PHASE 2 RANDOMIZED CONTROL TRIAL OF ARGININE THERAPY FOR PEDIATRIC SICKLE CELL DISEASE PAIN

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Study Title: Phase 2 Randomized Control Trial of Arginine Therapy for Pediatric Sickle Cell Disease Pain (IND#66,943)

Short Tile: Arginine Therapy for SCD Pain (RCT)

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ABSTRACT

The purpose of this study is to determine the effects of IV L-arginine hydrochloride therapy in children with sickle cell disease (SCD) and vaso-occlusive pain events (VOE). Specifically, the impact on total opioid use (mg/kg) in morphine equivalents over the duration of their emergency department (ED) visit and hospital stay will be evaluated.

Sickle cell disease is an inherited hemolytic anemia that affects approximately 100,000 individuals in the US (7), primarily African-Americans, and is therefore an orphan disease. It represents a group of genetic disorders variably characterized by anemia, severe pain, and potentially life-threatening complications such as bacterial sepsis, splenic sequestration, acute chest syndrome, stroke and chronic organ damage. Vaso-occlusive painful episodes in SCD are common, debilitating, & the leading cause of hospitalizations, ED visits, missed school, & are associated with an increased mortality rate (8). No current therapies specifically target vaso-occlusion, with symptomatic interventions limited to analgesia & hydration. Nitric oxide (NO), produced by the 5-electron oxidation of L-arginine, is a potent vasodilator and exerts pleiotropic effects on vascular and circulating blood cells, including the inhibition of platelet aggregation, down-regulation of adhesion molecules, and modulation of ischemia-reperfusion injury, all pathways adversely affected during VOE (3). We and others have found that SCD patients with VOE have acutely depleted L-arginine levels (4, 9). Additionally, we have now completed a single-center randomize, double-blinded, placebo-controlled trial (RCT) of arginine therapy in 54 children with VOE requiring hospitalization (1). **We observed a reduction in total narcotic usage by 54% and lower pain scores at discharge in children who received up to 5 days of 100 mg/kg/dose, three times a day (TID), intravenous (IV) L-arginine therapy compared to placebo (1).** We propose to extend these results in a phase II trial of L-arginine for VOE in children with SCD to further assess the efficacy and safety of this therapy and determine whether a loading dose of IV L-arginine at presentation provides additional benefits.

Dysregulation of the arginine metabolome contributes to complications in SCD including VOE and early mortality (2, 4, 6, 10, 11) . In transgenic mouse models of SCD, L-arginine supplementation has been shown to inhibit the red cell Gardos channels (12), reduce red cell density (12, 13), improve perfusion, and reduce lung injury, microvascular vaso-occlusion and mortality (14-17). Arginine also increases erythrocyte glutathione levels in both mouse (15) and human trials (18), and may down-regulate inflammatory pathways (19). The impact of arginine therapy on glutathione has not yet been studied in children with SCD during an acute pain event. In addition, arginine is a key substrate in creatine synthesis, an important metabolic pathway not yet sufficiently studied in SCD that may be impacted by an arginine- deficient state. Although mechanisms of arginine dysregulation are complex and multifactorial (3, 10), they can be overcome through arginine supplementation, a phenomenon known as the “arginine paradox” (20). **We therefore hypothesize that L-arginine is a safe and inexpensive intervention with narcotic-sparing effects in pediatric SCD patients with VOE that will decrease pain scores and contribute to a decreased length of hospital stay. By providing a loading dose of L-arginine (twice the usual dose) early on during an acute VOE in the emergency department (ED) or clinic setting, when patients are known to experience a sudden drop in plasma arginine levels (5), we hypothesize that global arginine bioavailability and pain will improve compared to placebo. The potential of arginine therapy to impact ED discharge will also be studied for the first time in this clinical trial.**

STUDY OBJECTIVES

The primary hypothesis of this study is that arginine therapy will decrease total narcotic use and improve pain scores in children with sickle cell disease (SCD) and vaso-occlusive pain episodes (VOE) compared to

placebo. We also predict that a one-time loading dose of 200 mg/kg IV L-arginine at presentation for acute pain will be more effective than the standard dose of 100 mg/kg on clinical and biological outcomes studied.

We anticipate that arginine therapy will impact multiple biological pathways beyond nitric oxide. We hypothesize that treatment will improve global arginine bioavailability and erythrocyte redox biology; in particular it may increase erythrocyte glutathione concentration, decrease oxidative stress, inflammatory biomarkers and endothelin-1, and ultimately improved mitochondrial function. We will also monitor arginase activity, nitric oxide metabolites (NO_x), exhaled nitric oxide (NO), and hemolytic rate in order to evaluate potential study-drug mechanisms of action.

Specific Aims

1. **To determine the efficacy of IV arginine therapy on the primary endpoint, total parenteral opioid use (mg/kg of morphine equivalents) in children with SCD and VOE and evaluate whether a loading dose provides additional benefits compared to placebo. Important secondary outcome measures including length of hospital stay, time to pain crisis resolution, pain scores and discharge rate from the emergency department/clinic will also be evaluated. (*Efficacy)***

2. **To monitor for potential toxicities associated with a loading dose and repeated administration of IV L-arginine. *(Safety)***

3. **To characterize alterations in the arginine metabolome in children with SCD and VOE and evaluate how it is impacted by IV arginine therapy. *(Exploratory)***

INTRODUCTION AND BACKGROUND

Mechanisms of Arginine Dysregulation

Vaso-occlusion is believed to be the root cause of sickle cell pain. NO is a free radical & a potent vasodilator (21) that regulates vascular homeostasis & plays a role in vaso-occlusive complications of SCD (1, 3, 22-27). NO has properties that can impact every aspect of SCD, from decreasing platelet activation & adhesion receptor expression on the vascular endothelium, to decreasing vascular smooth muscle proliferation, limiting ischemia-reperfusion injury, modulating endothelial proliferation, & regulating inflammation. Given the crucial role of NO depletion in endothelial dysfunction (28), it is not surprising that NO dysregulation is a common denominator among varied mechanisms of sickle vasculopathy (3, 10, 27). NO is produced in the endothelium from its obligate substrate L-arginine, which is converted to citrulline by a family of enzymes, the NO synthases (NOS) (21). Although NOS expression & activity is increased, SCD is characterized by a state of NO resistance, NO inactivation, & impaired NO bioavailability (27, 29, 30). Under conditions of increased hemolysis, inflammation or oxidative stress, the compensatory upregulation of NO likely becomes overwhelmed & ineffective. Vascular dysfunction is the end result, due to complex & multifactorial interactions **(Fig 1) (2).**

Normal arginine metabolism is impaired in SCD (2, 4, 31-34) for many reasons. We found that adults with SCD are arginine deficient at steady-state (3, 4), **while children have plasma levels that are similar to normal controls (4).** The altered arginine metabolome differs in children vs. adults (4). An arginine deficiency develops with age & is influenced by acute events & chronic end-organ damage. However plasma arginine concentration decreases significantly in both adults & children with VOE & acute chest syndrome (ACS), and is associated with low NO_x levels (4). Lowest arginine levels were found in children requiring admission for VOE **(Fig 2A)**(4)**,** with **arginine levels returning to baseline during convalescence in the hospital (Fig 2B).** Of interest, **low plasma arginine alone was a sensitive predictor for admission** (4)**,**

while NO_x levels were not, suggesting a function for arginine bioavailability in VOE severity that goes beyond NO. Arginine bioavailability may represent a novel objective metabolic biomarker of pain severity worth exploring further.

Low global arginine bioavailability (GAB) is associated with both early mortality in SCD (2) & VOE (4, 9). Increased arginase activity from both inflammatory triggers & more significantly from erythrocyte-arginase release during hemolysis (2), intracellular arginine transport inhibition, renal dysfunction (which impairs the major route for endogenous arginine biosynthesis) (3, 10), elevated endogenous NO synthase (NOS) inhibitors like asymmetric dimethylarginine (ADMA) (35-37), competitive inhibitors of arginine transport & all NOS isozymes (38), uncoupled NOS (17) & other consequences of oxidative stress (15, 16, 39, 40) lead to low GAB in SCD. These mechanisms may impact an individual's response to arginine therapy. **Although mechanisms of arginine dysregulation are complex & multifactorial (3, 10), they can be overcome through arginine supplementation, a phenomenon known as the "arginine paradox" (20).** Arginine represents a novel NO-based therapy (1, 33) that may have greater therapeutic potential than NO gas because of its multi-faceted nature that extends beyond its function as the obligate NO substrate (41).

Why Arginine if NO gas does not treat VOE? Question raised from the DeNOVO trial (41). Hemolysis will drive arginine consumption, which will ultimately exacerbate NO sequestration & decreased NO synthesis (3). Under conditions of hypoxia, high ADMA, low arginine, or low essential NOS cofactors (42), NOS will uncoupled, producing reactive oxygen species in lieu of NO, further reducing NO bioavailability & adding to the milieu of oxidative stress. An imbalance between eNOS-derived NO & superoxide generation exists in SCD (43). Upregulation of NOS would therefore enhance oxidative stress when the local milieu favors NOS uncoupling. Indeed, studies in transgenic SCD mice demonstrate that NOS activity is paradoxically increased & uncoupled while NO bioavailability is low (17). With NOS uncoupling, inhaled NO gas will be rapidly sequestered by superoxide, forming peroxinitrite known to cause lung damage & cell death. **It is plausible that the provision of NO excess in SCD may lack therapeutic benefit in the absence of sufficient arginine bioavailability. This is a potential pitfall of sildenafil therapy as well as inhaled NO gas (41, 44).**

Therapeutic Potential of Arginine Therapy in SCD. In transgenic mouse models of SCD, L-arginine supplementation inhibits the red cell Gardos channels (12), reduces red cell density (12, 13), improves perfusion, & reduces lung injury, microvascular vaso-occlusion & mortality (14-17). Arginine increases erythrocyte glutathione levels in both mouse (15) & human trials (18). Independent of SCD, low global arginine bioavailability is associated with major adverse cardiovascular events including mortality in patients screened for cardiovascular disease (45), mortality risk in malaria (46), & is associated with pulmonary hypertension risk (11, 33, 47). Rapid healing of leg ulcers was reported with IV ***arginine-***butyrate in both SCD & thalassemia (48). Short term arginine therapy improved pulmonary hypertension in SCD (6), & acutely increases both plasma & exhaled NO when administered to ethnically matched normal controls & patients with VOE (5, 49). When arginine is given to SCD patients at steady-state, a paradoxical **decrease** in NO_x occurs **(Fig 3A)** that is not overcome by higher doses **(Fig 3B)(5),** clearly indicating that arginine is metabolized differently in SCD compared to controls. However, when arginine is given during VOE **(Fig 3B**), a robust dose-dependent increase in NO_x is observed. **(Fig 4)(5)** This indicates that arginine is also metabolized differently in SCD at steady-state compared to times of acute illness including pain and acute chest syndrome (4, 5, 49). These early observations may account for the negative outcome of the Comprehensive Sickle Cell Centers' (CSCC) arginine trial**,** particularly since the primary outcome measure of that study was an increase in plasma NO_x levels, when published data in fact

demonstrated a decrease in NO_x with arginine supplementation at baseline. Ultimately nutritional therapy like arginine may only be beneficial during a deficient state such as VOE.

Arginine effect is dose-dependent in VOE: Rational for a loading dose in the ED. Low dose arginine therapy is likely to be subtherapeutic in SCD, & may represent an additional flaw in the CSCC prophylactic arginine trial design, as doses used were close to placebo based on the cardiovascular literature (50, 51). Previous studies have shown that low-dose arginine is unlikely to impact NO synthesis (51), an observation confirmed in the CSCC study. The capacity of arginine to increase NO_x production in SCD is dose-dependent (5). **Higher levels of plasma arginine are likely needed to overcome multi-factorial effects including impact of arginase & ADMA on global arginine bioavailability, & accelerated arginine consumption during VOE compared to baseline (Fig 5B).** However, the long-term safety of doses > 100 mg/kg/dose TID is unknown in SCD, although a 1-time dose of 30 grams IV is safe & commonly used for growth hormone stimulation testing (52, 53). Since the arginine formula is L-arginine hydrochloride, the wisdom of higher doses over time is questionable given the potential to induce acidosis & must be taken into consideration. However, using a one-time loading dose upon initial presentation for pain should be safe based on endocrine experience with growth hormone stimulation testing using 500 mg/kg/dose for children, would avoid risks of repeated higher doses, and may theoretically be more efficacious by quickly overcoming effects of excess arginase and ADMA. This is the rationale behind investigating clinical effects of a loading dose of 200 mg/kg.

Although specific mechanisms contributing to changes in the arginine metabolome during VOE are unknown, hemolysis is unlikely to play a major role at least initially, as straightforward VOE is not typically associated with a lower than baseline hemoglobin (Hb), although our data shows a significant Hb drop within the first 24 hours after admission (1). A ≥1 gram drop in baseline Hb at presentation for pain can be a red-flag for impending ACS (54, 55). Our early studies revealed a falling arginine level reaching a nadir within 24 hours of a positive CXR **(Fig 5A),** days into the VOE admission, a pattern that differed from uncomplicated VOE **(Fig 2B)** (4)**.** Dysregulation of arginine metabolism appears to be different in VOE vs. ACS (4), & mechanisms for arginine consumption in VOE should be sought out in future arginine trials since it may impact response to arginine therapy. Insight into these paradigms may allow us to develop novel targeted therapies. L-arginine supplementation inhibits the red cell Gardos channels (12), reduces red cell density (12), improves perfusion, and reduces lung injury, microvascular vaso-occlusion and mortality (14-17). Arginine also increases erythrocyte glutathione levels in both mouse (15) and human trials (18), and may down-regulate inflammatory pathways (19). However, none of these mechanisms of action have been studied during VOE in children with SCD. In addition, arginine is a key substrate in creatine synthesis, an important metabolic pathway not yet sufficiently studied in SCD that may be impacted by an arginine- deficient state. Although the role of NO in SCD has become controversial (56, 57), these studies demonstrate that the mechanistic impact of arginine goes beyond NO. We speculate, however, that improved NO bioavailability with arginine therapy may also be a contributing factor in children with vaso-occlusive pain.

Based on preliminary pharmacokinetic studies (5, 49), peak plasma arginine concentration after oral arginine (0.1 g/kg) is significantly higher during SCD steady-state compared to VOE **(Fig 5B),** although levels are similar by 4 hours. Normal controls reach a peak arginine level between 1-2 hours that is maintained at 4 hours, & does not trend down as in SCD (5). **Accelerated arginine metabolism or consumption occurs during VOE compared to steady-state despite the same oral arginine dose given.**
Results of a single-center randomized, double-blinded, placebo-controlled trial of arginine therapy for children with VOE requiring hospitalization (1). A total of 56 children >3 years old admitted to CHRCO for VOE were enrolled between 2000-2008 in this NIH-supported arginine trial **(NHLBI K23 award HL-04386-**

05). Patients were consented within 24 hours of admission. Patients with significant liver, renal or neurological dysfunction, >10 VOE admissions/year or a history of narcotics dependence were excluded. A standardized treatment & monitoring program for VOE was followed. Average age was 13.9 ± 4 years (range 3.6-19 years), and 52% were female. 73% of the patients had Hb-SS, 18% had Hb-SC, and 9% carried S-β thalassemia. Patients received IV or oral arginine (0.1 gram/kg TID, N=28) or placebo (N=28) for 5 days or until discharge from the hospital. Two patients randomized to the arginine arm did not receive parenteral opioids and were excluded from the analysis. Narcotic records for 2 patients (randomized to placebo arm) were incomplete & were not included in the narcotic use analysis.

RESULTS: Age was equally distributed between treatment & placebo groups. 57% of the arginine treatment group & 46% of the placebo group were female. The overall time between triage in the emergency department or presentation to clinic and delivery of first randomized study drug dose was 20.4±11 hours, with no difference between the placebo and treatment arm. **A significant reduction in narcotic use (defined as total opioid use over the course of the hospital stay in mg/kg) by 54% was observed in the treatment arm receiving IV or oral arginine compared to placebo (mean ± SEM: 1.9 ± 0.4 mg/kg; n=26 vs. 4.1 ± 0.8mg/kg; n=26, *p=0.02*; Fig 6A).** Pain scores based on a 10-cm visual analog scale of 0-10 reported in the ED or in clinic during initial presentation were similar in both the arginine and placebo group (Fig 1B). As expected, pain scores decreased significantly in both the arginine and placebo arms at time of discharge compared to initial evaluation. However, **pain scores were significantly lower at discharge in the arginine arm compared to placebo (Mean±SD: 1.9±2.4 vs. 3.9±2.9, *p=0.01, Fig 6B*).** Comparing the differences in the change from presentation (day 1) to day of discharge adjusting for day 1 values between the placebo and arginine arms, a larger drop in pain scores was also observed in the arginine arm compared to placebo (*p=0.01*). Average length of hospitalization was 4.5 ± 0.4 days, & there was no significant difference between the 2 groups (4.1±0.3 vs. 4.8±0.5 days, *p=0.27*; arginine vs. placebo arm), although a clinically relevant trend favored patients treated with arginine. **Total opioid use (mg/kg) correlated strongly to length of hospital stay (r=0.86, *p<0.0001*), and represents a surrogate biomarker of LOS to evaluate study drug efficacy.** Four episodes of ACS occurred during the study, 3 in the treatment arm and one in the placebo arm. One patient experienced clinical deterioration associated with ACS requiring emergent transfusion & a transfer to the pediatric intensive care unit (PICU) in the placebo arm. No clinical deterioration or PICU transfers occurred in the arginine arm. Five in the treatment arm received transfusion vs. 4 in the placebo arm. No drug-related adverse events were observed. No significant differences were observed between pre & post therapy liver or renal function, or hematological parameters in the arginine treatment group vs. placebo. Arginine is a safe, efficacious and inexpensive intervention with narcotic-sparing effects that
should be considered as a potential adjunct to standard therapy for VOE (1, 10, 50).

Details of Adverse Events with RCT of IV Arginine Therapy in SCD. Among 56 pain events treated, a total of 3 SAEs and AEs were reported. No serious drug-related adverse events were observed, although one AE and one SAE occurred that led to a discontinuation of study drug, and a decision by the principal investigator to withdraw the participant from the study without breaking the blinded study drug code until the completion of the trial. One patient (randomized to the arginine arm) developed hives during the study drug infusion. Although the patient had a history of allergy to paper tape, and paper tape had been inadvertently used, study drug was discontinued and the patient was withdrawn from the trial due to an AE that was possibly related to study drug. A second patient (randomized to the placebo group) was withdrawn from the study after experiencing an acute clinical deterioration during the evolution of ACS, requiring an emergency red blood cell transfusion, and transfer from the ward to the intensive care unit. A second SAE was also reported (in a patient randomized to the placebo group), involving a clinically relevant rise in liver function enzymes (ALT rose from 44 on admission to 197 U/L on day 4 while AST increased from 64 to 200 U/L). The clinical team felt this was likely related to SCD, but could possibly be

related to study drug, so an SAE was reported to the IRB & Data Safety Monitoring Board, but the patient continued participation in the study and was closely monitored. No other patients experienced clinical deterioration, & no other adverse events occurred. Arginine & placebo were well tolerated.

STUDY SIGNIFICANCE: The importance of novel approaches to vaso-occlusive pain in sickle cell disease

Pain is the clinical hallmark of SCD, and a significant problem in emergency medicine (58). Vaso-occlusive painful episodes (VOE) are common, debilitating, and a true medical emergency. VOE are the leading cause of hospitalizations, emergency room visits, missed school, and are associated with an increased mortality rate (8). There is no effective therapy that targets the underlying mechanisms of VOE. Symptomatic relief with analgesics and hydration is the only currently available treatment, and this has not changed in decades. Episodic periods of severe pain lead to high use of health care resources (59), with high readmission rates even in patients hospitalized for pain management (8). A recent health care utilization report published in 2010 revealed that 20% of patients with SCD experienced 3 or more encounters per year (8). This is remarkably higher than the 5.2% with 3-19 visits per year published in 1991(60). Hospital admission rates are high for children with SCD (61) presenting to ED with VOE, and commonly > 60% (62). Although the reason for high pediatric admission rates are unknown, many children with SCD live with daily pain to some extent that their families try to control at home through various methods. It is when the pain becomes acutely worse, and unbearable, that they present to the ED, often in acute distress**. Novel approaches to treatment of acute pain for SCD that can be utilized in the ED as well on the hospital ward are critically needed. Interventions that target underlying mechanisms of sickle cell pain in addition to providing symptomatic relief would be ideal and are worth pursuing. This study has the potential to alter standard care for children with this orphan disease.**

We have now completed a single-center randomize, double-blinded, placebo-controlled trial of arginine therapy in 54 children with VOE requiring hospitalization (1). **We observed a marked reduction in narcotic usage by over 54%, lower pain scores at discharge, and a clinically relevant trend in reduced length of hospital stay of approximately 17 hours in children who received L-arginine therapy compared to placebo (1)**. We propose an intervention trial for pain in children with SCD that goes beyond symptomatic treatment and will provide valuable mechanistic insight. In addition, the use of a one-time loading dose may help overcome the multi-factorial limitations on global arginine bioavailability in SCD. This proposal will therefore provide essential data for product development including the planning of a future multi-center Phase 3 trial, which will ultimately help gain product approval for an orphan disease targeting the indication of decreased length of hospital stay.

Critical Barriers to Care for Patients with Sickle Cell Disease and Pain in the Emergency Department

Acute care of patients with SCD and pain in the ED is a neglected area of sickle cell research. Nationally, 78% of the nearly 200,000 annual ED visits for SCD are for a complaint of pain (63). Given the high ED utilization rate for pain in both adults & children with SCD (8, 59, 63), ED-based research has not received the amount of attention and funding that it deserves. Unlike other types of pain, experts point out that research support & progress in understanding SCD pain followed a sluggish & almost stagnant, path (64). Funding support for pain research that begins in the ED will help to remedy this disparity. Interventions designed to minimize SCD complications and avoid hospitalization will also reduce the significant economic burden of this disease (65).

Negative attitudes towards patients with painful sickle cell crises. The negative attitudes toward patients with painful sickle crises have been compounded by racial stereotypes, the effects of the disease in limiting educational & employment opportunities, suboptimal medical coverage, & the large doses of

opioids often required to obtain pain relief (64). Most studies find that ED doctors misunderstand SCD pain (66, 67). Studies indicate that 53% of ED physicians believe that over 20% of SCD patients are addicted to opioids, with 22% of ED physicians believe that over 50% are addicted to opioids (68). A recent study of 232 patients with SCD that kept detailed pain diaries found 35.5% to be high ED utilizers with 3 or more ED visits a year (69). However, these patients had lower hemoglobin, lower quality of life, more pain & distress, more pain days, more crises, & more transfusions than those not high ED users. Indeed, controlling for the severity of their illness, high utilizers **did not** use more opioids any more often (69). In short, they were sicker patients. Improved ED physician education is essential to improve negative attitudes commonly perceived by families dealing with SCD when seeking emergency care. This sort of advocacy will begin with research in the ED setting, with information filtering to national Emergency Medicine meetings, as data is presented in emergency medicine targeted forums. The majority of SCD research is presented in venues that target hematologists. Despite high ED utilization & the role of the ED physician in treating the most common complication of SCD, the ED physician has for the most part been left out of the loop. A pivotal clinical trial to address pain in the ED is a step in the right direction, as it extends SCD research into the ED arena, promoting awareness of SCD among a group that most commonly manage the disorder, but often misunderstands the disease.

The chaotic nature of the ED. Although disparities specific to pain management practices in the ED for children with SCD have not been identified (70), ethnic disparities in ED care have been reported (71-73), & adults with SCD experience longer delays in the initiation of analgesics compared to other patients with pain (74). However, time to initiation of treatment in the ED is often delayed as a result of random events that are beyond anyone's control, such as high patient volumes (35) & acuity of other patients in the ED, even when policies are in place for immediate triage of patients with SCD & pain. In a recent study of children with SCD, median time from arrival to analgesia administration was 90 minutes, with high ED census as the biggest culprit for delays (35). Barriers to rapid care in the ED are common across the country, including overcrowding, nursing ratios, insufficient staff coverage, inadequate funding, & slow flow of patients from the ED to the wards in addition to patient acuity. These also represent significant barriers to performance of successful research in the ED, where emergent clinical care appropriately takes priority over research endeavors. Pediatric emergency medicine (PEM) champions are needed to work closely with SCD experts in the field to help establish & disseminate best practices for the treatment of pain in the ED that leads to rapid assessment, intervention, & relief of suffering. They are also essential to the successful development & implementation of any clinical trial that targets sickle cell care in an ED, given their familiarity with the pitfalls of research in this hectic environment & knowledge of means to overcoming these barriers. The approach & clinical style of an ED-trained physician differs from that of a hematologist, & close cooperation between these 2 sub-specialties is essential to change the current paradigm & barriers to ED care described above. Such collaborations are also essential to successful clinical trials that address pediatric sickle cell pain in the ED. SCD experts working outside the ED setting often have unrealistic impressions of the ED routine & functional capacity & may be unfamiliar with common hurdles known to the PEM physician. Close collaboration between the ED and Aflac hematology group at Emory University will insure the success of this clinical trial.

The results of this proposal may significantly impact patient care & change clinical practice for children with SCD in both the ED & hospital wards. It offers greater insight into the pathophysiology of SCD, & it is also the foundation for a future multi-center trial. It holds the potential to improve quality of life for patients with SCD.

Innovation

There are several innovative components to the planned research.

- **We propose a novel therapy to target the leading cause of ED visits & hospitalizations for patients with SCD**, supported by promising data demonstrating significant narcotic-sparing effects, decreased pain scores at discharge and a trend towards a reduction in length of hospital stay. Early initiation of arginine therapy in the ED may impact duration of VOE & total narcotic use further. Arginine is a promising therapy for both hospitalized & ED patients with SCD. **Acute care of patients with SCD & pain in the ED is a neglected area of research.** This proposal moves toward remediating the paucity of ED-based SCD research. Insight into metabolic mechanisms linked to pain will also help change the prevalence of negative attitudes toward pain in SCD.

- Our drug candidate is selected based on an impressive back-bone of basic science, pre-clinical efficacy, and extensive human safety experience. **Arginine has demonstrated marked biological plausibility for multiple beneficial effects in SCD, including decreased total narcotic use in a recently published randomized, placebo controlled trial at CHRCO (1).** Therapies that target underlying mechanisms of vaso-occlusive pain are innovative and superior to those targeting symptomatic pain relief alone.

- This study expands upon the positive outcomes of a recently published pilot arginine trial (1), evaluates the effects of a loading dose of arginine compared to standard dosing, & includes enrollment at 3 high-volume children's hospitals within the CHOA network to insure sufficient recruitment potential.

- This study will for the first time evaluate multiple aspects of the arginine metabolome during VOE and strives to identify the key mechanisms of action of arginine therapy in SCD. Although the exact mechanisms remain unknown & are likely multifactorial, those that have been implicated through mouse & human pilot studies include vasodilation from increased nitric oxide production & decreased endothelin-1, impact on global arginine bioavailability & overcoming effects of excess arginase activity & ADMA, decreased inflammation, increased erythrocyte glutathione levels and improved RBC redox balance, and decreased oxidative stress. The end result would be altered mitochondrial function. Rather than the common model of looking at individual mechanisms in isolation, Dr. Morris has gathered together a team of experts who can collaboratively view the bigger metabolic picture.

STUDY DESIGN AND METHODS

The research plan is to institute a phase II RCT of IV arginine therapy in children with SCD and VOE in order to further knowledge on efficacy and safety of this orphan drug. This study will also determine if arginine therapy is superior to placebo in clinical and biological outcomes described. In addition, this study will determine whether a one-time loading dose of arginine (200 mg/kg IV) followed by 100 mg/kg TID dosing is superior to 100 mg/kg IV TID alone without a loading dose. Our exploratory objective is to more fully characterize the arginine metabolome in children with SCD during VOE, and evaluate the effects of arginine therapy on global arginine bioavailability, vasodilation (NO metabolites, endothelin-1) arginase activity, hemolytic rate and inflammation, erythrocyte redox biology & oxidative stress, and mitochondrial function together with important clinical endpoints (pain scores, time to VOE resolution, hospital length of stay and admission rates from the ED/clinic) in children with SCD and VOE. This study will take place in CHOA emergency departments (Egleston, Hughes Spalding and Scottish Rite) and/or on the hospital inpatient ward. It will initiate at Egleston and open at other campuses as needed to achieve enrollment goals.

Specific Aim 1: *Efficacy*

To determine the efficacy of IV arginine therapy on the primary endpoint, total parenteral opioid use (mg/kg) in children with SCD & VOE and evaluate whether a loading dose provides additional benefits.

This is a Phase II RCT of IV arginine therapy in children with SCD with VOE presenting to an acute care setting or admitted for treatment of their pain. Children with SCD and moderate-to-severe pain that is related to their SCD and not attributable to other non-SCD causes, receiving parenteral opioids are eligible for participation. **This study will adhere to all investigational new drug and FDA regulations.**

Qualified IRB- approved study personnel who are knowledgeable about the protocol, familiar with the consent process and are delegated the responsibility of consenting will be allowed to consent eligible patients. All potential participants will discuss the study with the research study personnel. This may be in person or remotely (over the phone, video call etc.). They will be given an opportunity to discuss participation with an investigator or research staff member who has been delegated this responsibility. Candidate participants will be assured that participation is voluntary, and that participation will not affect or influence clinical or medical care. Permission for the child will be obtained by one parent/legally authorized representative. Subjects and/or their legally authorized representative will agree or decline participation in the study. Electronic consent and assent will be obtained, and this process will be documented electronically in REDCap. Electronic consent may be obtained in person (using a laptop, iPad, or other touch screen device etc). In the event of technology failures paper consent forms may be used. Either an electronic or paper copy of the consent form will be provided to the participant. If this copy is provided electronically this may be sent directly via REDCap to the participant or it will be sent to the participant via encrypted email.

Once informed consent is obtained, eligible children and adolescents age 3-21 years will be randomized to one of three study arms: Standard L-arginine dose (100 mg/kg IV TID), one-time L-arginine loading dose (200 mg/kg IV) + standard dose (100 mg/kg IV TID), or placebo (normal saline 1-2 ml/kg IV TID) up to a maximum of 7 days (21 doses) or until discharge, whichever comes first. The maximum loading dose of IV L-arginine will be 20 grams, with a standard dose maximum of 10 gms IV TID. A total of 114 patients will be treated/randomized in this protocol, 38 in each arm. Up to 150 patients may be enrolled, to account for potential screen failures and withdrawals. **Patients must be randomized within 24 hours of receiving their initial parenteral opioid in the ED or clinic/day hospital**. Even though a 24-hr window is established for feasibility, the ideal goal is to randomize and deliver study drug within 2-4 hours in the ED or clinic/day hospital. The average time to study drug delivery in the original Morris Arginine study was 20.4±11 hours (1). Although this original study was not sufficiently powered to detect a decreased length of hospital stay (LOS), the trending decrease by 17 hours is a clinically relevant improvement for patients and their families. A standardized treatment and monitoring protocol for VOE utilized by Emory/Children's Healthcare of Atlanta mutually agreed upon by both the ED and department of hematology will be followed. Pain scores (0-10 scale or FACES as appropriate) will be obtained at presentation to the acute care setting for pain prior to study drug administration and at least TID until discharge. If the patient is discharged from the ED or day hospital/clinic without a hospital admission, a discharge pain score will also be obtained. A discharge blood draw will be obtained whenever possible for those discharged from the ED but will be limited to research labs targeting the arginine metabolome (plasma and erythrocyte amino acids, arginase activity, NO_x and exhaled NO). Information on hydroxyurea use (HU) will be obtained. Randomization will be stratified for HU use to minimize potential confounding effects of HU use. Patients,

physicians, and staff involved in patient care will be blinded as to whether each patient receives arginine vs. placebo.
Patients already participating in the clinical trial may not be enrolled more than once.

IV L-Arginine-HCl: Patients will be randomized to IV arginine/placebo (normal saline). IV L-Arginine-HCl is obtained from Pfizer in a 10% Arginine-HCl injection formulation (R-Gene 10). See product information in active **IND# 66943** " **Impact Of Arginine Therapy On Pulmonary Hypertension And Biological Markers In Sickle Cell Disease"** for details on preparation, clinical pharmacology, toxicology, precautions & adverse reactions. The preparation will not be modified, and will be administered per manufacturer's recommendations, except that the dosage will be adjusted for this protocol. **There will be sufficient access to study drug for this clinical trial.** Study Drug Infusion & Strategy for Managing Infusion Complications: Arginine will be infused based on manufacturer's instructions (R-Gene 10, Pfizer)**, over 30 minutes. However, rates may be slowed to over 60 minutes for patients experiencing symptoms of flushing, nausea, vomiting or headache at research team discretion.**

It is provided as a ready-to-use solution and is not typically diluted further. Pediatric doses will be drawn up by the pharmacy. Patients with SCD undergoing growth hormone stimulation test have tolerated 500mg/kg/dose over 30 minutes without complication. In practice, arginine infusion protocols are not typically modified for SCD. No complications with infusions were reported during the arginine trial recently published (1). Arginine compatibility is similar to that of total parenteral nutrition (TPN) and can be co-administered with normal saline/dextrose fluids, ketorolac, opioid analgesia/PCA and antibiotics. There are no issues with IV tubing, which can be changed according to CHOA policy. Although it is a hypertonic solution, the amount of fluid delivered with 1-2ml/kg is minimal and is not anticipated to trigger a pain crisis. In fact our data demonstrated that 1ml/kg of 10% Arginine-HCL given IV TID throughout hospitalization significantly decreased pain scores compared to placebo in children with SCD hospitalized for VOE (1). Past clinical trial experience with arginine did not support the need for a second IV placement specifically for the arginine infusion. Therefore, the clinical IV placed for parenteral opioid therapy will be utilized for study drug. If the IV site should become infiltrated, the IV catheter will be removed and the infiltration addressed clinically as needed, per hospital protocol for IV infiltration. A new IV catheter will be placed if the patient's clinical status mandates continued parenteral opioids or IV access is required for other reasons. If the patient loses his/her IV and it is no longer clinically needed, IV arginine will be discontinued per study protocol. Information on IV infiltration during arginine infusion will be collected and reported as an adverse event.

VOE resolution in patients discharged from the ED or day hospital/clinic and hospital will be defined as the time in hours, of study drug delivery to the time of the last parenteral opioid.

Length of Stay will be defined as the time in hours, of study drug delivery to the time that the discharge order is given in written or electronic form.

Pain Scores: Pain associated with VOE will be measured on a numeric pain rating scale of 0-10, with the ends representing the extreme limits of "no-pain" (0) and "worst pain" (10), or the FACES pain rating scale when appropriate. Pain scores will be obtained at presentation to the acute care setting for pain prior to study drug administration and at least TID daily, until discharge and at time of discharge.

Specific Aim 2: ***Safety monitoring***

To monitor for potential toxicities associated with a loading dose & repeated administration of IV L-arginine.

Vital signs including blood pressure, hemoglobin, liver and renal function testing will be obtained at the same time as the study blood draw for research labs. Rates of acute chest syndrome, blood transfusion, clinical deterioration, need for intensive care and hospital/ED returns within 72 hours and 30 days will be monitored in all enrolled patients and compared between study arms.

Primary Outcome Measure: *The primary outcome measure is total parenteral opioid use (morphine equivalents mg/kg) calculated from the initial dose of study drug, throughout the ED and/or hospital stay in the arginine arms compared to placebo.*

Secondary Outcome Measures:

Clinical Outcomes

1. Length of hospital stay from study drug delivery to discharge (time of discharge order)
2. Time to VOE resolution after study drug delivery
3. Pain scores at presentation vs. discharge
4. Pain score over time
5. Length of ED stay (time of ED triage to discharge or admission order)
6. ED discharge rate
7. Total opioid dose (mg/kg) in the first 8 hours after study drug delivery
8. Number of study drug doses
9. Rate of acute chest syndrome (not present before randomization)
10. Rate of blood transfusion
11. Change in oxygen saturation and use of supplemental oxygen during admission. PROMIS pediatric Pain and Fatigue short forms (daily for up to 1st 7 days and then on day of discharge: collaboration with Nitya Bakshi/Carlton Dampier)
12. Symptoms questionnaire
13. 72-hour returns and 30-day return visits to the ED or in-patient hospitalizations
14. Diffusion correlation spectroscopy

Study Labs: Clinical, Research and Safety Monitoring

1. Clinical and toxicity monitoring: complete blood count, reticulocyte count (retic), comprehensive metabolic panel, Urine dipstick (monitoring for proteinuria) on study entry (day 0), day 1, day 7 and or discharge (see table p 17.) With the ability to use discarded samples saved up to 72 hours for research tests.
2. Urine or serum pregnancy test will be done at enrollment in girls ≥12 years if not already done as part of standard of care within 24 hours of randomization
3. The arginine metabolome and global arginine bioavailability: plasma arginine, ornithine, citrulline, arginase, ADMA, NO metabolites (Brown lab - Emory), exhaled NO.
4. Urine amino acid metabolites (to search for possible renal wasting or arginine as cause of deficiency)
5. Biomarkers of oxidative stress (glutathione, isoprostanes, malonyldialdehyde, peroxynitrite, B12, methymalonic acid and its derivatives. (Brown lab)
6. Inflammation: white blood cell count, CRP, Th-1 and Th-2 cytokines (Brown lab)
7. Hemolysis: Lactate dehydrogenase (LDH), Cell-free hemoglobin (Hb), retic, indirect bilirubin, (Brown Lab)

8. Mitochondrial function (Shiva mito platelet panel-UPMC)

Specific Aim 3: *(Exploratory)*
To characterize alterations in the arginine metabolome in children with SCD and VOE and evaluate how it is impacted by IV arginine therapy.

The mechanism of action for arginine therapy during VOE is UNKNOWN, although we have shown that children are metabolically different during VOE compared to baseline with respect to the arginine metabolome. Several biochemical & cellular effects of L-Arginine administration will be evaluated in order to provide mechanistic insight into potential benefits of arginine therapy based on pre-clinical data supporting each potential mechanism. Since no single mechanism occurs in isolation, multiple mechanism are likely. Blood will be drawn, and urine will be obtained, processed and stored for future analyses at the time of presentation prior to study drug delivery (day 0), on day 1 (drawn with routine am labs 12-36 hr after initiation of study drug) and then on day of 21st dose (day 7) and/or prior to discharge (or ED discharge, whichever comes first). Exhaled NO levels will be obtained at presentation and daily in children mature enough to perform the procedure based on ATS guidelines (76), or prior to discharge in the ED for those not admitted. Additional information on important clinical outcomes will also be collected. Bloodwork for safety/toxicity monitoring will also be obtained with study blood draws (see Specific Aim #2).

Potential Mechanisms Impacted by Arginine Therapy

- **Global arginine bioavailability (GAB) and the arginine metabolome:** (arginine, ornithine, citrulline, arginase, ADMA, arginase activity/concentration, creatine, urinary arginine/amino acids).

We have established that the arginine metabolome is dysregulated in SCD (Figure 1) (2, 3), low arginine concentration is associated with VOE (4), that arginine supplementation can increase NO_x when given during VOE (5), and that arginine therapy improves clinical outcomes like pain scores & total opioid use (1). The etiology of an acute arginine deficiency during VOE is unknown. Excess renal loss of amino acids may occur during VOE, though it has never been evaluated. Urinary arginine/amino acids will be evaluated together with plasma levels. ADMA is elevated in SCD (35-37) & will impact GAB (3), and also contributes to NOS uncoupling and mitochondrial dysfunction. Recent evidence suggesting a direct role for ADMA in NOS uncoupling and mitochondrial dysfunction that is attenuated by treatment with arginine (77, 78) may be of particular interest in SCD although this paradigm has not yet been explored in hemolytic anemias. Our proposal is the first to evaluate impact of arginine therapy on the arginine metabolome, oxidative stress, hemolysis, inflammation and subsequent mitochondrial dysfunction in SCD.

- **Vasodilation:** (*Nitric oxide (NO); Endothelin-1 (ET-1*).

Since L-arginine is the obligate substrate for NO production, an impact on this potent vasodilator is intuitively plausible and important to investigate. Our early work demonstrated a dose-dependent impact of arginine therapy on NO_x production in SCD and VOE (4). The clinical implications of NO_x levels remain to be determined and can be effected by confounders like diet. Therefore, NO_x is <u>not</u> an ideal biomarker in isolation but may provide valuable data when combined with information on the arginine metabolome including arginase concentration & ADMA levels, peroxynitrite concentration and mitochondrial superoxide production. Other studies have shown that IV arginine infusions can decrease levels of ET-1 (79), a potent vaso-constrictor that may play a role in vaso-occlusive events. The ability for IV arginine to improve endothelial dysfunction was associated with elevated ADMA levels (79). Our proposal will investigate the impact of arginine therapy on both NO_x, ET-1 and ADMA. One limitation is that values of any metabolite in the plasma do not necessarily reflect levels in organ systems or distinct pools. We will

also follow daily measurements of exhaled NO to evaluate NO bioavailability from the lungs and compare this to NO_x, mitochondrial superoxide production and peroxynitrite levels (the consequence of NO + superoxide).

- **Oxidative stress** (rbc glutathione, GSH/GSSH, peroxynitrite, isoprostanes)

Oxidative stress plays a pivotal role in the pathophysiology of SCD (39). Of interest to this project, the erythrocyte may be a major determinant of the global redox environment. The sickle erythrocyte has increased concentrations of the reactive oxygen species compared with normal red blood cells (30, 80-82). In the presence of alterations in the glutathione buffering system that occurs in these hemoglobinopathies (40, 81-88), it is likely that these erythrocytes are incapable of handling the increased oxidant burden and this might predispose them to hemolysis. Reduced glutathione (gamma-glutamyl-cysteinyl-glycine; GSH) is the most abundant low-molecular weight thiol (89) and the principal thiol redox buffer in erythrocytes (90, 91).

The intracellular GSH concentration is the final result of a dynamic balance between the rate of GSH synthesis and the combined rate of intracellular GSH consumption & efflux. GSH is readily oxidized to glutathione disulfide (GSSG) by free radicals and reactive oxygen and nitrogen species. GSSG efflux from cells contributes to a net loss of intracellular GSH (89). **The GSH/GSSG ratio is often used as an indicator of the cellular redox state;** it is typically ~100 under normal physiological conditions (89). GSH/GSSG is the major redox couple determining the antioxidative capacity of cells (89, 92, 93). Arginine also increases erythrocyte glutathione levels in both mouse (15) & human trials (18), and may down-regulate inflammatory pathways (19).
Dr. Lou Ann Brown's lab has published vastly on the topic of GSH and the cellular redox state.

To estimate oxidative stress, this study will measure GSH together with its amino acid precursors, NO_x, peroxynitrite, isoprostanes, aconitase (biomarker of superoxide production - increased in SCD, and the bi-product of NOS uncoupling that is influence by low GSH, low arginine bioavailability and/or high ADMA) and ultimately mitochondrial function (Shiva lab). Alterations in the erythrocyte redox environment can contribute to both increased oxidative stress and hemolysis, and represents a mechanistic model bridging two critical pathways that may be impacted by arginine therapy.

- **Inflammation** (CRP, WBC, Th-1/TH-2 cytokines)

Archer et al found that arginine down-regulates inflammatory pathways (19) in the sickle cell transgenic mouse model. In particular C-reactive protein (CRP) and interleukin-6 were decreased in mice fed a high protein diet. CRP and a panel of inflammatory cytokines will be evaluated pre and post therapy to determine if this observation translates to human SCD.

- **Hemolysis** (LDH, Hb, retic count, bilirubin, cell-free Hb).

Hemolysis contributes significantly to an altered arginine metabolome in SCD (2, 29, 94-96) and will be evaluated. Whether arginine has any impact hemolytic rate is unknown, however the degree of hemolysis in individual patients presenting with VOE may impact the severity of their acute arginine deficiency and response to therapy. Past studies demonstrating an effect of arginine therapy on erythrocyte GSH levels could impact degree of hemolysis. Our preliminary data demonstrated a trend towards lower retic count in the arginine arm compared to placebo (1), and should be followed.

- **Exhaled nitric oxide**

Exhaled nitric oxide is a non-invasive procedure that is considered to be an indirect measurement of airway inflammation. It is also impacted by supplemental arginine. Subjects will be instructed to take in a deep breath and blow air out at a constant pressure as directed by personnel through a standardized method. One measurement will be taken and recorded at Day 0 and daily during hospitalization.

- **Cerebral and Muscle Blood Flow**

Subjects will have a sticky sensor gently secured to the forehead and/or arm or leg to look at blood flowing to the brain or painful arm/leg and blood flow to the arm or leg that does not hurt before study drug is delivered, and then after study drug is delivered for up to 8 hours on the first day, and then for 5-10 minutes daily (20 minutes maximum). A blood pressure sensor will be secured to the subject's upper arm and fore finger during this time.

- **Mitochondrial function**

Mitochondrial dysfunction in the platelets of SCD patients. In a pilot study, we isolated platelets from the blood of adult SCD patients & healthy African American volunteers. Comprehensive analysis of mitochondrial function demonstrated that SCD patients manifested mitochondrial dysfunction characterized by decreased complex V activity compared to healthy African American subjects (Fig 8). A decrease in complex V activity, the ATPase required for ATP generation by oxidative phosphorylation, potentially results in increased mitochondrial reactive oxygen species production (through electron buildup in complexes I-IV) and decreased ATP generation. Consistent with this, we have measured increased mitochondrial ROS generation in SCD platelets compared to healthy controls (Fig 9A-C).

Mitochondrial dysfunction in SCD patients can be mimicked by oxidative stress. To determine whether oxidative stress causes mitochondrial dysfunction in mitochondria of SCD patients, healthy human platelets were exposed to free hemoglobin in vitro, a generator of oxidants through auto-oxidation of heme. Free hemoglobin (100uM) caused a decrease in mitochondrial complex V activity, which was prevented when an antioxidant (TEMPOL; 250uM) was included in the incubation (Figure 10).
Collectively, these data suggest that platelets from SCD patients manifest mitochondrial dysfunction that is potentially propagated by oxidative stress. Information on ADMA and mitochondrial dysfunction will also provide insight into these 2 overlapping mechanisms.

****Blood volume obtained from children with SCD may sometimes vary due to difficult IV access. Studies run for research labs will be prioritized to arginine metabolome. Additional secondary tests will be analyzed based on available blood sample. Use of discarded samples saved up to 72 hours will also be used to run research specified labs only if needed.***

PARTICIPANT SELECTION

The research coordinator/study team will review the ED track board and daily admission logs, and approach patients for enrollment who meet the following inclusion criteria:

Inclusion Criteria

- Established diagnosis of sickle cell disease (all SCD genotypes)
- Age 3-21 years of age
- Pain requiring medical care in an acute care setting (ED, hospital ward, day hospital, clinic)

not attributable to non-sickle cell causes, that is moderate-to-severe treated with parenteral opioids

Exclusion Criteria

- Decision to discharge home from acute care setting.
- Hemoglobin less than 5 gm/dL or immediate need for red cell transfusion anticipated within the next 12 hours.
- Hepatic dysfunction: SGPT (ALT) > 3X upper value
- Renal dysfunction: Creatinine >1.0
- Mental status or neurological changes
- Acute stroke or clinical concern for stroke
- Pregnancy
- Allergy to arginine
- Two (2) or more ED visits within the last 7 days prior to CURRENT ED visit
- Hospital discharge < 14 days prior to presentation to ER.
- Previous randomization in this arginine RCT (patient consented and screen failed before receiving study drug or placebo remains eligible for future participation).
- Patient refusal
- Use of inhaled nitric oxide, sildenafil or arginine within the last month
- Acidosis with $CO2 \leq 16$
- Hypotension requiring treatment with clinical intervention
- Not an appropriate candidate in the investigator's judgement
- Newly started on HU for < 3 months
- PICU admission from the emergency department

Patients will be given or sent electronically a standard consent form prior to study enrollment approved by the institutional review board (IRB). This consent form will provide a brief summary of the study protocol and will provide families with contact information for the principle investigator and IRB personnel should they have any questions/concerns about the study. Both informed parental consent and patient assent, where appropriate, will be obtained electronically, and when appropriate a paper consent can be obtained. A copy of the consent and assent, where appropriate, will be placed in the patient's medical chart. If a copy of the consent form is sent electronically to a patient, this will be sent either via REDCap or via encrypted email, as such the patient's electronic mailing address will be captured.

A study binder will be kept by the research coordinator/enrollers that will record the names and unique identification code of each patient approached for enrollment in an enrollment log. The names and demographic information (age, gender, Hb-genotype) of those who were approached and decline enrollment and the reason for declining (if known) will be recorded as approached/not enrolled. Age, gender and Hb-genotype information on patients screened by the study team/coordinator but found to be not eligible will also be collected. All study binders will be kept in a locked cabinet in the research office of the PED or Aflac coordinators and electronic files with any private health information (PHI) will be kept in a password protected CHOA supported division sharepoint site that is backed up nightly. Only members of the study team will have access to the study folder.

Randomization and Arginine/Placebo Administration

Arginine/Placebo will be administered in a blinded fashion. Using randomization lists prepared by the

research pharmacist, the research pharmacist at Egleston Children's Hospital will perform a block randomization for all CHOA sites, and study drug will be allocated to the patient TID until discharge. **Randomization will be stratified for hydroxyurea use to insure an equal number of patients on HU in all arms to minimize this as a potential confounder.** The patient, nurses, and physicians caring for the patient will be blinded as to which medication the patient is receiving.

Once written informed consent is obtained, and eligibility is confirmed by study staff, children and adolescents age 3-21 years will be randomized to one of following three study arms:

1) Standard L-arginine dose (100mg/kg IV TID)
2) One-time L-arginine loading dose (200mg/kg IV) plus the standard dose (100mg/kg IV TID)
3) Placebo (normal saline 1-2 ml/kg IV TID)

Upon enrolment, the patient's medical record is reviewed for CMP and creatinine testing in the past month in the past 12 months.

- If there are no measurements within the past 12 months or if either SGPT/ALT or creatinine were elevated in the past 12 months (meeting exclusion criteria), the current CMP results must be reviewed prior to dose administration. (Patient may be randomized; however, delivery of study drug should be held until CMP results are reviewed and no exclusion criteria are noted).
- If CMP and creatinine levels were normal enough to not meet exclusion criteria in the past 12 months, the patient can receive the first dose of study drug and the CMP and creatinine labs reviewed when available.
- Patients with a CMP after randomization that meet exclusion criteria will have study drug stopped for toxicity monitoring. With an intention-to-treat analysis, these patients will remain on study. Blood analysis and exhaled NO_x measurements will continue per protocol, but arginine/placebo supplementation will be terminated.

Administration of Analgesics to Study Patients

Patient Controlled Analgesia (PCA) devices will be used in admitted patients whenever possible according to standard practice at CHOA. Intravenous ketorolac, oral NSAIDS and oral opioids with acetaminophen may be used in addition to parenteral opioids. Intranasal fentanyl may be administered at presentation in the ED per protocol. Dosing of the PCA will follow a standard protocol of pain management established by the Aflac sickle cell center.

Monitoring During Study

Patients will be identified from the over 1400 ED SCD patient visits/year for VOE in children followed by the staff at the Aflac Sickle Cell Center of CHOA/Emory University School of Medical. They will be examined daily by the clinical team and will undergo routine vital signs including blood pressure per clinical protocol. A chart review will be performed, and pertinent data extracted. A pain score will be obtained and recorded daily (at least 3 times a day, once per shift) and at time of discharge. Standard of care pain scores will be utilized. A symptom questionnaire to assess for potential side effects of arginine will be completed daily by the study coordinator (see appendix). Information for the symptom's questionnaire evaluating for potential adverse events should be obtained by direct questioning of the patient/family in addition to the medical record. Use of parenteral narcotics will be documented every shift by nursing staff per standard protocol and will include narcotics delivered by the PCA device as well as any additional intravenous, intranasal and/or oral opioids used. Data on total daily opioid use will be collected by study team coordinator.

Laboratory monitoring will be undertaken according to the schedule below:

	DAY 0	DAY 1	Day 7/Discharge*
CBC with retic count (SOC)	X	X	X
Comprehensive metabolic panel	X	X	X
Urinalysis for protein (urine to Brown lab)	X	X	X
BHCG (girls > 12 yrs)	X		
RESEARCH LABS **See Specific Aim 3** **Arginine Metabolome**	X X	 X	X X

Total research Blood draw/day < 20 cc (less than 2 tablespoons)

*Day 0 pre-study drug delivery and Day 1 is the first day >12 hours after study drug initiation. If routine daily labs occur within < 12 hours of the first study drug dose, day 1 labs may be rescheduled to the following day. *Discharge bloodwork will be obtained on Day 7 of arginine therapy for prolonged admissions AND day of discharge. Day 7 is the day that the last dose # 21 will be given for patients who have not yet been discharged. Discharge labs may be obtained with routine daily blood draw whenever possible and may be drawn on days when the research and clinical team suspect the patient may be discharged, to avoid multiple venipuncture. In the event the patient is not discharged on that date, discharge labs should be drawn again prior to discharge. Tests may be run on discarded samples saved up to 72 hours if needed.*

Toxicity Monitoring/ Study Drug Stopping Rules

Patients will be removed from the study if they meet the following criteria:

1) Neurological dysfunction or development of stroke while on protocol
2) Increase in SGPT to > 6X ULN value
3) Increase in creatinine to >3X value at study entry or >2.0
4) Allergic reaction to study medication
5) Patient's request
6) Acidosis with CO2 ≤ 16
7) Hypotension requiring treatment with clinical intervention
8) Concern by study team and sickle cell clinical team that a serious adverse event is related to study drug

Data from patients removed from the study will be collected and analyzed for potential side effects or toxicities of arginine. Blood analysis and exhaled NO_x measurements will continue per protocol, but arginine/placebo supplementation will be terminated. Patients who ALT values increased >6X ULN, the ALT will be monitored daily with a study CMP until the values are no longer increasing, then once weekly or until patient is clinically stable for discharge, whichever is first.

ACS severity definition

ACS is defined as a new pulmonary infiltrate associated with fever, chest pain or respiratory symptoms. ACS is a clinical diagnosis and will be determined based on discharge diagnosis by the hematology team. Severity of ACS for the purposes of this study will be defined as mild, moderate or severe based on the

criteria below, based on a definition created for other trials in SCD.

- **Mild:** +CXR with no oxygen use or transfusion required
- **Moderate:** +CXR associated with oxygen use or rbc transfusion
- **Severe:** +CXR and PICU transfer, intubation, and/or death

Study Compensation

Each consent participant will receive $30 after randomization and completion of at least 1 study drug infusion and a second $30 if they complete the discharge study requirements. Patients that do not complete the entire study will be paid for the days they have completed. Each participant will receive a total of $60, if all study requirements are completed.

STATISTICAL ANALYSIS

Over a 1-year period, >1400 visits to CHOA EDs were recorded for children with SCD presenting for acute management of VOE. Over 500 of these visits through 2012 occurred between the hours of 7am and 3pm. Therefore, there is more than sufficient access to an eligible patient population due to the large volume of patients receiving care at the Aflac sickle cell center and its associated pediatric emergency departments.

The primary goal of this analysis is to compare the standard arginine treatment with placebo. The power analysis is based on this comparison. This sample size calculation is based on a two-sample two-sided t-test, with the objective of detecting a 55% reduction in opioid use from its mean value (4.1) in the placebo group based on the preliminary data generated from the recent Morris arginine trial (1). Based on these findings, the standard deviations are 2.0 and 4.1 (days) in the arginine and the placebo groups, respectively. A sample size of 33 subjects per group has 80% power to detect the treatment effect stated above on a two-sided test at the 0.05 significance level. A secondary goal is to compare the other arginine treatment arm (with a loading dose) to the placebo arm. Finally the two arginine treatments will be compared to determine if there is any difference or trend towards a difference when a loading dose is used that is superior to the standard arginine dose used with success in prior studies. Based on past experiences with arginine therapy, few patients withdrew from the study after the therapy was switched from oral to parenteral. Up to 150 patients will be enrolled, allowing for screen failures and study withdrawal, in order to insure randomization and study completion of 108 patients (36 per group) allowing for a 7% withdrawal rate. We did not adjust the sample size calculations for multiple comparisons as the power calculation only corresponds to the primary goal involving a single comparison of patients receiving the standard treatment or the placebo. We anticipate that the "loading dose" arm will be either the same or better than the standard dose. We will evaluate the efficacy and safety of this loading dose compared to the placebo group.

Each of the comparisons mentioned above will be conducted via a general linear model analysis. Predictors will include the treatment indicator, use of HU, age group, gender and the interactions of each of the last three covariates with the treatment indicator. By including interactions in the model we will be able to determine whether the treatment effect varies across subgroups of patients.

Assistance with statistical analysis and power calculation was provided by the Department of Biostatistics and Bioinformatics at the Rollins School of Public Health, Emory University, by Professor Michael Haber PhD, who will be the biostatistical consultant on this project.

Sample size power analysis for LOS as outcome: *Goal for a future phase 3 RCT*

This proposal will help provide more accurate data to generate a sample size power analysis for decreased LOS as the primary outcome measure in a multi-center Phase 3 RCT in the future.

If the true difference (based on the Morris et al (1)) between groups were 17 hours for LOS, n=150 subjects would be required in each group to have 80% power to detect a difference, assuming a 5% type 1 error rate and estimated standard deviation of ~ 50 hours. Given > 20 hour delay in administration of study drug from time from ED presentation for most patients in the Morris study (1), study drug administration within < 12 hours of ED presentation (ideally < 4 hours) will improve our power to detect a difference in LOS, likely requiring a smaller sample size. See below calculations and **Figure 11**. If the true decrease in LOS were closer to 24 hours (theoretically achievable with the elimination of delay in study drug delivery), a sample size of 76 patients in each group (n=152 total) is needed, approximately half the size required based on our current data. This proposed study is therefore a sound investment to insure the best design for a future phase 3 trial that will advance this orphan drug towards FDA regulatory approval.

Two-Sample T-Test Power Analysis

Numeric Results for Two-Sample T-Test

Null Hypothesis: Mean1=Mean2. Alternative Hypothesis: Mean1≠Mean2

The standard deviations were assumed to be unknown and unequal.

Power	N1	N2	Allocation Ratio	Alpha	Beta	Mean1	Mean2	S1	S2
0.80251	150	150	1.000	0.05000	0.19749	0.0	17.0	43.0	60.0
0.80277	134	134	1.000	0.05000	0.19723	0.0	18.0	43.0	60.0
0.80154	120	120	1.000	0.05000	0.19846	0.0	19.0	43.0	60.0
0.80007	108	108	1.000	0.05000	0.19993	0.0	20.0	43.0	60.0
0.80385	99	99	1.000	0.05000	0.19615	0.0	21.0	43.0	60.0
0.80254	90	90	1.000	0.05000	0.19746	0.0	22.0	43.0	60.0
0.80045	82	82	1.000	0.05000	0.19955	0.0	23.0	43.0	60.0
0.80361	76	76	1.000	0.05000	0.19639	0.0	24.0	43.0	60.0

Power Analysis Summary Statements

Group sample sizes of 150 and 150 achieve 80% power to detect a difference of -17.0 between the null hypothesis that both group means are 0.0 and the alternative hypothesis that the mean of group 2 is 17.0 with estimated group standard deviations of 43.0 and 60.0 and with a significance level (alpha) of 0.05000 using a two-sided two-sample t-test.

Site Monitoring Plan

The study coordinator will notify the Principle Investigator (PI) of all adverse effects. The PI will review the Adverse and Serious Adverse Event reports within 24 hours of being reported. All Serious Event Reports linked to study drug will be submitted to the FDA within the required time frame and the IRB will be notified per protocol. Summaries of all Adverse Event Reports will be provided to the FDA quarterly and IRB annually. After the occurrence of 2 Serious Adverse Events related to study medication, the study will be halted until a modification in design can be considered and submitted for IRB and DSMB PCRC Advisory board approval before continuing the study.

Data Safety Monitoring Plan

An Independent Data Safety Monitoring board, which is a group of experts not connected to the study, will be reviewing the data from this research throughout the study. Participants will be informed about new information from this or other studies that may affect their health, welfare, or willingness to stay in the study. The study Principle Investigator (PI) will review the Adverse and Serious Adverse Event reports within 24 hours. All Serious Event Reports linked to study drug will be submitted to the FDA within 24 hours and the IRB will be notified per protocol. Summaries of all Adverse Event Reports will be provided to the FDA quarterly, and IRB annually. After the occurrence of 2 Serious Adverse Events related to study medication, the study will be halted until a modification in design can be considered and submitted for IRB and DSMB Advisory board approval before continuing the study. A Data Safety Monitoring Board will be established from individuals not involved in this project, consisting of 1 pediatric hematologists from the Aflac Sickle Cell Center, one pediatric emergency medicine physician from Children's Healthcare of Atlanta, one individual with SCD expertise and past DSMB experience from outside Emory, and one biostatistician to be identified from the Data Coordinating Center. Drs. Clint Joiner MD, PhD, Director of Hematology, Aflac Cancer and Blood Disorders Center, Emory University School of Medicine, Harold Simon MD, MBA, Marcus Professor and Vice Chair, Department of Pediatrics, Emory University School of Medicine, and Elizabeth Klings MD, Associate Professor of Medicine, Director of the Pulmonary Hypertension Program, Boston Medical Center, Boson University School of Medicine, have all committed to participation in the DSMB. The DCC biostatistician will be Curtis Travers MPH from the Emory Department of Pediatrics. The arginine arms of this study will be performed under an active IND for arginine therapy in sickle cell disease held by Dr. Morris amended to reflect this study design.

Adverse Events

Adverse Event Definition

As defined by the International Conference on Harmonization (ICH) E-6 Guidelines for Good Clinical Practice, an adverse event is any untoward medical occurrence in a subject administered an investigational product and 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 an investigational product, whether or not related to the investigational treatment.
In this study collection of adverse events will begin at the time informed consent is obtained in the ED at the time they present for a pain crisis.

Pre-existing conditions which worsen during a study are to be reported as AEs.

Adverse Event Assessment

Adverse events should be assessed for intensity, relationship to the investigational product, expectedness, and seriousness. The investigator team is responsible for the assessment of all adverse events. During the clinical trial all adverse events will be reported on the AE eCRF.

Intensity

Intensity will be graded as mild, moderate, severe, or life-threatening as defined below:

- Mild: Discomfort noticed but no disruption of normal daily activity
- Moderate: Discomfort such that normal daily activity is affected
- Severe: Inability to work or perform normal daily activity

- Life Threatening: Represents an immediate threat to life

The term severe relates to the intensity of the adverse event. An event may be assessed as "severe" but not meet the criteria for a Serious Adverse Event as outlined in Section on Seriousness, below.

Relationship to Study Treatment / Procedures

Relationship to investigational product refers to the cause of the event and will be assessed using the following scale:

- Definitely Related: Event clearly related to investigational product.
- Probably Related: Event is likely to be related to the investigational product.
- Possibly Related: Event is unlikely or doubtful to be related to the investigational product.
- Not Related: Clearly not related to the investigational product.

Expectedness of Adverse Event

All adverse events will be evaluated as to whether their occurrence was expected (anticipated) or unexpected (unanticipated).

Unexpected:

An unexpected adverse event or adverse treatment reaction is one for which the nature or intensity is not consistent with either:

- Known information associated with the procedures and described in the protocol, consent form, or product brochure, and other relevant sources of information, or
- The expected natural progression of any underlying disease, disorder, or condition of the subject(s) experiencing the adverse event and the subject's predisposing risk factor profile for the adverse event.

Expected:

An event is considered expected if it is known to be associated with the study treatment or the expected natural progression of the subjects' underlying disease or condition.

Expected events related to opioid administration include the following:

Table 1. Expected Adverse Events Related to Opioid Administration

Anorexia	Headache
Apnea	Hypotension
Biliary tract pressure	Miosis
Blurred vision	Nausea
Bradycardia	Palpatations
Bruising	Pruritus
Cardiac arrest	Rash
Confusion	Respiratory arrest
Cramps	Respiratory depression
Diaphoresis	Sedation
Diplopia	Shock
Dizziness	Tinnitus

Drowsiness	Urinary retention
Euphoria	Urticaria
Flushing	Vomiting

Expected events related to sickle cell disease progression include the following:

Table 2. Expected Adverse Events Related to Sickle Cell Disease Progression

Acute chest syndrome	Jaundice
Angioedema	Leukocytosis
Aplastic crisis	Meningitis
Aplastic crisis/anemia	Metabolic acidosis
Arthralgia	Osteomyelitis
Appendicitis	Pyleonephritis
Bone Infarction	Pain, joint
Cardiomegaly	Pain, long bone
Cerebrovascular accident	Pain, severe abdominal
Cholecystitis, hepatic sequestration	Priapisim
Cranial nerve palsy	Pulmonary embolism
Decreased kidney function	Pulmonary hypertension
Decreased lung function	Pulmonary parenchymal infiltrates on CXR
Delayed growth/puberty	Pneumonia
Depresses ESR	Rhabdomyolysis
Fever	Renal Failure
Headache	Renal Insufficiency/albuminuria
Hematuria	Renal papillary necrosis
Hemiplegia	Reticulocytosis (10%-20%)
Hemolysis	Retinal disease
Hepato/splenomegaly	Retinal hemorrhage
Hyperplastic bone marrow	Skin ulcers
Hyposthenuria	Splenic sequestration
Hypoxia (PO2 < 85 mm Hg)	Stroke
Hyperkalemia	Sepsis
Hypotension	Upper respiratory infection
Infection, pneumococcal	Urinary tract infection

Seriousness

An adverse event is considered serious if it meets at least one of the following criteria:

- Results in death; (Note: Death is an outcome not an event).
- Is life-threatening (places the subject at immediate risk of death from the event as it occurred);
- Results in inpatient hospitalization or prolongation of existing hospitalization;
- Results in a persistent or significant disability/incapacity;
- Results in a congenital anomaly/birth defect; or
- Based upon appropriate medical judgment, is medically significant or requires intervention to prevent one of the outcomes listed above.

Adverse events meeting the SAE criteria listed above will be assessed as to intensity, relationship to the investigational product and expectedness as outlined in Section F.2.2.

Reporting of Serious Adverse Events

Any serious adverse event occurring within the course of the clinical trial related or probably related to study drug must be reported to the DCC within one working day of the investigator learning of the event. The investigator team is responsible for notifying the local Ethics Committee/IRB of a SAE in writing as soon as possible and in compliance with local and international laws and regulations.

This study adheres to the definition and reporting requirements of ICH Guidelines for Safety, Data Management, Definitions and Standards for Expedited Reporting, Topic E2. Complete information can be found in the Manual of Procedures.

Outcome of Adverse Events (AE)

Every AE must be followed to a definitive outcome or stabilization of the event, even when this requires a time period beyond the scope of the study (this is particularly applicable to serious adverse events (SAEs)).

Outcome is defined as information on recovery and any sequelae; what specific tests and/or treatment may have been required and their results. For a fatal outcome, cause of death and a comment on its possible relationship to the suspected reaction should be provided.

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

- **Ongoing**
- **Resolved without sequelae**
- **Resolved with sequelae**
- **Death**

Actions taken in response to an AE and follow-up results must be recorded in the subject's medical record (this includes follow-up laboratory results). Any treatment administered for the adverse event must be recorded in the subject's electronic case report form (eCRF). 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.

Post-Study Procedures for Adverse Events

All adverse events unresolved at the time of the subject's termination from the study will be followed by the investigators until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained or has stabilized. Any death or other clinically serious adverse event that may be related to the study treatment and that occurs at any time after a subject has discontinued study treatment or terminated study participation will be reported.

PHARMACEUTICAL INFORMATION

See drug label insert (attachment).

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