dexamethasone treated arm to those in the placebo treated arm. The symptom/hospitalization duration for subjects with ACS will be summarized by treatment group, severity strata, and age strata. The effect of treatment on symptom/hospitalization duration will be tested using a Generalized Linear Mixed Model (GLMM) with the natural log of symptom/hospitalization duration as the outcome variable. The SAS® procedure MIXED will be used to fit the GLMM. The GLMM will include treatment, age group, and severity as fixed effects. The GLMM will not specify any variables as random effects or repeated effects, but it will use the GROUP option in the REPEATED statement to estimate separate error variances for each treatment (i.e., GROUP = "treatment"). The SAS® procedure MIXED will be used to fit the GLMM.

The GLMM model specified above is necessary for the primary analysis because pilot data give evidence that the length of stay (LOS) in the hospital (which is assumed to be similar to duration of symptoms) for ACS has a lognormal distribution. If the LOS for ACS is distributed as lognormal in each treatment group, then the model of the natural log of LOS must allow for differences in treatment group variances as well as for treatment group mean differences. By allowing for differences in treatment group variances and treatment group means, the treatment group specific distributions can be correctly estimated under the lognormal assumption, resulting in a correct comparison of treatment group means.

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As a secondary analysis, parametric survival analysis techniques will be used to estimate distributional parameters and model the hazard function of symptom duration/LOS/ hypoxemia/fever for each treatment group. The time to the event of interest (e.g., symptom duration) will be described using a Kaplan-Meier plot stratified by treatment.

The effects of dexamethasone on secondary clinical endpoints of duration of hospitalization, supplemental oxygen therapy (or hypoxemia), and fever will be summarized by treatment group, severity strata, and age strata and evaluated by using a similar GLMM as the primary analysis. The secondary clinical endpoints of the amount of opioid usage, the number/type of transfusions, pulmonary function and pulmonary radiograph findings, the number of rebound hospitalizations, and pain scores, will be summarized by treatment group, severity strata, and age strata. The effects of dexamethasone treatment on these outcome variables will be evaluated using:

- GLMM with treatment, age group, severity, and timepoint as fixed effects and subject as a random effect for the longitudinal pain scores;
- Wilcoxon Rank test for rebound hospitalizations and number of transfusions;
- T-Test (or Wilcoxon Rank test if assumptions are not met) for the amount of opioid usage and all pulmonary function outcomes;
- Cochran-Mantel-Haenszel Chi-square test for radiographic findings (resolved, improved, unchanged, worse).

The secondary endpoints of the laboratory variables ($VCAM_1$, $ICAM_1$, P-selectin, L-selectin, vWF multimers, endothelial and monocyte microparticles, WBTF, NO, and $sPLA_2$) will be summarized by measurement timepoint, treatment group, severity strata, and age strata. The overall change from baseline for each of the collected measurements over time will be modeled using a random effects longitudinal mixed model, with treatment, baseline measurement, age group, and severity as fixed effects and subject as a random effect. This class of model allows us to control for the correlation induced by having multiple measurements on an individual. Estimates of the treatment difference over time will be depicted by displaying the estimated means of each treatment group over time. Descriptive p-values for comparisons of interest will also be produced.

All meaningful subject data collected in the CRFs will be provided in data listings. All statistical analyses will be performed using the SAS® System.

13.2.3 Safety Analyses

Safety analyses will be performed on all randomized and dosed subjects (i.e., the ITT population). An analysis of safety will include a summary of AEs, SAEs, vital signs, clinical laboratory tests (CBC and reticulocyte count), physical examination, urine output and daily fluid balance, blood culture, peripheral oxygen saturation, and baseline and interim history. All safety outcomes will be summarized by treatment, age strata, and measurement time point (if appropriate).

Treatment-emergent AEs, drug-related AEs, SAEs, and drug-related SAEs (as determined by the investigator) will be classified using the Medical Dictionary for Regulatory Activities (MedDRA, Version 6.0 or later) preferred terms. A treatment-emergent AE is one that started or worsened in severity after initial administration of study drug. All treatment-emergent AEs and SAEs will be classified with regard to severity and duration of the event. These data will be summarized by treatment group, age strata, body system, and preferred terms within a body system. AE comparisons across treatment groups, stratifying on age group, will be made using a Chi-square test on any AEs that occur in more than 10% of the subjects. Each occurrence of an AE (classified on the basis of MedDRA terminology) will be reported separately for a given subject.

13.2.4 Interim Analyses

Two formal interim analyses will be performed on the primary efficacy variable. The first interim analysis will take place when 56 subjects have resolved signs and symptoms of ACS *or* have been discharged from the hospital. To test for differences between the treatment arms, the same F-test from the GLMM specified in the primary analysis will be used with a Peto stopping boundary of $p<0.001$.[78] The study will be stopped if significant efficacy is identified. The second interim analysis will be performed when 112 subjects have resolved signs and symptoms of ACS *or* have been discharged from the hospital. The primary efficacy hypothesis will be tested at the $\alpha = 0.01$ level on the total subjects,

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and the 0.05 level for the children. If significant efficacy will be identified for total subjects and for children, the study will be stopped. Otherwise, the study will continue enrollment to 112 children, after which the primary efficacy hypothesis will be tested on the $\alpha = 0.039$ level. By utilizing these alpha levels for each test and a step-down procedure for the pediatric sub-population, the overall study alpha level for the test of the primary hypothesis remains at the 0.05 level.

In order to ensure that future subjects are not exposed to study drug unnecessarily, the DSMB will also be provided with the conditional probability of completing a successful trial at the interim analyses under three scenarios: (1) the treatment difference continues to be the same as in the first portion of the trial; (2) the treatment difference is zero for the next portion of the trial; (3) the treatment difference is 30 hours for the next portion of the trial. Based on this information, the DSMB can make an informed decision about the futility of the trial.

13.3 Statistical Considerations

13.3.1 Covariates

Age group and severity group will be used as covariates in the primary analysis. Baseline laboratory measurements will be controlled for when analyzing laboratory outcomes. Center will be included in the models as permitted by sample size. Other covariates may be considered in the analysis of clinical, laboratory, and safety outcomes as needed.

13.3.2 Multi-center Studies

Randomization will be stratified by severity group, center and age group to preserve a balance across treatment groups. Center effects will be examined as permitted by sample size.

13.3.3 Multiple Comparisons and Multiplicity

One primary hypothesis will be tested and other reported p-values will be considered descriptive. Hence, no multiple comparisons adjustments will be made.

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13.3.4 Examination of Subgroups

Analyses may be stratified by severity of ACS strata (see Section 8.3). If warranted, exploratory analyses may examine the treatment effect in severity of ACS subgroups.

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. The validity of the primary analysis will depend on the assumed distribution and minimal censoring. If a higher number of deaths or withdrawals prior to discharge occur than expected, the secondary survival analysis will provide an important sensitivity analysis. All longitudinal analyses (e.g., pain scores and laboratory measurements) will utilize models that do not require eliminating the subject for intermittent missing data.

13.4 Sample Size

At least 56 subjects will be enrolled into this trial. Subjects will be randomized to one of two treatment groups: placebo or dexamethasone.

The measurement of primary interest is the duration of ACS signs and symptoms/hospitalization. Previous studies using dexamethasone to treat ACS reported hospital length of stay (LOS) instead of duration of symptoms. Since subjects are generally not discharged until their symptoms are resolved and are discharged relatively quickly after the resolution of symptoms, the LOS is also a surrogate endpoint for the purposes of calculating an appropriate sample size for the duration of symptoms. It is anticipated that LOS will be more variable than duration of symptoms due to the impact of administrative considerations (e.g. subjects not being discharged in the middle of the night). Therefore, we expect that any sample size calculations based on LOS data will be conservative for the endpoint of symptom duration, helping ensure that the study will examine enough subjects to test the primary hypothesis.

The raw data from the follow-up study by Huh et al (2004), as described in Section 5, and the published statistics from the Bernini et al (1998) study were available to use for

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sample size calculations. The average LOS for ACS in children together with the standard deviation as observed in the two pilot studies are presented in Table 13.1.

Table 13.1 Effect of dexamethasone on duration of hospitalization for ACS in children

	Bernini et al, (1998) Duration of hospitalization (hrs)	Huh et al (2004) Duration of hospitalization (hrs)
Placebo	80 (SD=50)	87 (SD=70)
Dexamethasone	47 (SD=16)	60 (SD=37)

Based on these preliminary findings, we anticipate that dexamethasone will decrease the LOS by 30 hours (compared to 33 hours for Bernini and 27 hours for Huh) and we will therefore power our tests to detect a 30-hour difference. The data from Huh et al (2004)[48] gives evidence that the LOS for ACS may have a lognormal distribution, but the clinical hypothesis makes a statement about differences in mean of LOS not the natural log of LOS. Assuming the LOS in each treatment group is distributed as lognormal, normal distribution based statistical analysis methodologies can be used for analysis of the natural log of LOS. With the lognormal assumption, the mean LOS for each treatment group is a function of both the mean and variance of the natural log of LOS, so the following sample size calculations had to consider possible lognormal distributions and the mean difference on the untransformed scale.

Table 13.2 presents the lognormal transformed results of Table 13.1, which we used in the sample size calculations.

Table 13.2 Effect of dexamethasone on natural log of the duration of hospitalization for ACS in children

	Bernini et al, (1998) Ln(Duration of hospitalization)	Huh et al (2004) Ln(Duration of hospitalization)
Placebo	4.217 (SD=0.574)	4.216 (SD=0.707)
Dexamethasone	3.795 (SD=0.331)	3.954 (SD=0.560)

The hypothesis tested is: shortened duration of ACS signs and symptoms/hospitalization by at least 30 hours for children with SCD who are hospitalized for the treatment of ACS and receive dexamethasone. Using LOS as a surrogate, we used a two group Satterthwaite t-test of equal means (unequal variances, equal n's) to test the difference in natural log of the hospitalization duration between treatment groups. We additionally

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assumed a two-sided alpha of 0.05 and 90% power. Several scenarios were explored in which we varied the lognormal variance parameter and lognormal mean parameter for the two treatment groups according to the ranges found in the two pilot studies.

If we assume that

- we are testing a two-sided hypothesis,
- the treatment effects seen in children are the same in adults,
- the lognormal population standard deviation for the placebo group is 0.6,
- the lognormal population standard deviation of the dexamethasone group is 0.3,
- the lognormal mean of the placebo group is 4.25,
- the lognormal mean of the dexamethasone group 3.95, (corresponding to a mean difference in LOS between the two treatment groups of 30 hours),
- the Type I error rate is 0.05,
- and the power is 90%,

then the necessary amount of subjects needed per treatment group is 54. We do not anticipate many dropouts prior to the assessment of the primary endpoint because the population should remain in the hospital under observation until that time. However, we will add two more subjects to each treatment arm to compensate for potential withdrawals, so the total number of subjects needed per treatment group is 56.

We have also explored alternative scenarios and concluded that 56 subjects per treatment group is sufficient under the following conditions:

- the lognormal population standard deviation for the placebo group ≤ 0.6 (and thus follows the Bernini[47] study), and
- the lognormal population standard deviation of the dexamethasone group ≤ 0.5 (and thus is in the range of both the Bernini and Huh study), and
- the lognormal mean of the placebo group is 4.0, 4.1, 4.2, or 4.3, and

- the lognormal mean of the dexamethasone group is 3.6, 3.7, 3.8, 3.9, or 4.0, and
- the mean difference in LOS between the two treatment groups is ≥ 30 hours.

If we wish to vary assumptions, we would see differences in the number of subjects needed.

14 HUMAN SUBJECTS PROTECTION

14.1 Discontinuation of Study

NHLBI reserves the right to discontinue the study at any time for administrative reasons. Safety will be monitored by the NHLBI-designated DSMB, which can recommend suspension or termination of the trial for safety reasons at any time. The DSMB will receive expedited reports for deaths or unexpected SAEs and regular summaries as described in Section 10. Imbalances in the numbers of SAEs and AEs between treatment groups are monitored regularly and reported to the DSMB as well. Subjects can discontinue for any reason at any time during the trial, with no consequences to the quality of their treatment. Investigators will be reimbursed for reasonable expenses incurred to the date of discontinuation on the basis of completed subjects.

14.2 Ethics

14.2.1 Good Clinical Practice and IRB Review

Compliance with GCP guidelines for the conduct and monitoring of this clinical trial will occur through observation of the ethical and regulatory requirements presented in ICH E6, *Good Clinical Practice: Consolidated Guideline*. By signing this protocol, the investigator agrees to adhere to these requirements. The study (protocol, informed consent, advertisements, Clinical Investigator Brochure, subject information sheets, Investigator CV and credentials) should be reviewed and approved by the IRB or ethics committee. Changes to the protocol will be initiated by the NHLBI and approved by the IRB. Subjects must sign written informed consent prior to being screened and before undergoing any study procedures.

The investigators and institutions affiliated with this study will permit trial-related monitoring, audits, IRB/IEC review, and regulatory inspection(s) by providing direct access to source documents.

14.2.2 Informed Consent

Informed consent is an ongoing process that includes the signing of an informed consent document. Subjects are required to sign an informed consent prior to being screened and before undergoing any study procedures or assessments, in accordance with ICH E6; 4.8, "Informed Consent of Trial Subjects." When substantial modifications are made to the informed consent form, the DSMB or IRB may require all subjects currently enrolled in the study to be re-consented; ICH E6; 4.8 guidelines would still apply. A sample informed consent form will be provided to the sites for modification to comply with the requirements of their local IRB. The informed consent form will adhere to the guidelines in ICH E6; specifically, it will contain the elements as specified in Section 4.8.10 of that guideline. The CSCC Regulatory Specialist and NHLBI will review substantive changes suggested by the IRB to assure that all participants are adequately informed prior to participating in the study.

The site should follow the sample informed consent as closely as possible. The informed consent can be modified to include IRB required language; the template language should not be deleted. The SDMC and NHLBI will review each site's informed consent to ensure that all of the key issues are appropriately covered in each site's informed consent.

Subjects will be provided with a copy of the informed consent and printed materials that explain the purpose of the study, the medication(s) used in the study, procedures, and assessments. Subjects will also be provided with the telephone numbers of the investigator and qualified personnel who can assist with their questions and concerns.

14.2.3 Confidentiality

Subject confidentiality will be maintained by the investigator, the investigator's associates and co-workers, and by all administrators who are part of the CSCC project. Confidentiality will be maintained according to ICH E6; 4.8.10, Part O: "Records

identifying the subject will be kept confidential and, to the extent permitted by the applicable laws and/or regulations, will not be made publicly available. If the results of the trial are published, the subject's identity will remain confidential."

14.3 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.

14.4 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 as outlined by the CSCC-CTC and publication guidelines.

15 SUBJECT COMPENSATION

Subjects will be paid $25 dollars at the time of the first follow-up visit to reimburse them for their time and for parking fees. Subjects will be paid $50 dollars at the time of the second follow-up visit to reimburse them for the additional time this visit requires to complete all of the study procedures as well as their parking fees.

16 PROTOCOL SIGNATURE PAGE

I, [Insert name of Site Investigator here], agree to conduct:

"Randomized Trial of Oral Dexamethasone for Acute Chest Syndrome".

I understand that no deviations from this protocol, version 7.0, dated 01/29/2008, 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.

Signature: _____

Date: _____

17 LIST OF INVESTIGATOR (S) AND RESEARCH LABORATORY (S)

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19 APPENDIX 1

Biomarker Assays (Laboratory of Drs. Stuart and Setty)

Subject samples will be identified by their unique CSCC subject identifier, specimen number, and date of specimen collection. Subject names will not be used.

Biomarkers of Endothelial and White Cell Activation, and Red Cell Adhesion include:

1. Plasma sVCAM-1, sICAM-1, sP-selectin, sL-selectin and vWF panel.
2. Total circulating microparticles, endothelial-derived microparticles (VCAM-1 and TF-positive) and monocyte-derived microparticles (TF-positive).
3. Whole Blood Tissue Factor (WBTF).
4. Nitric Oxide metabolites.

Assays and Quality Control

1). Measurements of sVCAM-1, sICAM-1, sP-selectin and sL-selectin will be measured using commercially available ELISA Kits (R&D Systems, Minneapolis, MN). While these assays are not routinely performed in a hospital lab, our laboratory has published values for normal controls and determined intra-assay variation[1,2]. General quality control measures will include pipette calibrations to meet standard laboratory guidelines with logs kept on these calibrations. Samples will be batched and run, with appropriate standard curves performed on day of sample measurement ($r \geq 0.99$).

2). Total, endothelial-derived and monocyte-derived circulating microparticles will be assessed as previously described with a microbead standard deviation for inter-assay and intra-assay variability of 10% and 4% respectively3. All general flow cytometry procedures will be followed, including routine daily, weekly and monthly maintenance. The FACS and ultracentrifuge will be maintained via standing service contracts. When not in use, all antibodies and reagents will be refrigerated with stock reagents frozen according to manufacturers' recommendations. All runs will be done by technicians (2) familiar with the requirements for gating and assessing microparticles. Hard copies of all

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data will be printed daily and raw data stored on the hard drive of the FACS, as well as being backed up on CD-ROM in secure files.

3). Whole blood tissue factor (WBTF) will be assayed as previously described[4]. Samples will be batched and run, with appropriate standard curves using relipidated human brain TF (1pg of standard = 1u of TF PCA) versus clotting time (($r \geq 0.99$). Intra-assay variability was 5.7% for pooled normal samples. 4 normal and 4 abnormal samples will be aliquoted and saved, and 1 normal and abnormal sample aliquot will be assayed in each batched run over the 36-month period of the study to double-check TF standardization. General quality control measures will include pipette calibration with logs kept on these calibrations.

4). Measurement of NO metabolites (nitrate and nitrite) with an intra-assay variability of 6% will be performed as we have previously described[1]. Standard curves from 10 to 1000 pmol will be performed during each batched assay ($r \geq 0.99$). General quality control measures will include pipette calibrations to meet standard laboratory guidelines, and reagent testing monthly.

Blood Sample Preparation Requirements (total of 7.5ml per blood draw)

TUBE A:

1. **First 4.5ml** from a free-flowing blood draw will be transferred into a **blue-top tube (3.2% Sodium citrate).** After 30 minutes at room temperature centrifuge at 2500g for 15 minutes, divide supernatant into 2 thick-walled plastic microfuge tubes and freeze at -70°C. Batch and send **every 2 weeks on dry ice** to Biomarker Laboratory.

TUBE B:

2. **Next 2.5ml** will be placed into a **green-top pediatrics heparin tube** and gently shaken. Spin at room temperature within 60 minutes at 1500g for 10 minutes. Place supernatant in a thick-walled plastic microfuge tube and respin at 13,000g for 10 minutes. Place supernatant in a thick-walled plastic microfuge tube, and freeze at -70°C. Batch and send once **every 2 weeks on dry ice** to Biomarker Laboratory.

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TUBES C:

3. **Final 0.4 to 0.5ml of whole blood** will be placed in a **small purple-topped (EDTA) bullet** with gentle shaking. Withdraw blood with either a pipette or syringe with large gauge needle and place in a thick-walled empty plastic microfuge tube, and immediately freeze at -70°C. Specimen can be **batched** and sent **once every 2 weeks on dry ice** to Biomarker Laboratory.

Address for Overnight FedEx Shipment to Biomarker Laboratory:

(Monday thru Thursday Shipments Only)

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20 APPENDIX 2

Rehospitalization after Acute Chest Syndrome ("rebound") is a significant safety concern with this study. The current study has been designed to monitor this concern every three months (quarterly) after the first subject has been enrolled. Specifically, the percent of subjects with a rehospitalization would be compared between the two treatment groups and if the Dexamethasone arm had a significantly higher percentage than the placebo arm, the DSMB would be alerted immediately to review the data in detail and decide whether the difference warranted a change to the study or stopping the study. Please note that this is similar to the comparisons made on other safety measures, such as serious adverse events and all adverse events. It is not clear, however, how often these suspension guidelines would be invoked under different rates of rebound. The purpose of this appendix is to describe the characteristics of the suspension guidelines in terms of probability of stopping early under different assumed rates of rebound for each treatment arm.

Previous studies (Huh (XXXX) and Bernini (XXXX)) found that treatment with Dexamethasone increased the percentage of subjects with rebound by a factor of six. Specifically, the Bernini study found that 5% of subjects treated with only standard of care (placebo arm) had a rehospitalization, while 27% of subject treated with Dexamethasone had a rehospitalization. Similarly, the Huh study found 6% in the placebo arm and 31% in the Dexamethasone arm had rehospitalizations. The tapering dosing schedule specified in this study is expected to reduce the number of rebounds in the Dexamethasone arm, so we wish to look at a range of percentages for the Dexamethasone arm and assume 5% of the placebo subjects have a rebound. The following table presents five "scenarios" that could be reasonably expected to be observed in the trial.

Table X. Assumed percentage of rebounds for each scenario considered.

	% Rebound		Expected total number of rebounds
Scenario	Placebo	Dexamethasone	
A	5%	25%	17
B	5%	30%	20
C	5%	10%	8
D	5%	5%	6
E	5%	2.5%	4

Using the percentage of rebound from each of these scenarios, we then construct simulations of the trial conduct, with evaluations every three months. The trial is expected to enroll a total of 112 subjects over a 15 month period, which corresponds to approximately 22 subjects every three months and four interim evaluations (n=22, 44, 66, 88) prior to the end of the study. Although oversimplified, this provides us a framework to examine the characteristics of the study using simulations.

Each simulated trial will take the assumed percentage of rebounds from the corresponding scenario and generate a random number of subjects with rebound from the number of subjects enrolled prior to that interim evaluation. Evaluations will occur at 22, 44, 66, and 88 subjects, with half enrolled into the placebo arm and half enrolled in the Dexamethasone arm. Each interim evaluation will then generate a p-value from the chi-square statistic testing no difference between the treatment arms. If that p-value is less than 0.05, an alert will be considered triggered. One thousand trials are simulated for each scenario and the percentage of these trials that have an alert triggered are reported in the following table.

	Assumed % Rebound		% of Trials with Alert
Scenario	Placebo	Dexamethasone	
A	5%	25%	68%
B	5%	30%	94%
C	5%	10%	19%
D	5%	5%	%
E	5%	2.5%	%

The first two scenarios correspond approximately to what was observed in the two previous studies. If the true underlying percentage of rebound is 30% in the Dexamethasone arm and only 5% in the placebo arm, this suspension guideline would alert the DSMB prior to the end of the study in 94% of the studies. If the true percentage were 25%, the guideline would alert the DSMB in 68% of the studies. If, however, the true underlying percentage of rebound in the Dexamethasone group is only slightly higher than the placebo group, (i.e. 10% vs. 5%) then the DSMB would be alerted in only 19% of the trials.

There is a danger of a “false positive” alert with this guideline. In the case where both treatment arms have the same low (5%) percentage of rebounds, the DSMB would still be alerted to a possible imbalance X% of the time. And even in the case where the percentage of rebounds in the Dexamethasone treated group is lower than the placebo treated group (i.e. 2.5% vs. 5%) the suspension guideline would trigger an alert in X% of the studies.

21 APPENDIX 3

Acute Chest Syndrome Assessment

Instructions:

1. The ACS Assessments are completed every 4 hours during hospitalization and at hospital discharge. The first 24 hour period begins at the time of the first dose of study drug on Day O. i.e. First dose of study drug given on 05JAN07 @ 08:00, therefore Day 0 is from 05JAN07 08:00 – 06JAN07 07:59. The first Q 4 Hour ACS assessment begins as close as possible to 4 hours after first dose of study drug and the next routine nursing assessment. i.e. First dose given at 08:00, next routine nursing assessment is 13:00, therefore begin Q 4 Hour ACS assessments at 13:00 and time the following assessments from this time point. Even if an assessment is missed, this form should be completed and the appropriate expected time point should be selected.

2. Complete in the order the assessments are listed on the ACS Assessment worksheet.

3. Rate the respiratory rate and work of breathing **BEFORE** taking the oxygen off for one minute to check the O_2 saturation.

4. If subject is ventilated or if it is deemed unsafe to trial subject off O_2, then record "NA" (not applicable).

5. If steady state value is not known, enter 92%.

6. Enter a separate pain score for Thoracic and Non-thoracic pain with either the 10 point NRS or the Oucher (subjects 5 – 10 years).

7. **Invasive** ventilatory support means the use of an artificial airway (tracheal intubation) to support breathing. **Noninvasive** support means the use of positive pressure ventilation ***without*** the need for an invasive artificial airway, e.g. CPAP or BiPAP. The use of incentive spirometry or IPPV alone is **NOT** a form of noninvasive support.

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Acute Chest Syndrome Assessment

Date of Assessment: _____
Time of Assessment: _____

Element of Index		Endpoint Criteria	Endpoint Met (Y/N)
1. Respiratory rate			
a. Current rate:	_____	a ≤ b	
b. Upper limit of normal[1] (bpm) plus 2:	_____		
2. Work of breathing			
a. Retractions (Y/N)	_____	a = No	
b. Nasal flaring (Y/N)	_____	b = No	
c. Use of accessory muscles (Y/N)	_____	c = No	
3. Pain			
a. Thoracic pain scale[4]	_____	a ≤ 4	
b. Non-Thoracic pain scale	_____		
4. S_pO_2 in room air[2]			
a. Current value:	_____	a ≥ b	
b. Steady-state value[3] (%) minus 2:	_____		
5. Medical intervention			
a. Supplemental O_2 (Y/N)	_____	a = No	
b. Invasive or noninvasive ventilatory support (Y/N)	_____	b = No	

[1]Age 5-11.99 y, 30 bpm; age 12-17.99 y, 25 bpm; age ≥18 y, 20 bpm.
[2]If subject is ventilated or if it is deemed unsafe to trial subject off O_2, then record "No" for endpoint met.
[3]If steady-state value is not known, enter 92% and do not subtract 2.
[4]Enter value of a 0-10 point NRS or Oucher pain scales or the value of a 0-5 point scale multiplied by two.

The study endpoint is met when the answer to each element is "Yes" OR when the subject is discharged (whichever occurs first).

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22 APPENDIX 4

Secretory Phospholipase A_2 Assays (Laboratory of Dr. Kuypers)
Subject samples will be identified by their unique CSCC subject identifier, specimen number, and date of specimen collection. Subject names will not be used.

Secretory Phospholipase A_2 Assays include:

1). Measurements Secretory Phospholipase A_2 activity will be measured in a fluorescent assay described previously[1]. All general calibration and maintenance of the fluorometer will be kept current. Samples will be batched and with appropriate negative and positive controls will consistently be maintained within 10% of the expected values.

2). Measurements Secretory Phospholipase A_2 concentration will be measured using commercially available ELISA Kits (Cayman Chemical, Ann Arbor, MI), which is 100% specific for type IIA $sPLA_2$. The intra and interassay CV is $\leq$10%. General quality control measures will include pipette calibrations to meet standard laboratory guidelines. Samples will be batched and run, with appropriate standard curves performed on the day of sample measurement ($r \geq 0.99$).

Blood Sample Preparation Requirements (total of 8.5ml per blood draw)

TUBE D: **1.0ml** from a free-flowing blood draw will be transferred into a **red-top tube** with no anticoagulant. After 30 minutes at room temperature centrifuge at 2500g for 15 minutes, place supernatant in a thick-walled plastic microfuge tube and freeze at -70ºC. Study personnel will batch and send on dry ice every two weeks to Dr. Stuart's Biomarker Laboratory. Dr. Stuart's laboratory will batch and send every month on dry ice to Red Cell Biology Laboratory.

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