Study Protocol

Sickle Cell Disease Treatment with Arginine Therapy (STArT) Trial

Protocol Date: December 1, 2020

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Sickle Cell Disease Treatment with Arginine Therapy (STArT) Trial
PECARN Protocol Number 050
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Pediatric Emergency Care Applied Research Network

Protocol Version 1.00
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PROTOCOL TITLE:

Sickle Cell Disease Treatment with Arginine Therapy (STArT) Trial

Short Title: STArT
PECARN Protocol Number: 050

Lead Investigator and Author:
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Emory University
IND#66943

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I confirm that I have read this protocol, I understand it, and I will conduct the study according to the protocol. I will also work consistently with the ethical principles that have their origin in the Declaration of Helsinki and will adhere to the Ethical and Regulatory Considerations as stated. I confirm that if I or any of my staff are members of the Institutional Review Board, we will abstain from voting on this protocol, its future renewals, and its future amendments.

Principal Investigator Name: _____

Principal Investigator Signature: _____

Date: _____

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Abstract

Background: Vaso-occlusive painful episodes (VOE) in sickle cell disease (SCD) are the leading cause of hospitalizations, emergency room (ED) visits, and missed school, and are associated with an increased mortality rate. There are no current therapies to relieve vaso-occlusion, with interventions limited to hydration and analgesia. 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. In previous trials it has been found that pediatric SCD patients admitted with VOE have depleted plasma L-arginine levels. Additionally, a single-center randomized, double-blinded, placebo-controlled trial of arginine therapy in 54 children with VOE requiring hospitalization was completed. A reduction in total opioid use (mg/kg) by 54% and significantly lower pain scores at discharge in children who received 5 days IV L-arginine therapy every 8 hours compared to placebo, as well as a clinically relevant trend in reduced length of hospital stay of approximately 17 hours was observed. In pharmacokinetic studies, it was found that IV arginine induced a dose-dependent improvement in mitochondrial function in children with SCD hospitalized for pain.

Design and Methods: It is now proposed that these results are extended to a pivotal phase 3 trial of L-arginine for VOE. It is hypothesized that arginine is a safe intervention with opioid-sparing effects in pediatric patients with SCD and VOE that will decrease the time children experience severe pain. Aim 1 of this study will determine the efficacy of IV arginine therapy on the primary outcome, time-to-crisis resolution, as well as total parenteral opioid use (mg/kg) and pain scores in children with SCD and VOE compared to placebo (Efficacy). Aim 2 will monitor for safety of IV L-arginine (Safety). Aim 3 will characterize alterations in the arginine metabolome and mitochondrial function in children with SCD and VOE, and evaluate how it is impacted by IV arginine therapy (Exploratory).

Significance: This proposal will provide essential data for product development and FDA regulatory approval for use of arginine in SCD. Acute care of patients with SCD and pain in the ED is a neglected area of research. The results of this study may ultimately lead to change in clinical practice for children with SCD in both the ED and inpatient hospital wards. ED-based studies and novel therapies that target mechanisms of vaso-occlusion and pain are needed in SCD.

1 Study Summary

1.1 Synopsis

Table 1: Synopsis

Title:	Sickle Cell Disease Treatment with Arginine Therapy (STArT) Trial
Study Description:	The trial is designed as a double-blind, placebo controlled, randomized, phase 3, multi-center trial of IV arginine therapy in children with SCD and VOE to further knowledge on efficacy and safety of this orphan drug. The 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 and mitochondrial function together with important clinical outcomes (time to VOE resolution, pain scores, total parenteral opioid use, Patient-Reported Outcomes (PROs), and hospital length of stay) in children with SCD and VOE
Objectives:	**Objective 1:** • To determine the efficacy of IV L-arginine, when added to standard therapy, on the time-to-crisis resolution in children with SCD and VOE compared to placebo. Important secondary and tertiary outcomes include length of hospital stay, total parenteral opioid use (mg/kg morphine equivalents), pain scores, and patient reported outcomes (PROs) for pain in children with SCD and VOE.
	Objective 2: • To determine the safety profile of IV L-arginine in the treatment of acute VOE in children with SCD. In particular, rate of acute chest syndrome, requirement for blood transfusion, oxygen use during study drug delivery, clinical deterioration and 72-hour and 28-day return visits to the ED or hospital admission will be analyzed in those receiving arginine compared to placebo.

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	Objective 3: • To further characterize alterations in the arginine metabolome and mitochondrial dysfunction in children with SCD and VOE, and evaluate how it is impacted by arginine therapy. • Establish a SCD-VOE biorepository to explore future mechanistic studies.
Outcomes:	**Primary Outcome:** time-to-crisis resolution **Secondary and Other Outcomes:** Length of hospital stay, total parenteral opioid use, pain scores, Patient Reported Outcomes (PROs), oxygen saturation at discharge, rate of acute chest syndrome, ED or hospital readmission rates, requirement for blood transfusion, and pain medication MQS.
Study Population:	This study will enroll 360 patients in the United States that are 3–21 years old with an established diagnosis of sickle cell disease (any genotype) that are requiring medical care in an acute care setting (ED, hospital ward, day hospital, clinic) for pain not attributable to non-sickle cell causes.
Phase:	Phase 3
Description of Sites/Facilities Enrolling Participants:	10 sites total (United States): • Children's Hospital Los Angeles • Children's Healthcare of Atlanta at Egleston • Children's Healthcare of Atlanta at Hughes Spalding • Children's Hospital of Philadelphia • Children's National Medical Center • Medical College of Wisconsin/Wisconsin Children's Hospital • Nationwide Children's Hospital • Texas Children's Hospital/Baylor College of Medicine • UCSF Benioff Children's Hospital • Washington University/St. Louis Children's Hospital

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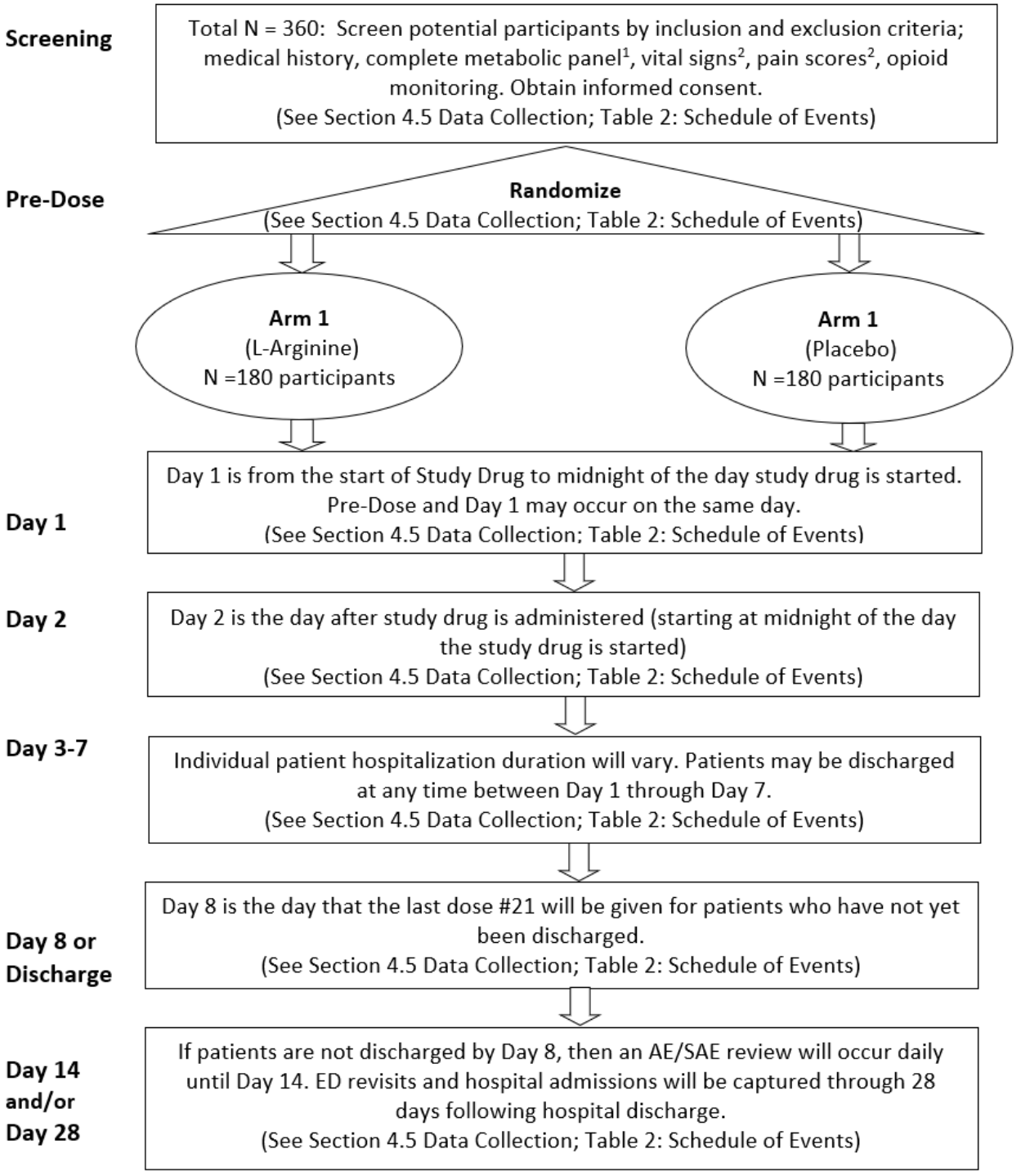
Description of Study Intervention:	Arginine is a safe nutritional supplement that is FDA approved in parenteral form for growth hormone stimulation testing, with nearly 50 years of safety experience through its common use by endocrinologists. **Route of administration:** IV **Dose:** Participants will be randomized to one of two study arms: • A one-time L-arginine loading dose (200 mg/kg IV) + standard dose (100 mg/kg IV TID); OR • Placebo (normal saline) loading dose (2ml/kg IV) + 1ml/kg IV TID.
Study Duration:	6 years
Participant Duration:	28 days after discharge

1.2 Schema



1 - Screening CMP from a clinically ordered CMP or the last CMP in the medical record within the last 12 months will be reviewed. If there are any values within higher than protocol defined cut offs for study drug withdrawal, then a CMP is to be drawn and reviewed prior to randomization. For participants with normal past CMP, a CMP will be done on day 2.
2 - The first set of Vital Signs (including oxygen saturation) and Pain scores will be captured when the patient first presents to the Emergency Department.

This phase 3 randomized controlled trial will investigate the efficacy and safety of IV arginine for the treatment of children with Sickle Cell Disease (SCD) and acute pain. Pain is the clinical hallmark of SCD, and is the leading cause of hospitalizations, emergency room visits, missed school, and is associated with an increased death rate. Arginine is a promising new therapy that could change the way we treat acute pain in children with SCD.

1.3 Hypotheses

The hypotheses of this study are:

1. Arginine therapy will decrease time-to-crisis resolution in children with an acute sickle cell vaso-occlusive pain episode compared to placebo.
2. Arginine therapy will lower total parenteral opioid use (mg/kg) compared to placebo.
3. Arginine therapy will improve pain scores compared to placebo.
4. Arginine will have a similar safety profile as placebo.
5. Arginine therapy will improve global arginine bioavailability and mitochondrial activity.

1.4 Specific Aims

This project has the following Specific Aims:

Specific Aim 1. To determine the efficacy of IV L-arginine, when added to standard therapy, on the time-to-crisis resolution in children with SCD and VOE compared to placebo. Important secondary and tertiary outcomes include length of hospital stay, total parenteral opioid use (mg/kg morphine equivalents), pain scores, and patient reported outcomes (PROs) for pain in children with SCD and VOE.

Specific Aim 2. To determine the safety profile of IV L-arginine in the treatment of acute VOE in children with SCD. In particular, rate of acute chest syndrome, requirement for blood transfusion, oxygen use during study drug delivery, clinical deterioration and 72-hour and 28-day return visits to the ED or hospital admission will be analyzed in those receiving arginine compared to placebo.

Exploratory Specific Aim 3.

3a.To further characterize alterations in the arginine metabolome and mitochondrial dysfunction in children with SCD and VOE, and evaluate how it is impacted by arginine therapy.

3b. Establish a SCD-VOE biorepository to explore future mechanistic studies.

1.5 Subject Eligibility, Accural and Study Duration

The trial will enroll up to 360 participants with VOE, requiring up to 6 years of accrual.

1.6 Eligibility Criteria

Eligible participants will be identified by study staff.

Inclusion criteria are:

1. Age 3–21 years of age, inclusive; AND
2. Established diagnosis of sickle cell disease (any genotype); AND
3. Pain requiring medical care in an acute care setting (ED, hospital ward, day hospital, clinic) not attributable to non-sickle cell causes, treated with parenteral opioids.

Exclusion criteria are:

1. Responds to 2 doses of IV opioids sufficiently for outpatient management
2. Greater than 12 hours from first dose of intravenous opioids to treat current pain in acute care setting
3. Hemoglobin less than 5 gm/dL or emergent need for red blood cell transfusion for hemodynamically unstable patient; OR
4. Ketamine use in the emergency department for treatment of VOE; OR
5. Glutamine within 30 days; OR
6. New Hydroxyurea (HU) use $<$ 3 months or HU dose change $<$ 3 months; OR
7. Acute mental status or neurological changes; OR
8. Acute stroke or clinical concern for stroke; OR
9. Three or more ED visits for sickle cell related pain receiving parenteral opioids in previous 7 days (not including current ED visit); OR
10. Hospital discharge within previous 7 days; OR
11. Hypotension requiring clinical intervention; hemodynamic instability; septic shock; OR
12. Previous randomization in this arginine phase 3 RCT; OR
13. Use of inhaled nitric oxide, sildenafil or arginine within the last month; OR
14. Non-English speaking; requires translator for clinical care; OR
15. Pregnancy; OR
16. Allergy to arginine; OR
17. PI/clinical team concerns for compliance/issues that may adversely impact study participation/outcome.

2 Rationale and Background

2.1 The importance of novel approaches to vaso-occlusive pain in sickle cell disease (SCD)

Pain is a clinical hallmark of SCD, and a significant problem in emergency medicine.[1, 2] Vaso-occlusive painful episodes (VOE) are common, debilitating, and a medical emergency. VOE are the leading cause of hospitalizations, emergency department (ED) visits, missed school, and are associated with an increased mortality rate.[3] Symptomatic relief with analgesics and hydration are the only currently available treatments, and these have not changed in decades. Episodic periods of severe pain lead to high use of health care resources, with high readmission

rates.[3] A 2010 health care utilization report revealed that 20% of patients with SCD experienced $\geq$ 3 ED encounters per year.[3] Hospital admission rates for VOE are $\sim$ 60% for children with SCD[4] and VOE.[5] Many children with SCD also 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 in acute distress. **A significant evidence gap exists for best treatment of VOE**. Novel approaches to SCD/VOE that can be utilized in the ED and hospital ward are critically needed. Interventions that target underlying mechanisms of SCD pain in addition to providing symptomatic relief are worth pursuing.

A single-center, double-blinded, randomized, placebo-controlled trial (RCT) of arginine therapy in 54 children with VOE requiring admission[6] was completed. A marked reduction in parenteral opioid usage by 54%, decreased pain scores at discharge and a clinically relevant trend in reduced length of hospital stay of $\sim$17 hours in children treated with 5 days of 100 mg/kg/dose, three times a day (TID), L-arginine therapy compared to placebo[6] was observed. In pharmacokinetics (pK) studies, it was also demonstrated for the first time, a dose-dependent increase in platelet mitochondrial activity in children with SCD-VOE receiving IV arginine.[7] A definitive phase 3 RCT is now indicated.

2.2 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.[8] Given the high ED utilization rate for pain in both adults and children with SCD,[2, 3, 8] ED-based research has not received the amount of attention and funding that it deserves.[9] Funding support for pain research that begins in the ED will help to remedy this disparity and potentially reduce the significant economic burden of this disease.[10]

Negative impact of opioids over time. Emerging data supports long-term multi-organ side effects of opioids in SCD not appreciated years ago, and the recurrent/chronic use of large doses of opioids compounds the problem further. Opioid-induced endothelial-, mast cell-, renal mesangial-, and epithelial-cell-specific effects and proinflammatory signaling has been reported. Experimental and clinical studies suggest that opioids may exacerbate existent organ damage and also stimulate pathologies of their own.[11] **Opioid-sparing pain therapies are needed; decreasing opioid use is a clinically important outcome of SCD-related morbidity.**

2.3 Mechanisms of Arginine Dysregulation

Vaso-occlusion is believed to be the root cause of sickle cell pain. Nitric oxide (NO) is a free radical and a potent vasodilator[12] that regulates vascular homeostasis and plays a role in SCD vaso-occlusion.[6, 13–19] NO has properties that can impact every aspect of SCD, from decreasing platelet activation and adhesion receptor expression on the vascular endothelium,

to decreasing vascular smooth muscle proliferation, limiting ischemia-reperfusion injury, modulating endothelial proliferation, and regulating inflammation. NO dysregulation is a common denominator among varied mechanisms of sickle vasculopathy.[19–21] 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). Although NOS expression and activity is increased, SCD is characterized by a state of NO resistance, NO inactivation, and impaired NO bioavailability.[18, 22, 23] Under conditions of increased hemolysis, inflammation or oxidative stress, the compensatory upregulation of NO likely becomes overwhelmed and ineffective. Vascular dysfunction is the end result, due to complex and multifactorial interactions (Figure 1).[19]

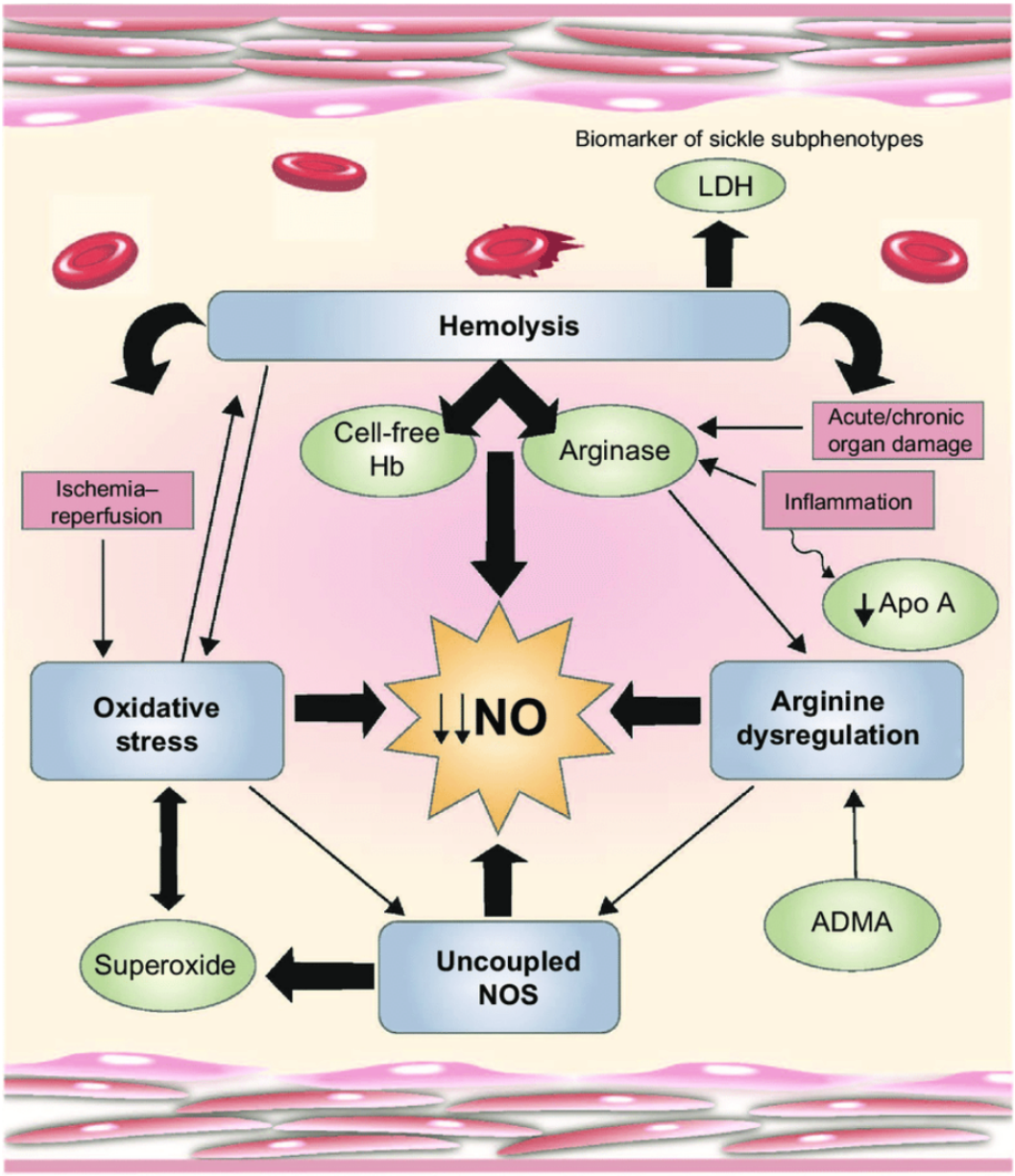


Figure 1: Mechanisms of Vasculopathy and Dysregulation of the Arginine Metabolome

SCD is an arginine deficiency syndrome.[20, 24–26] Normal arginine metabolism is impaired[27–32] for many reasons (Figure 1). Plasma arginine concentration decreases significantly in both adults and children with VOE and is associated with low NOx levels.[29] It was observed that lowest arginine levels were found in children requiring admission for VOE,[29] with arginine levels returning to baseline during convalescence in the hospital. Of interest, **low plasma arginine concentration alone was a sensitive predictor for admission**,[29] while NO_x levels were not, suggesting a function for arginine bioavailability in VOE severity that goes beyond NO. Although adults with SCD are arginine deficient at steady-state,[19, 29] children have plasma levels that are similar to normal controls.[29] Alterations in the arginine metabolome differ in children vs. adults. An arginine deficiency develops with age and is influenced by acute events and chronic end organ damage that worsens over time. Children may therefore be more responsive to arginine therapy during an acute pain event compared to adults.

L-glutamine was recently FDA approved to treat adults and children with SCD, and is an arginine prodrug.[20, 26, 33] Since glutamine supplementation improves arginine bioavailability,[34] similar mechanisms of action may contribute to decreased pain for both arginine and glutamine therapy.[26] Low global arginine bioavailability (GAB) as defined by the ratio of arginine to ornithine+citrulline is associated with both early mortality in SCD[27] and VOE.[29, 35] Increased arginase activity from both inflammatory triggers and more significantly from erythrocyte-arginase release during hemolysis,[27] intracellular arginine transport inhibition, renal dysfunction (which impairs the major route for endogenous arginine biosynthesis),[33] elevated endogenous NO synthase (NOS) inhibitors like asymmetric dimethylarginine (ADMA),[36–38] competitive inhibitors of arginine transport and all NOS isozymes,[39] uncoupled NOS[40] and other consequences of oxidative stress[41–44] lead to low GAB in SCD. These mechanisms may impact an individual's response to arginine therapy. **Although mechanisms of arginine dysregulation are complex and multifactorial,[24–26] they can be overcome through arginine supplementation.[45]** Arginine represents a novel NO-based therapy[6, 31] 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.[46]

2.4 Why Arginine if NO gas does not treat VOE?

Hemolysis will drive arginine consumption, which will ultimately exacerbate NO sequestration and decreased NO synthesis.[19] Under conditions of hypoxia, high ADMA, low arginine, or low essential NOS cofactors,[47] NOS will uncouple, producing reactive oxygen species in lieu of NO, further reducing NO bioavailability and adding to the milieu of oxidative stress. An imbalance between eNOS-derived NO and superoxide generation exists in SCD.[48] 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 and uncoupled while NO bioavailability is low.[40] With NOS uncoupling, inhaled NO gas will be rapidly sequestered by superoxide, forming peroxinitrite known to cause lung damage and cell death. **It is plausible that the provision of NO 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.[46, 49]**

2.5 Therapeutic Potential of Arginine Therapy

Arginine is a safe nutritional supplement that is FDA approved in parenteral form for growth hormone stimulation testing,[50, 51] with nearly 50 years of safety experience through its common use by endocrinologists. Experience with both oral and parenteral arginine therapy in sickle cell disease is growing.

In transgenic SCD mouse models, arginine supplementation inhibits the red cell Gardos channels,[47] reduces red cell density,[52, 53] improves perfusion, and reduces lung injury, microvascular vaso-occlusion and mortality.[40, 43, 44, 54] Arginine increases erythrocyte glutathione levels in both mouse[43] and human trials.[55] Independent of SCD, low global arginine bioavailability is associated with major adverse cardiovascular events including mortality in patients screened for cardiovascular disease,[56] mortality risk in malaria,[57] and is associated with pulmonary hypertension (PH) risk.[31, 58, 59] Rapid healing of leg ulcers was reported with IV ***arginine***-butyrate in both SCD and thalassemia.[60] It was found that short term arginine therapy improves PH in SCD,[61] and acutely increases both plasma and exhaled NO when administered to healthy controls and patients with VOE.[62, 63] When arginine is given to SCD patients at steady-state, a paradoxical decrease in NO_x occurs that is not overcome by higher doses (Fig 2A),[62] clearly indicating that arginine is metabolized differently in SCD compared to controls. However when arginine is given during VOE, a robust dose-dependent increase in NOx is observed. (Fig 2 B and C)[62] This indicates that arginine is also metabolized differently in SCD at steady-state (baseline) compared to times of acute illness including pain.[29, 62, 63] 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 NOx levels, when published data in fact demonstrated a decrease in NO_x with arginine supplementation at baseline. Ultimately, nutritional therapy like arginine may be most beneficial during a deficient state such as VOE.

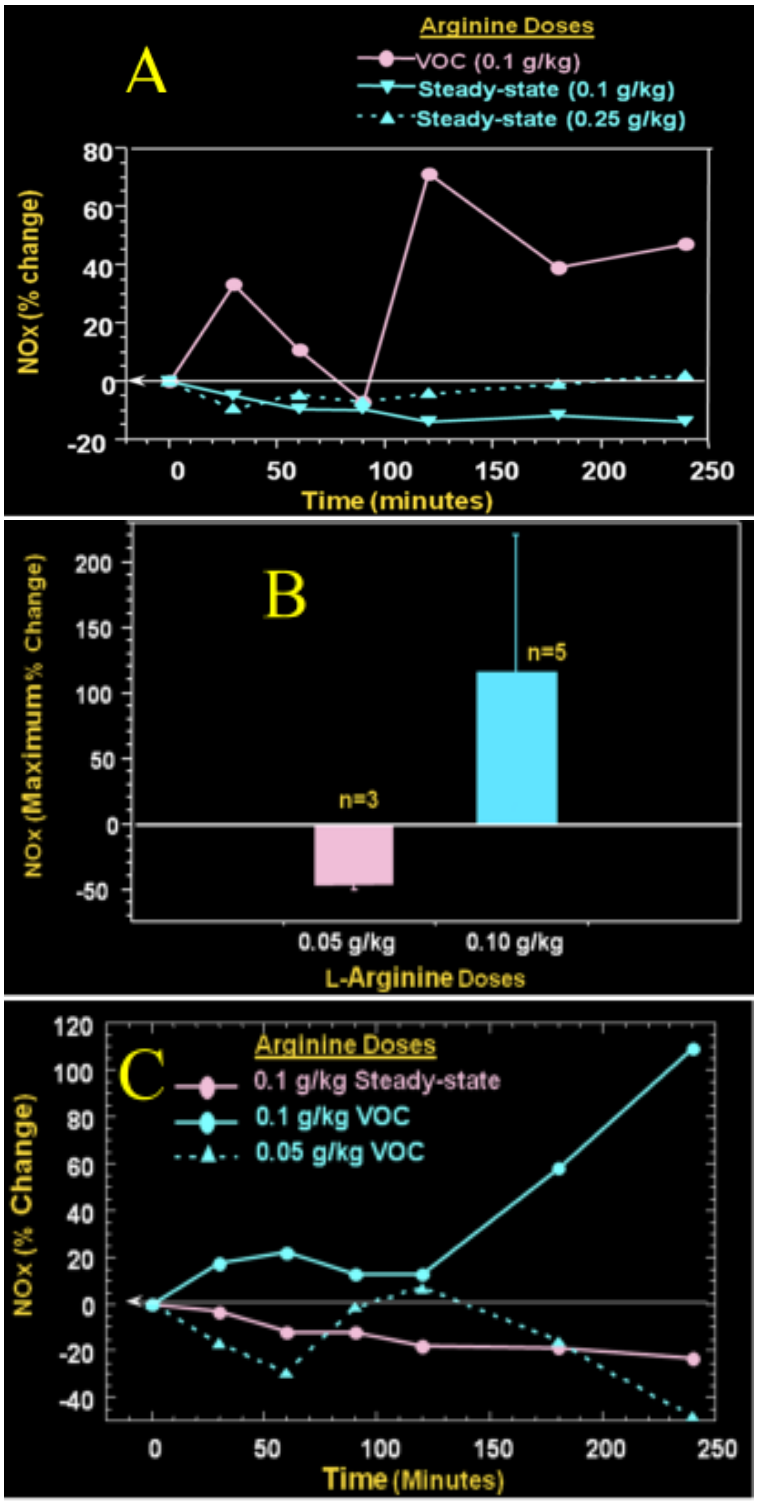


Figure 2: Maximum % change in NO_x in **A**. SCD at steady-state vs. VOE, **B**. VOE after low vs. standard dose L-Arginine, and **C**. Arginine dose response in representative adolescent SCD patient during steady-state versus two (2) separate VOE events.

2.6 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, and may represent an additional flaw in the CSCC prophylactic arginine trial design, as doses used were close to placebo based on the cardiovascular literature.[64] Previous studies have shown that low-dose arginine is unlikely to impact NO synthesis,[64] an observation confirmed in the CSCC study. The capacity of arginine to increase NOx production in SCD is dose-dependent.[62] **Higher levels of plasma arginine are likely needed to overcome multi-factorial effects including impact of arginase and ADMA on global arginine bioavailability, and accelerated arginine consumption during VOE compared to baseline (Fig 2 B and C)**. A 1-time dose of 500 mg/kg or 30 grams IV arginine is safe and commonly used for growth hormone stimulation testing.[65, 66] Since the arginine formula is L-arginine hydrochloride, the safety of multiple higher doses over time is unknown given the potential to induce acidosis, and must be taken into consideration, although no such adverse events have occurred in the Morris arginine trials. 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. In addition, our R34-supported pK study reveals a greater increase in mitochondrial activity utilizing a loading dose (Fig 2).[7]

Based on our preliminary pK studies,[62, 63] peak plasma arginine concentration after oral arginine (0.1 g/kg) is significantly higher during SCD steady-state compared to VOE, although levels are similar by 4 hours. Normal controls reach a peak arginine level between 1-2 hours that is maintained at 4 hours, and does not trend down as in SCD.[62] **Accelerated arginine metabolism or consumption occurs during VOE compared to steady-state despite the same arginine dose given**, likely contributing to an acute nutritional deficiency.

2.7 R34 pharmacokinetic study[7]

Twelve children with SCD hospitalized for VOE were randomized to treatment utilizing one of 3 dosing schemes of L-arginine: 1) 100 mg/kg IV three times/day (TID; n=4); 2) loading dose (200 mg/kg) then 100 mg/kg TID (n=4); or 3) loading dose (200mg/kg) followed by continuous infusion (300mg/kg/day) until discharge (n=4). Platelet rich plasma was isolated and stored 81 at baseline and discharge for each subject and mitochondrial electron transport complex activities measured (Fig 3 on the following page).

Results: Mean age was 14±3 years, 75% had Hb-SS, 67% were male and all were on Hydroxyurea (HU). Compared to SCD patients in steady state,[7] all subjects with VOE had a significantly decreased complex V activity (Fig 3 A). Notably, complex V activity was increased at discharge in children with VOE treated with Arginine in all 3 dosing schemes. However, the increase in complex V activity was greatest with loading dose arms compared to

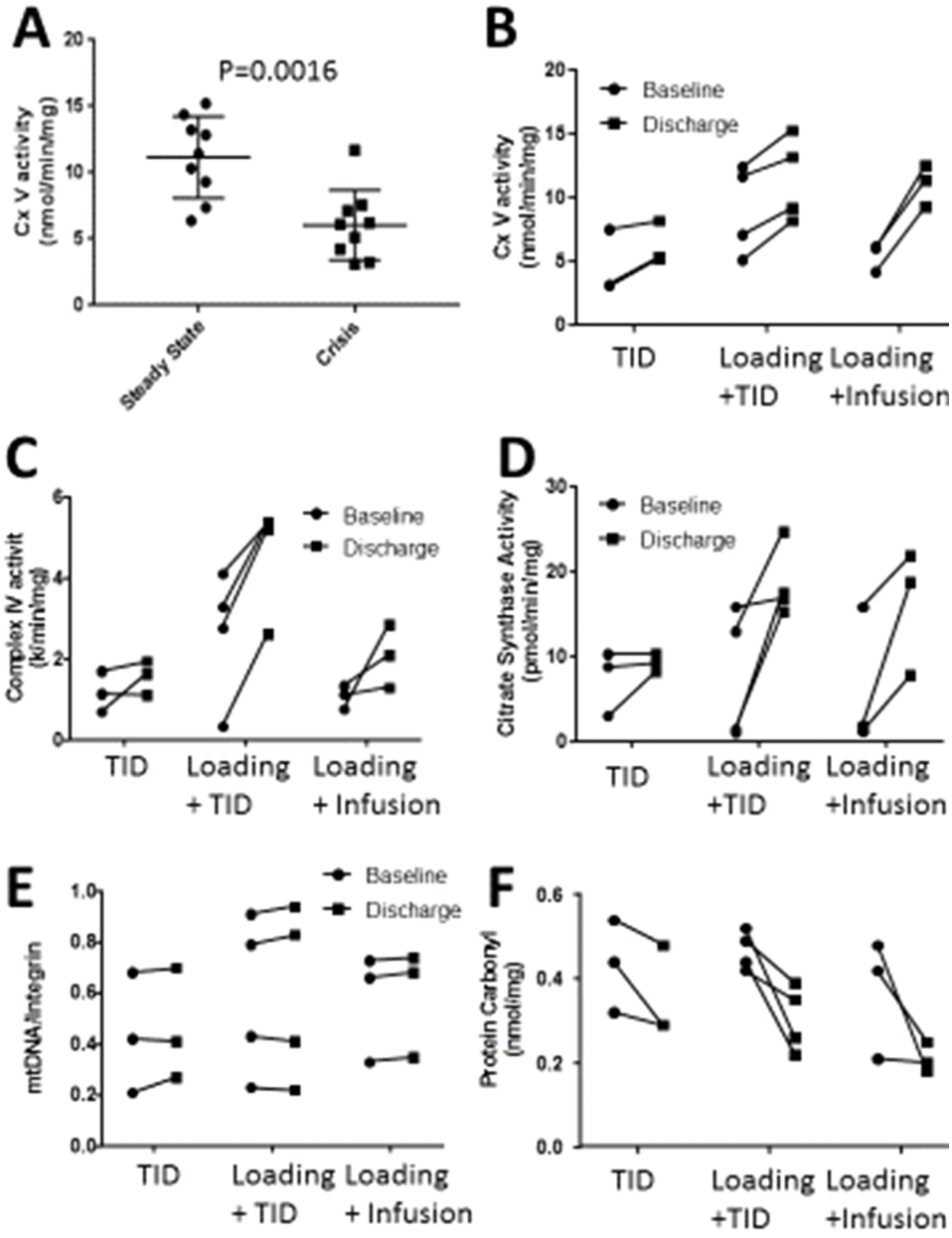


Figure 3: Arginine impact on mitochondrial activity

standard dose (Fig 3 B). While complex IV and citrate synthase activities were not changed in platelets from subjects with VOE vs. steady state (*data not shown*), the activities of these enzymes were also significantly increased in VOE subjects after Arginine treatment (loading dose then continuous infusion, Fig 3 C–D). These changes with arginine are not due to increased mitochondrial number as quantification of mitochondrial DNA before and after arginine were similar (Fig 3 E). IV Arginine significantly decreased levels of protein carbonyls in the platelet-rich plasma, suggesting a decrease in oxidative stress (Fig 3 F). **Conclusion:** Promising reports of oral and IV arginine use for complications of mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes (MELAS) have been described.[67–73] Our data demonstrate for the first time that arginine supplementation increases mitochondrial activity in SCD/VOE, which may mechanistically contribute to decreased pain and less opioid requirement after IV arginine, as well as have further implications for improved metabolism and oxidative signaling in these patients. Although this pilot work did not include a placebo arm, the significant dose-dependent effect suggests a differential effect of L-arginine in SCD.

In total, our data suggest higher arginine consumption during VOE compared to steady-state, that perhaps can be circumvented by a loading dose of parenteral L-arginine at initial presentation for treatment of VOE.

2.8 Results of a single-center, double-blinded, RCT of arginine therapy for children with VOE

A total of 56 children $>$ 3 years old admitted to for VOE were enrolled in this NIH-supported arginine trial *(NHLBI K23 award HL-04386-05).* Mean age was 13.9 $\pm$ 4 years (range 3.6–19 years), and 52% were female; 73% had Hb-SS, 18% Hb-SC and 9% carried S-β thalassemia. Patients received IV arginine (100mg/kg TID) or placebo during hospitalization. **RESULTS:** Patient characteristics were similar between treatment and placebo groups. Mean time between ED triage and delivery of first randomized study drug dose was 20.4 $\pm$ 11 hours, with no difference between the placebo and treatment arm. **A significant reduction in opioid use** (defined as total opioid use in morphine equivalents during hospitalization in mg/kg) by 54% was observed in the arginine treatment arm compared to placebo (mean $\pm$ SEM: 1.9 $\pm$ 0.4 mg/kg vs. 4.1 $\pm$ 0.8mg/kg; p=0.02; Fig 4 A). Pain scores were similar at presentation, but significantly lower at discharge in the arginine arm vs. placebo (Fig 4 B). A clinically relevant trend of decreased hospital length of stay (LOS) by 17 hours favored patients treated with arginine. Total IV opioid use (mg/kg) correlated strongly to LOS (r=0.86, p $<$ 0.0001), and represents a surrogate biomarker of LOS to evaluate study drug efficacy (Fig 4 C). Rates of ACS, transfusion and AEs were similar in both arms. No drug-related AEs were observed. No significant differences were observed between pre and post therapy liver or renal function, or hematological parameters in the arginine group vs. placebo.

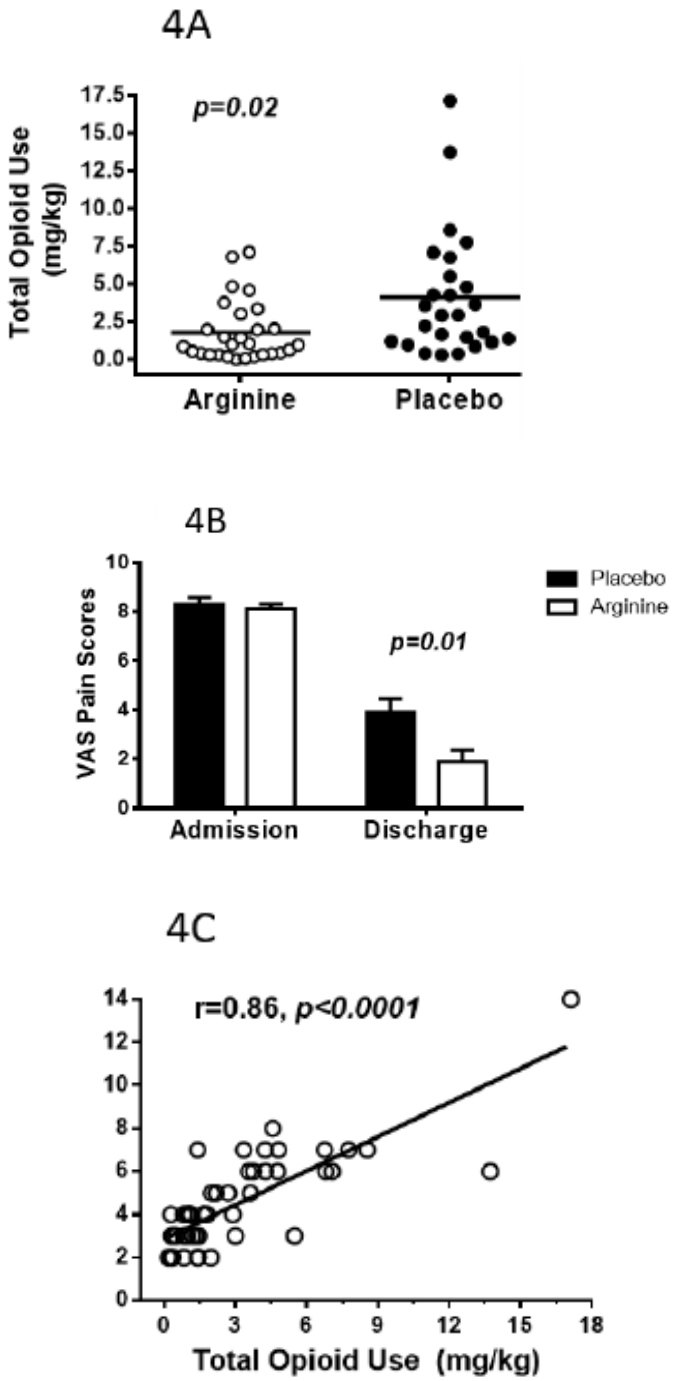


Figure 4: Impact of arginine therapy on total opioid use, pain scores, & LOS. **A**. Arginine supplementation (unfilled circles) led to a significant & clinically relevant reduction in total opioid use by 54% over the course of the hospital stay compared to the placebo group (filled circles). The difference remains significant even when the 2 outliers with the largest total opioid use in the placebo arm are excluded from the analysis ***(p=0.04)***. **B**. Pain scores were significantly lower at discharge in the arginine group compared to placebo by 2 cm.[6] **C**. Correlation between Total Opioid Use (mg/kg) and Length of Hospital Stay (Days).

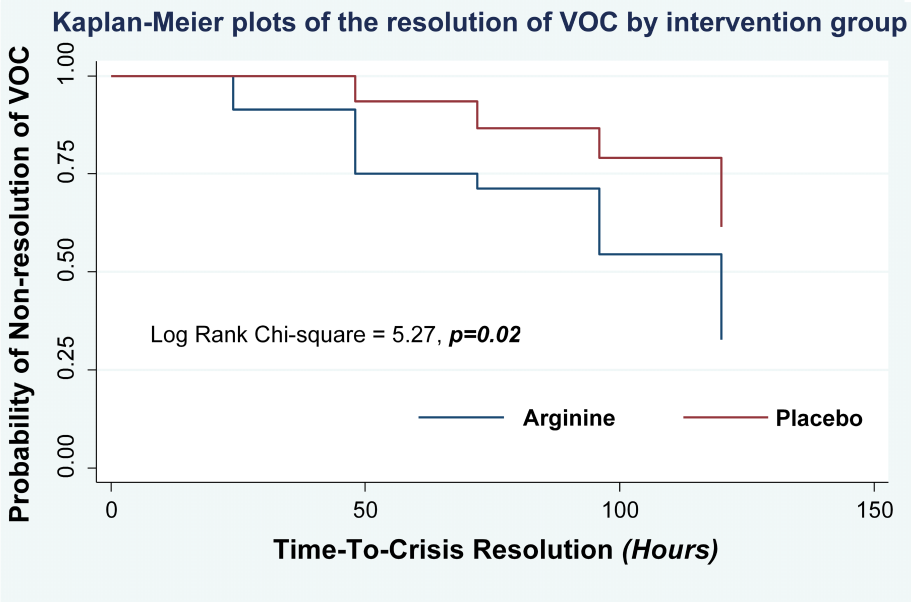
Safety data from 83 children enrolled in a phase 2 RCT protocol utilizing this IND#66943 (Sponsor-Morris), registered with ClinicalTrials.gov (NCT02536170), found no drug-related SAEs occurred and SAE/AE rates are similar across study arms, providing further support for the safety of arginine therapy in children with SCD.[74] Re-hospitalization rates within 72-hours are 6%, which is less than the 20% previously reported in the literature. ED-based recruitment staff available to obtain consent prior to admission is essential for successful enrollment into acute VOE studies, since guardians are often not available once the child is transferred to the inpatient unit. Arginine is a safe, efficacious and inexpensive intervention with narcotic-sparing effects.[6]

2.9 Results of a phase 2 RCT of oral arginine in children with SCD/VOE in Nigeria

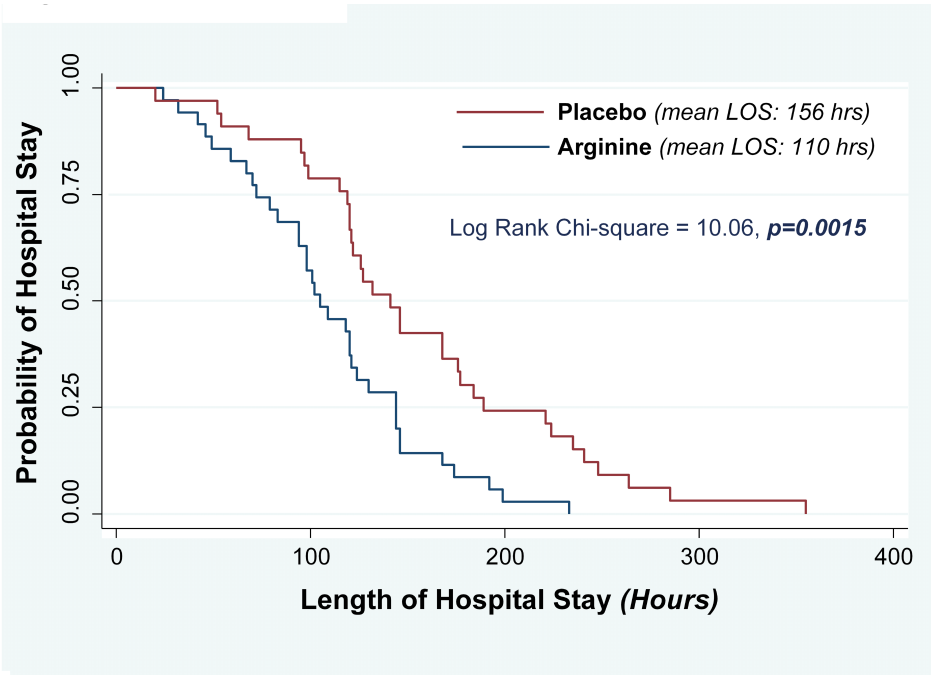
Results of a phase 2 RCT of oral arginine therapy in 68 Nigerian children were reported as the "Best of ASH" in December 2019.[75] Mean age was 10.6±0.4 years, 38% were male and all participants had Hb-SS. The authors found than arginine deficiency plays a role in acute pain requiring hospitalization in Nigerian children with sickle cell anemia, similar to what has been reported in the US. Plasma arginine levels significantly increased with arginine supplementation, and improved global arginine bioavailability was inversely associated with total analgesia and opioids used in VOE management. Total mean analgesia use and pain scores were lower, while time-to-crisis-resolution (Figure 5 A on the next page) and LOS (Figure 5 B on the next page) were shorter in children treated with arginine compared to placebo. No serious adverse events occurred in the arginine arm, while rates of adverse events were similar to placebo, providing further support for the safety of arginine therapy in children with SCA. Oral arginine is a promising adjuvant therapy for sickle cell anemia-VOE management.

3 Overall Study Design

The trial is designed as a double-blind, placebo controlled, randomized, phase 3, multi-center trial of IV arginine therapy in children with SCD and VOE to further knowledge on efficacy and safety of this orphan drug. 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 and mitochondrial function together with important clinical outcomes (time to VOE resolution, pain scores, total parenteral opioid use, PROs, hospital length of stay) in children with SCD and VOE. All patients will receive standard of care treatment for VOE based on NHLBI guidelines.[76] This study will utilize the PECARN network.[77–79]



(a) Probability of Non-resolution of VOC vs. Time-To-Crisis Resolution (Hours)



(b) Probability of Hospital Stay vs. Length of Hospital Stay (Hours)

Figure 5: Crisis Resolution = Pain Score < 4

4 Study Procedures

4.1 Screening and Enrollment

Each of the sites identified by the PECARN Steering Committee and the Study PIs have access to their respective ED Electronic Health Record (EHR) for real time screening along with in person coverage in house for 5–7 days per week and from 18–24 hours each day. The RCs may also be contacted by pager or cell phone should the clinical care team identify any patients that were not identified using the EHR or by the onsite RC. Research staff will approach parents of children and or patients between the ages of 3 and 21 years old in the ED or inpatient ward (by phone or in person) to gauge interest in participation in the study. Recruitment and study procedures will occur during "down" times when the patient is not actively engaged in treatment (e.g. waiting for testing to commence and/or results to return) to avoid interfering with clinical care.

Patient information will be reviewed and eligibility assessed for all patients as appropriate. Research staff will approach eligible patients for consent (see Protections of Human Subjects section for consent procedures). No study procedures will be performed until informed consent is obtained.

4.2 Randomization

Subjects will be randomized to receive either IV arginine or placebo. Permuted-blocks, stratified by clinical center and age group (<12 vs. ≥ 12), will be used. Equal allocation randomization tables will be provided by the Data Coordinating Center to the site research pharmacies. Randomization numbers will be delivered to the clinical center staff using a web-based system. This system will use each enrolled patient's age group (<12 vs. ≥ 12) to deliver the next assigned treatment. Each site research pharmacy will use a randomization number to look up the treatment assignment and prepare the appropriate study drug. The randomization number will be recorded in the database.

4.3 Study Drug Administration

4.3.1 Preparation, Storage & Labeling and Accountability

Study drug will be prepared and blinded from clinical staff and patients by each site research pharmacist. All sites will be responsible for documentation of drug accountability including storage and dispensing information.

4.3.2 Dosing Schedule

Patients age 3–21 years will be randomized to one of two study arms:

- A one-time L-arginine loading dose (200 mg/kg IV) + standard dose (100 mg/kg IV TID); OR
- Placebo (normal saline) loading dose (2ml/kg IV) + 1ml/kg IV TID.

The maximum loading dose will be 20 gm, with a standard dose maximum of 10 gm IV TID, which will continue until ED or hospital discharge, or for 21 doses, whichever comes first. (Dosing can be ordered every 8 hours, but there is TID flexibility)

4.3.3 Discontinuation of Study Drug

Study drug will be stopped if a patient meets any one of the following criteria:

1) Neurological dysfunction or development of stroke while on protocol
2) Allergic reaction to study medication
3) Patient's request
4) Abnormal lab results:
    - Hepatic dysfunction: SGPT (ALT) >6X upper value; OR
    - Renal dysfunction: Creatinine >1.2; OR
    - Acidosis with bicarbonate <16

Arginine/placebo will be terminated, but clinical data and research blood collection will continue.

NOTE: This is different than withdrawal from the study. The clinical or research teams may stop study drug administration for development of AEs or SAEs as described above without withdrawing from the study. The participant may also decide to stop study drug without withdrawing consent to participate. See section 4.6 on page 32 (Withdrawal from Study) for additional information

4.3.4 Maintaining Blinding during the Study

In the event of adverse events thought to be related to arginine (see above) providers should assume that the subject is receiving active drug (arginine) and take appropriate steps. The clinical team and investigators should not attempt to unblind themselves.

4.4 Sample Collection and Processing

Blood (for plasma, erythrocytes and platelets) will be drawn, processed and stored for future batched analyses at the time of presentation prior to study drug delivery (pre-Dose), on day 1 and prior to discharge (or ED discharge, whichever comes first) (Table 2 on the following page). The impact of IV arginine therapy on global arginine bioavailability will be determined and compared to the placebo arm. Mitochondrial respiratory complex activities will be measured as an indicator of mitochondrial function in platelets; aconitase activity will be measured as an index of mitochondrial oxidative damage.[80] These samples will also serve to establish a SCD-VOE biorepository to explore future mechanistic studies.

Urine or serum pregnancy test results will be reviewed prior to randomization for female participants 13 years or older.

Sample collection, processing, storage, and shipping instructions will be detailed in a separate document.

Table 2: Laboratory monitoring will be undertaken according to the schedule below

	Pre-Dose[1]	Day 2[2]	Day 8 or Discharge[3]
CBC with retic count (clinical)*	X	X	X
Comprehensive metabolic panel	X**	X	
BHCG (girls $\geq$ 13 yrs)	X		
Research Labs (Aim 3)	X	X	X

1 – Pre-dose is before the start of study drug.

2 – Day 2 is the day after study drug is administered (starting at midnight of the day the study drug is started)

3 – Day 8 is the day that the last dose #21 will be given for patients who have not yet been discharged

***when available clinically

****Last CMP in medical record will be reviewed prior to randomization (up to 12 months): if abnormal (meeting criteria for discontinuation of study drug) or not available, a CMP will be obtained Pre-Dose and reviewed before randomization. Patients with CMP obtained at pre-dose that meet criteria for discontinuation of study drug will be excluded from participation. All other patients will have a CMP obtained on day 2. Patients with ALT > 3X upper limit, creatinine >1.0 or bicarb of 16 pre-dose or day 2 will have CMP repeated in 24 hours.

4.5 Data Collection

Primary Outcome Measure: *Time-to-crisis resolution*, defined as the time in hours from study drug delivery to time of last dose of parenteral opioid delivery.

- Data being collected: date and time of first study drug administration and last IV opioid treatment

Secondary Outcome Measures:

1) Total parenteral opioid use (morphine equivalents, mg/kg)
   - Data being collected: time of IV placement, opioid monitoring
2) Pain scores at presentation vs. discharge; Daily highest and lowest pain scores recorded
   - Data being collected: Pain Scores (0–10 scale)
3) Three Patient Reported Outcomes (PROs),[81–83] performed within 12 hours of study drug delivery and on the day of discharge.
   (a) PROMIS Pain Interference
   (b) PROMIS Pain Behavior
   (c) PROMIS Fatigue

Tertiary Outcome Measures:

1) Patients ages 8 years of age and older and parents of patients ages 3–17 years of age will answer a general health question noted below:

   At time of discharge from the hospital:

   "Since you left the emergency department, how has your pain been? "No pain since in the Emergency Department, "Much better, "A little better, "The same, "A little worse, "Much worse

2) Oxygen saturation at discharge

   - Data being collected: Vital Signs

3) Medication Quantification Scale (MQS)[84] pre-dose and on day of discharge; daily.

4) Other Patient Outcomes not listed as secondary outcomes

Patient Reported Outcome (PRO) Data Collected:

1) Pediatrics - Ages up to and including 17 years old

   - **Pediatric PROMIS:** (35 items)
     To be completed by patients ages 8–17 years of age and parents of children ages 5–17 years of age.
     (a) pain behavior (8 items),
     (b) pain interference (8 items),
     (c) pain intensity (1 item),
     (d) physical stress experiences (8 items), and
     (e) fatigue domains (10 items)

   - **Peds QL SCD module:** (19 items)
     To be completed by children ages 5–17 years of age and parents of children ages 3–17 years of age
     (a) Pain and Hurt (9 items) and
     (b) Pain Impact (10 items) domains

   Total Pediatric PRO items to complete: 54 + 1 general health question = 55 items

2) Adults – 18 years of age and older

   (a) **PROMIS:**(25 items)
   (a) pain behavior (8 items),
   (b) pain interference (8 items),
   (c) pain intensity (1 item), and
   (d) fatigue (8 items)

(a) **PROMIS ASCQ-ME** (48 items)
   (a) Emotional Impact Short Form v2.0 (5 items),
   (b) Pain Episode Frequency and Severity (5 items),
   (c) Pain Impact Short Form v2.0 (5 items),
   (d) Sickle Cell Medical History Checklist (9 items),
   (e) Sleep Impact Short Form v2.0 (5 items),
   (f) Social Functioning Impact Short Form v2.0 (5 items), and
   (g) Stiffness Impact Short Form v2.0 (5 items)

Total adult PRO items to complete: 64 items + 1 general health item = 65 items

4.5.1 Schedule of Events

The Schedule of Events (table 3) summarizes data collection for each time point in the study.

Table 3: Schedule of Events

	Screening	Pre-Dose[1]	Day 1[2]	Day 2[3]	Day 3–7[4]	Day 8 or Discharge[5]	Day 14 and/or Day 28[6]
I/E	X	X					
Demographics and Genotype		X					
Study Treatment Assignment		X					
Medical History	X	X					
Pain Crisis Start Date and time[a]		X					
Time of Last IV opioid/Pain Crisis End date and time [b]						X	
Number of ED visits/hospitalizations in last year (excluding current)		X					
ED admission information		X					
Time of IV placement		X					
Conmeds		X	X	X	X	X	
CMP[d]	X[d]	X[d]		X[d]			
BHCG[e]		X					
Research Labs		X		X		X	
Vital Signs[f]	X		X	X	X	X	
Pain Score[f]	X		X	X	X	X	
PROs (PROMIS/Peds) QL/ASCQ-Me (adults)		X (within 12 hours)				X	
MQS Score		X	X	X	X	X	X
Symptoms Questionnaire[g]		X		X	X	X	X
Opioid Monitoring[h]	X	X	X	X	X	X	X
Maintenance Fluid Monitoring[h]		X	X	X	X	X	
AE[i]		X	X	X	X	X	X
SAE[i]		X	X	X	X	X	X
Study Drug Administration[j]			X	X	X	X	

Continued on next page...

Table 3 – *continued from previous page...*

1 – Predose is all information that is collected before the start of study drug.

2 – Day 1 is from the start of Study Drug to midnight of the day study drug is started. Pre-Dose and Day 1 may occur on the same day.

3 – Day 2 is the day after study drug is administered (starting at midnight of the day the study drug is started).

4 – Individual patient hospitalization duration will vary. Patients may be discharged at any time between Day 1 through Day 7.

5 – Day 8 is the day that the last dose #21 will be given for patients who have not yet been discharged.

6 – If patients are not discharged by Day 8, the an AE/SAE review will occur daily until Day 14. ED revisits and hospital admissions will be captured through 28 days following hospital discharge.

a – Pain Crisis start date/time is defined as the date/time the patient started experiencing pain (this could be before ED visit).

b – Pain crisis end date/time is defined as the date/time of last dose of parenteral opioid delivery.

c – Research labs: will be obtained with routine daily blood draws whenever possible and may be drawn on days when the research and clinical team suspect the patient may be discharged to avoid multiple venipunctures. In the event the patient is not discharged on that date, discharge labs should be drawn again prior to discharge. Baseline labs to be drawn before the start of study drug.

d – Screening CMP from a clinically ordered CMP or the last CMP in the medical record within the last 12 months will be reviewed. If there are any values within higher than protocol defined cut offs for study drug withdrawal, then a CMP is to be drawn and reviewed prior to randomization. For participants with normal past CMP, a CMP will be done on day 2.

e – Pregnancy test obtained at Pre-Dose for females 13 years and older.

f – The first set of Vital Signs (including oxygen saturation) and Pain scores will be captured when the patient first presents to the Emergency Department. The highest and lowest pain scores will be recorded from Day 1 through Day 8 (or within 6 hours of Discharge). The first set of vitals signs after 6 am will be collected up until Day 8/Discharge, whichever comes first. Ensure oxygen saturation is captured at Pre-Dose and within 6 hours of Discharge.

g – Symptoms Questionnaire will be completed daily through Day 14 or Discharge.

h – Use of Parenteral Opioids will be documented every shift by nursing staff per standard protocol, including opioids delivered by the PCA device as well as any intravenous, intranasal and/or oral opioids used. Data on daily opioid use will be collected and recorded.

i – AEs and SAEs will be monitored daily through discharge or through 14 days of hospitalization; Return ED visits and hospitalizations will be followed for 28 days after hospital discharge.

j – Patients will be randomized within 12 hours of their initial dose of parenteral opioid in the ED or ward and Arginine will be administered TID.

4.6 Withdrawal from Study

As noted in Section 4.3.3 on page 27, this is a distinct concept from stopping the study intervention. Patients and/or guardians may decide to stop participating in the study at any time. If someone says they want to stop being in the study, they USUALLY mean they want to stop the intervention. Efforts should be made to clarify their specific preferences (withdrawal from the intervention, withdrawal from follow-up, withdrawal from data collection, etc.) Attempts should be made to continue safety data collection.

5 Data Analysis

All analyses will be undertaken by the intention-to-treat (ITT) principle. Patients who drop out (discontinue study drug) will be followed and included in the ITT analysis. All statistical tests of hypotheses will be two-sided.

The hypotheses of this study are:

1. Arginine therapy will decrease time-to-crisis resolution in children with an acute sickle cell vaso-occlusive pain episode compared to placebo.

2. Arginine therapy will lower total parenteral opioid use (mg/kg) compared to placebo.
3. Arginine therapy will improve pain scores compared to placebo.
4. Arginine will have similar safety profile than placebo.
5. Arginine therapy will improve global arginine bioavailability and mitochondrial activity.

5.1 Specific Aim Analyses

Specific Aim 1: **To determine the efficacy of IV L-arginine, when added to standard therapy, on the time-to-crisis resolution in children with SCD and VOE compared to placebo.**

The primary outcome (time-to-crisis resolution) will be analyzed by a Van Elteren test, stratified by clinical center and age group. The significance level for the primary comparison will be set at 0.05. The total length of stay in the hospital will also be analyzed, and this is expected to show the same result as the analysis of the primary outcome.

In addition to the assessment of the statistical significance of the treatment effect, the distribution of the primary outcome in each treatment arm using standard summary measures and an estimated difference in means between the two arms with a two-sided 95% confidence interval will be described.

The overall significance level for statistical tests on the secondary outcomes will be set at 0.05. Holms method will be used to adjust for multiple comparisons.[85] The numeric secondary outcomes (opioid use, change in pain, and PRO measures) will be analyzed in the same manner as the primary outcome.

Specific Aim 2: **To determine the safety profile of IV L-arginine in the treatment of acute VOE in children with SCD.**

Analysis of safety outcomes (acute chest syndrome, requirement of blood transfusion, oxygen use, return visits, and clinical deterioration) will be conducted using Mantel-Haenszel chi-square tests, stratified by clinical center and age group.

Summaries of incidence rates (frequencies and percentages), intensity, and relationship to study of individual Adverse Events (AEs) by System Organ Class and Preferred Term (MedDRA) will be prepared. All AEs beginning after randomization through 14 days or until hospital discharge (whichever comes first) will be included. ED revisits and hospital admissions will be tracked through day 28 after hospital discharge. The occurrence of any adverse event will be considered as a dichotomous outcome and will be compared between groups using a Mantel-Haenszel test, stratified by clinical center and age group. Adverse events will be coded using the latest version of the Medical Dictionary for Regulatory Activities (MedDRA).

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

Arginine mechanisms of action are multifactorial and elusive in VOE, which is why incorporating an exploratory mechanism aim is important. However, it likely involves NO (Fig 1–2). SCD patients have an arginine deficiency that acutely worsens during VOE that should be assessed and treated. The benefit of arginine therapy may be in part- by simply replenishing a deficient nutrient. It was found that children are metabolically different during VOE compared to baseline with respect to the "arginine metabolome", which includes amino acids that are precursors to (i.e. glutamine) or byproducts of arginine metabolic pathways (ornithine, citrulline, polyamines etc.). Hemolysis releases erythrocyte-arginase, which contributes to arginine consumption (Fig 1). This showed that the degree of arginine deficiency correlates with pain severity and need for hospitalization.[5] It is essential from a mechanistic perspective to measure arginine levels in an arginine RCT pre and post-treatment. If arginine is working as a "drug", there may be effects for all patients. However if we are merely treating an acute nutritional deficiency that has consequences (i.e. vaso-occlusion), the most robust response may occur in patients who have the greatest deficiency.[86] Arginine bioavailability and the GABR will be determined pre and post treatment. GABR is associated with SCD complications and mortality risk,[11, 87] while others demonstrate a link to mortality risk and adverse events in cardiovascular disease[55] and malaria.[56] This mechanistic aim will determine whether GABR reflects an objective biomarker of pain severity, or one that identifies patients most likely to positively respond to arginine therapy. Subgroup analyses of clinical outcomes will be performed in patients from each tertile of GABR. Finding novel biomarkers that identify subgroup responders to drug therapies is an FDA and American Society of Hematology priority recently discussed at the SCD Clinical Endpoints Workshop.[88] It was also demonstrated that IV arginine therapy increases mitochondrial activity (Fig 3). This proposal offers an opportunity to study the impact of arginine on mitochondrial function in a placebo-controlled fashion, necessary to assign causality. Improved mitochondrial function may be associated with decreased oxidative stress, a concept supported by our pilot data showing decreased protein carbonyls levels. Arginine is already used clinically in mitochondrial disorders;[1, 67, 69–72] this would be an important novel mechanism of arginine in SCD to elucidate further, could lead to a new area of research and is easy to measure.[66, 89] Blood (for plasma, erythrocytes and platelets) will be drawn, processed and stored for future batched analyses at the time of presentation prior to study drug delivery (day 0), on day 1 and prior to discharge (or ED discharge, whichever comes first). The study will analyze the correlation between GABR and pain scores/opioid use, and the impact of IV arginine therapy on GABR compared to the placebo will be determined. Platelet mitochondrial respiratory complex activities will be measured as an indicator of mitochondrial function and aconitase activity as an index of mitochondrial oxidative damage.[66]

3b. This aim will establish a large SCD-VOE biorepository to explore future mechanistic studies. Currently a biorepository of pediatric SCD-VOE samples does not exist. Acute pain samples are challenging to acquire given the unpredictable nature of ED visits. Blood is already being collected in this protocol to measure GABR and mitochondrial

function; storing addition samples adds significant value to the proposal at minimal cost, to answer future mechanistic questions. Additional analyses may be of interest (like arginase, ADMA, NOx, creatine etc.), but would require additional funding. This project would create the largest biorepository of SCD/VOE samples in existence; it would be a missed opportunity to not bank additional sample for future mechanistic studies given the effort of evaluating 360 children with SCD and VOE as part of this protocol.

5.2 Sample Size Calculations and Statistical Power

Based on a previous small trial, the difference between arginine and placebo groups is approximately 0.7 days (length of stay, which parallels time to crisis resolution). A Type I error bound of 0.05 (alpha) for the primary hypothesis will be used and it will be assumed that age is not informative of outcome (a conservative bound for sample size). A simulation-based approach is used to determine the sample size required to detect a difference (shift in distribution) of 0.7 days with 85% power. In order to simulate placebo outcomes, time-to-crisis resolution data from the MAGiC trial[90](both arms combined, as the treatment effect did not reach statistical significance)was used. For the arginine arm, outcomes were simulated from the same distribution, shifted down by 0.7 days. 5,000 trials were simulated. This process resulted in a required sample size of 166 per arm. Accounting for withdrawal ($\sim$1%), interim efficacy monitoring (inflation of 2%), and crossover/non-adherence (3%, for example, arginine subjects who do not receive a sufficient amount of study drug) results in an overall inflation of about 8% or a total of 180 per arm. Prior to the first interim analysis (after approximately 100 subjects), the DCC and CCC principal investigators, along with the DSMB, will review, blinded to by-treatment results, summaries of the outcome distribution (mean, median, IQR with both arms combined – to compare with MAGiC data), withdrawal rate, and non-adherence rates and may adjust the target sample size accordingly, as funding allows.

Given a total sample size of 180 per arm, the effect size that will be detectable in subgroup analyses of the primary outcome, for example, a differential treatment effect based on gender or hydroxyurea use will also be reported. With 85% power, it will be possible to detect an effect size (difference in differences) of about 1.3 days, or approximately 1.9 times the main treatment effect size for which the study is powered. With 80% power, it will be possible to detect an effect size of 1.2, or 1.7 times the main treatment effect size.

5.3 Subgroups

The formal subgroups specified for this trial will be age group, hydroxyurea use, and sex. The interaction between assigned treatment and each subgroup factor in a linear model, at a significance level of 0.05/3 will also be tested. The model will include a main effect for treatment, a main effect for subgroup factor, and an interaction between the subgroup factor and treatment. Secondary outcomes in similar models will also be observed.

5.4 Missing Data

High levels of missing outcome data are not expected in the STArT trial, as the outcomes are quite short-term and readily available. In the event that a substantial number of subjects are withdrawn, baseline characteristics and available information on hospital course will be reviewed and compared to subjects not withdrawn, to assess empirically if these subjects differ from those remaining in the study for the scheduled treatment and data collection. Sensitivity analyses to assess whether the results are robust will also be performed.

5.5 Interim Analyses

The Data and Safety Monitoring Board and trial monitoring plan are discussed in detail in the Data and Safety Monitoring Plan. A two formal interim efficacy analyses of accumulating data, after approximately 150 and 250 subjects are enrolled, to be reviewed by the DSMB is proposed. Aside from these formal analyses, the DSMB will meet at least yearly to review safety data.

Early stopping will be considered if efficacy has been established. Two-sided O'Brien-Fleming boundaries, implemented using a flexible alpha-spending approach, will be used for efficacy monitoring. Specifically, the significance levels that will be used at the first and second interim analyses are 0.001 and 0.014, respectively. If the trial reaches the anticipated total sample size, a significance level of 0.046 will be used for the final test.

As the trial can provide valuable scientific information, even in the face of a non-significant primary result, early stopping for futility is not anticipated. However, the DSMB and NHLBI will evaluate all aspects of the trial and determine at any point in time whether futility should be considered. It is expected that an informal, conditional power approach will be used to provide statistical information to guide the futility analysis if and when needed. Specifically, the probability of the study finding a significant effect, if continued, will be calculated under a range of plausible true effect sizes. A conditional power less than 20%, given the most liberal plausible effect size will indicate futility. The DCC biostatistician will point out assumptions, advantages, and limitations of the futility analysis approach to the DSMB. The DSMB will consider other factors, including the burden of finishing the trial at its current point, in its deliberations.

6 Data Management

6.1 Clinical Site Data Management

The Data Coordinating Center will create the electronic data capture (EDC) system and worksheets that can be used by clinical site research coordinators and investigators.

Data will be entered via the Web into the EDC. Worksheets and study documents will be maintained in secure locations at each site. The site will maintain an Essential Documents

Binder, which may be in paper or electronic form. Copies of all informed consent documents will be kept on file and be available for site monitoring inspection (on site or remote).

6.2 Electronic Data Capture

Under the direction of the DCC PI and biostatistician, and in collaboration with study investigators, the DCC will be involved with the development of electronic data capture (EDC) systems to collect information on screening and enrollment, survey responses, and safety measures. The DCC uses a web-based interface designed specifically for clinical trials and observational studies. The DCC has developed a sophisticated software system for managing data discrepancy queries. This Query Management System allows for data checks on individual data fields (e.g., for missing or out-of-range data) in addition to the validation of data between different forms. This helps ensure that data will be complete and valid.

6.3 Data Coordinating Center

6.3.1 Data Center Description

Overview The Data Coordinating Center (DCC) in the Department of Pediatrics at the University of Utah School of Medicine provides data coordination and management services for a variety of national research networks. Anchoring these services is a state-of-the-art, energy efficient data center. The data center facility supports more than 3500 users around the country and provides a secure, reliable, enterprise-wide infrastructure for delivering mission critical DCC systems and services.

Facility, Hardware, Storage, Data Backup and System Availability The data center was built using industry standards and energy efficient cooling solutions. The data center is cooled by Liquid Cooling Package (LCP) inline cooling technology, providing the desired efficiency, redundancy and the modularity. The LCP utilizes a hot/cold aisle design that allows for even air distribution to minimize hot spots. The data centers electrical power system contains an uninterruptible power supply (UPS) with a diesel backup generator. The data center is protected with an FM-200 backed fire suppression system, which provides waterless fire suppression without leaving behind residue or particulate. Enhanced security measures are implemented to safeguard the equipment and the data within in it. Security measures are enforced 24 hours a day, 7 days a week, 365 days a year by a combination of on-premise security guards, University police officers and video surveillance.

The data center has a virtualized environment. This environment consists of more than 415 virtual servers across 25 host servers. Virtualization provides key advantages: High availability (HA) – in the event of hardware failure, virtual machines (VM) automatically restart on healthy resources, minimizing impact to end-users; Flexible infrastructure – compute and storage is seamlessly scaled as current needs change; Rapid deployment - new resources are provisioned on-demand.

Production servers running mission critical applications are clustered and configured for failover events across multiple clusters. Entire servers are backed-up to a dedicated infrastructure. The backup repositories are encrypted-at-rest using AES-256. The storage area networking (SAN) applications, clusters, and switch-to-switch links are highly redundant. Incremental backups of data occur Monday through Friday. A full data backup occurs weekly. Full backups are sent off-site on a weekly basis to a secure secondary location. The data center currently manages over 125 terabytes (TB) of data.

Information systems are available 24/7/365 unless a scheduled maintenance period or mitigation of an unexpected event is required. Critical systems availability has exceeded 99.9% for the past 5 years.

6.3.2 Security, Support, Encryption and Confidentiality

The data center coordinates the network infrastructure and security with University Information Technology (UIT) at the University of Utah. This provides us with robust firewall hardware, automatic network intrusion detection, and the expertise of dedicated security experts working at the University. Centralized authentication and communication over public networks are encrypted using transport layer security (TLS) and/or virtual private network (VPN) technologies. Direct access to data center machines is only available while physically on premise or via a VPN client.

All network traffic is monitored for intrusion attempts. Security scans are regularly run against data center servers and IT staff are notified of intrusion alerts. Security is maintained with Windows user/group domain-level security. Users are required to change their passwords every 90 days. All files are protected at user/group levels and database security is handled in a similar manner with group-level access to databases, tables, and views. Finally, all laptops used by faculty and staff in the DCC are whole-disk encrypted.

The data center uses monitoring tools to continuously monitor applications and servers. Environmental and network systems are also monitored to ensure up-time. System Administrators are on-call 24/7/365 to respond to urgent events.

All personnel involved with the DCC have signed confidentiality agreements concerning data encountered in the course of their daily work. All personnel (including administrative staff) have received Human Subjects Protection and Health Information Portability and Accountability Act (HIPAA) education. We require all users to sign specific agreements concerning security, confidentiality, and use our information systems before access is provided.

6.4 Records Access

The medical record and study files (including informed consent, permission, and assent documents) must be made available to authorized representatives of the Data Coordinating

Center, upon request, for source verification of study documentation. In addition, medical information and data generated by this study must be available for inspection upon request by representatives (when applicable) of the Food and Drug Administration (FDA), NIH, other Federal funders or study sponsors, and the Institutional Review Board (IRB) for each study site.

6.5 Study Monitoring

The investigators recognize the importance of ensuring data of excellent quality. Study monitoring is critical to this process. Monitoring has been a very effective tool for maintaining data quality in previous Pediatric Emergency Care Applied Research Network studies, and this process will be utilized to ensure excellent quality data in the proposed study. The DCC utilizes risk-based methodology to identify and correct problems that may arise at sites. The risk-based approach to monitoring focuses on oversight activities and preventing or mitigating key risks to data quality, as well as to processes critical to human subject protection and integrity of the trial or study.

Study monitors must be provided with appropriate access to study materials and the medical records for study subjects. If the medical records are in electronic form, the clinical investigator or an authorized individual must provide any assistance necessary to facilitate the study monitors review of data in the electronic medical record.

6.5.1 Site Monitoring Plan

A supplemental study-specific, risk-based monitoring plan, separate from the protocol will be completed which outlines specific criteria for monitoring. This plan may include the number of planned site visits, criteria for focused visits, additional visits or remote monitoring, a plan for chart review and a follow up plan for non-compliant sites. The monitoring plan also describes the type of monitoring that will take place (e.g., sample of all subjects within a site; key data or all data), the schedule of visits, how they are reported and a time frame to resolve any issues found.

6.5.2 Clinical Site Monitoring

Site monitoring visits will be performed by a trained site monitor during the study period to ensure regulatory compliance, patient safety, and to monitor the quality of data collected. Essential document binders, regulatory documents and data collection forms may be reviewed. Interim visits will take place depending on grant budget, site enrollment, and compliance issues identified. The site monitor will provide each site with a written report, and sites will be required to follow up on any deficiencies. It is anticipated that the study monitoring visits for this protocol will consist of a site initiation visit (prior to patient enrollment), interim visits, and a close out visit. The site initiation may take place as group training made up of site investigators and research assistants. Site monitoring visits may be conducted in-person or virtually, in conjunction with remote monitoring activities, depending on the specific monitoring activities being conducted.

6.5.3 Remote Monitoring

The Data Coordinating Center may also conduct remote monitoring activities. Remote monitoring involves detailed review of the data entered by the Clinical Center and consultations with the Clinical Center investigator and/or research coordinator to review safety and data quality. This may require uploading copies of medical records, patient study files, regulatory documentation, or other source documents for the monitor to review. Alternatively, other methods, such as remotely viewing source documentation, may be utilized. This helps assure protocol compliance and accurate data collection. The Data Coordinating Center may conduct more remote monitoring activities early in the trial to assure protocol compliance and identify any training issues that may exist. Remote monitoring documents will be retained in accordance with federal requirements. Safety of subjects will be monitored and ensured in accordance with the Data and Safety Monitoring Board (DSMB) plan.

6.5.4 Pharmacy Monitoring

The Clinical Center pharmacy must maintain adequate study drug accountability records of all dispensed study drug. Each pharmacy will be monitored and may be requested to send copies of these documents to the Data Coordinating Center. Since this study will use a central pharmacy, that pharmacy must also maintain adequate records and will also be monitored.

7 Protection of Human Subjects

7.1 Institutional Review Board (IRB) Approval

The Data Coordinating Center and each clinical center must obtain IRB approval prior to participating in the study. A Single IRB (sIRB) will be used for this study. The University of Utah IRB will serve as the IRB of record.

The Data Coordinating Center will track IRB approval status at all participating centers and will not permit subject enrollment without documentation of initial sIRB approval and local review sign-off. The Data Coordinating Center will also track the maintenance of that approval throughout subsequent years of the project.

7.2 Informed Consent

Parental Permission/Subject Consent

Written permission from parents or legal guardians will be required for participation for subjects who are eligible for this study and are under 18 years of age. Documentation of consent may be either in paper or electronic form due to safety precautions in place for the COVID – 19 Pandemic. Patients 18 years and older will consent for themselves. After determining that a subject is eligible, the site investigator or designee will approach the parent or legal guardian to offer participation for their child in the study. The parent or legal guardian will be informed about the objectives of the study and the potential risks and

benefits of participation. Subject will only be enrolled if their parent or guardian provides permission for their child to participate.

Child Assent

Children who are capable of giving assent and who are alert and competent, will be asked, following an age-appropriate discussion of risks and benefits, to give assent to the study or further study procedures. Assent will be waived if the child is too young, has a severely reduced mental age, decreased level of consciousness, psychological problems, is 6 years old or younger, or other legitimate reasons as judged by the Institutional Review Board.

7.3 Waivers Requested

Waiver of Authorization

A waiver of authorization is requested in order to be able to pre-screen/establish eligibility and medical review for subject prior to approaching, consenting, and enrolling a subject.

7.4 Potential Risks

The risks in this study are considered low. Loss of confidentiality of the subject is a potential risk of the study; however, safeguards are in place to protect against this.

All consented patients will undergo phlebotomy and IV placement as part of their clinical care. Patients admitted to the floor may undergo additional study blood draws before discharge. Blood samples for this study will be drawn at the time of other phlebotomies whenever possible to limit discomfort to the patient. Discomfort from venipuncture will be experienced, and bruising from the blood draws are a potential risk to the patient.

For patients randomized to study drug in the clinical trial, the risks of the study are confined to those associated with the arginine administration. As with any food or medication, the risk of an allergic reaction is a small but possible complication. Arginine is a nutritional supplement with a low toxicity and has been safely used in many human studies, including a number of studies in both adults and children with SCD.[51] Intravenous arginine is preferred over oral due to mild stomach discomfort may be experienced at higher doses, and issues with compliance with respect to ingesting a large number of pills. Intravenous arginine may cause flushing, headache or vomiting. Patients with known compromised liver or kidney functions will be excluded from participation. A significant overdose of L-arginine hydrochloride can cause acidosis and hyperkalemia particularly among patients with renal failure.[91] However, doses 5X higher than the standard dose used in these studies is typically used for growth hormone stimulation testing, and is considered safe.

There are no age-related risks associated with an arginine infusion. The majority of experience outside SCD for use of IV arginine is in younger children undergoing growth hormone stimulation testing. The amount of fluid a patient receives is based on weight, and

is 1-2ml/kg, which poses no risk for fluid overload.

All patients enrolled in the study will also receive treatment for vaso-occlusive pain episodes that has been standardized across all participating sites, based on 2014 NHLBI guidelines[76] and the expertise of hematology and emergency medicine specialists with vast sickle cell clinical experience.

7.5 Protections Against Potential Risks

Regarding loss/breach of privacy and confidentiality, all applicable parties (e.g. clinical sites, DCC) will be responsible for ensuring that appropriate data security procedures are in place.

Subjects enrolled in the study will be monitored carefully for the development of any potential complications as described above. Subjects will be evaluated frequently by the treating team as part of standard of care and medical records will be reviewed by the site investigator on a routine basis throughout each participants enrollment.

All subjects discontinued early from the study protocol will have a reason for the early discontinuation recorded on the appropriate case report form, and the circumstances leading to discontinuation will be briefly described. All adverse events (as described) leading to discontinuation of study interventions will be fully documented and followed up as appropriate.

7.6 Potential Benefits

The study may result in decreased pain and decreased length of hospitalization for the subjects involved. Furthermore, the information gained from the analysis may lead to further understanding of the physiology and treatment of sickle cell pain crisis. This could lead to new therapeutic options for future patients. Finally, this pediatric study may benefit adult patients in the future, as positive results from this study would be used to plan a future trial in adult patients.

8 Data and Safety Monitoring Plan

8.1 Data Safety Monitoring Board (DSMB)

The STArT Trial will utilize an independent NHLBI-appointed Sickle Cell Disease DSMB. The purpose of the DSMB is to advise the Federal funding agency (NHLBI) and the STArT scientific Principal Investigators regarding the continuing safety of study subjects and the continuing validity and scientific merit of the study. The DSMB is responsible for monitoring accrual of study subjects, adherence to the protocol, assessments of data quality, performance of individual clinical sites, review of serious adverse events and other subject safety issues. The Data Coordinating Center will send reports relating to these topics to the NHLBI prior to each DSMB meeting. It is anticipated that the DSMB will meet for two formal interim efficacy analyses, and review safety information at least yearly (by phone or in person), but

the DSMB will have the final say in determining meeting intervals.

The DSMB will meet once prior to the start of the STArT Trial, and will approve the final protocol prior to implementation. The DCC will draft a DSMB charter using the NHLBI template as a resource to guide its function for the trial and the charter will be approved by the DSMB. The charter will include rules of procedure, definitions of a meeting quorum, and information about meeting logistics and frequency. After the DSMB has approved its charter and the final protocol, the Data Coordinating Center will send this information to the single site IRB.

The DSMB can recommend whether or not to terminate enrollment in STArT because of potential safety concerns or study feasibility issues. The DSMB will also examine interim data analyses to make decisions as to whether or not to terminate based on high evidence of efficacy or low likelihood of finding an effect (futility). Technical details on the parameters around formal interim analysis can be found in the Statistical Analysis section.

Documentation of the DSMB meetings will follow the NHLBI processes for review and approval of the minutes and recommendations. After approval or modification by the NHLBI, the NHLBI Program Officer will forward the determination and recommendation to the DCC, and provide a memo for the sites and the sIRB. The DCC will forward the report to the single site Institutional Review Board (IRB). In the unlikely event that the DSMB recommends emergent cessation of enrollment in STArT because of safety concerns, this communication will be made during the debriefing segment of the DSMB meeting. If the NHLBI staff concur with this recommendation, the Data Coordinating Center will notify all STArT clinical sites to cease enrollment immediately.

8.2 Adverse Event Reporting

The site investigator is responsible for evaluating all adverse events at their Clinical Center and ensuring that they are properly reported. Adverse events that occur during this study will be recorded. The nature of each experience, date and time (where appropriate) of onset, outcome, course, and relationship to treatment will be documented.

8.2.1 Definition of Adverse Event and Serious Adverse Event

Adverse Event (AE) means any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention-related (21 CFR 312.32 (a)). An event constitutes a disease, a set of related signs or symptoms, or a single sign or symptom.

Serious Adverse Event (SAE): A serious adverse event (SAE) for this population is an adverse event that:

- results in death; or
- is life-threatening (the patient was, in the view of the site investigator, in immediate danger of death from the event as it occurred); or

- requires inpatient hospitalization or prolongs an existing hospitalization; or
- results in persistent or significant disability or incapacity; or
- results in congenital anomaly/birth defect; or
- any other event that, based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition.

8.2.2 Classification of an Adverse Event (Relatedness, Severity, and Expectedness

Relatedness: The suspected relationship between study interventions and any adverse event will be determined by the site investigator using the criteria below. *Relatedness must be assessed by an investigator and may **not** be assessed by a research coordinator.*

Not Related: The event is clearly related to other factors, such as the subject's clinical state, therapeutic interventions, or concomitant drugs administered to the subject.

Possibly Related: The event follows compatible temporal sequence from the time of beginning the assigned study intervention, but could have been produced by other factors such as the subject's clinical state, therapeutic interventions, or concomitant drugs administered to the subject.

Probably Related: The event follows a reasonable temporal sequence from the time of beginning the assigned study intervention, and *cannot be reasonably explained* by other factors such as the subject's clinical state, therapeutic interventions, or concomitant drugs administered to the subject.

Severity: The severity, which is a measure of intensity, of clinical adverse events and laboratory abnormalities will be recorded by the site investigator and categorized. The following guidelines will be used to describe severity.

Mild: The event requires minimal or no treatment and does not interfere with the participants daily activities.

Moderate: The event results in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.

Severe: The event interrupts a participants usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating. Of note, the term "severe" does not necessarily equate to "serious".

Expectedness of the Event: All adverse events, including serious adverse events, will be evaluated as to whether their occurrence was expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information described for the study intervention.

Expected: An event is considered expected if it is known to be associated with the underlying condition or is related to the study intervention and is mentioned in the protocol, informed consent, or other study documents. An event may be expected despite the study subjects clinical state immediately prior to the event.

Unexpected: An event is considered unexpected if there are no prior data linking this event with either the condition or intervention under study or an event that occurred unexpectedly in the course of treatment.

Expected complications of opioid administration and sickle cell anemia are summarized in Tables 4 and 5. Other expected adverse events are reactions known to occur with arginine administration, specifically nausea, vomiting, headache, flushing, warmth at the infusion site.

Table 4: Expected Adverse Events Related to Opioid Administration

Anorexia	Headache
Apnea	Hypotension
Biliary tract pressure	Miosis
Blurred vision	Nausea
Bradycardia	Palpitations
Bruising	Pruritus
Cardiac arrest	Rash
Confusion	Respiratory arrest
Cramps	Respiratory depression
Diaphoresis	Sedation
Diplopia	Shock
Dizziness	Tinnitus
Drowsiness	Urinary retention
Euphoria	Urticaria
Flushing	Vomiting

Table 5: Expected Adverse Events Related to Sickle Cell Disease Progression

Angioedema	Leukocytosis
Aplastic crisis	Meningitis
Aplastic crisis/anemia	Metabolic acidosis
Arthralgia	Osteomyelitis
Appendicitis	Pyelonephritis
Bone Infarction	Pain, joint
Cardiomegaly	Pain, long bone
Cerebrovascular accident	Pain, severe abdominal
Cholecystitis, hepatic sequestration	Priapism

Continued on next page...

Table 5 – *continued from previous page...*

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/spleno megaly	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

Treatment or Action Taken: For each adverse event, the site investigator will record whether an intervention was required:

- Medical or surgical procedure
- Concomitant medication: started, changed, or discontinued
- Other, specify
- No action taken

Outcome of Event: Finally, the site investigator will record the clinical outcome of each adverse event as follows:

- Death
- Recovered and the patient returned to baseline status
- Recovered with permanent sequelae
- Symptoms persist

8.2.3 Time Period for Adverse Events

For purposes of this study, events that occur following randomization through 14 days after study drug initiation or until hospital discharge (whichever comes first) will be reported as adverse events. ED revisits and hospital admissions will be tracked through 28 days following hospital discharge. Specifically, events that occur following parental permission to participate in the study, but prior to actual randomization, are not adverse events. These should be recorded as baseline conditions.

8.2.4 Data Collection Procedures for Adverse Events

After patient randomization all adverse events (including serious adverse events) will be recorded according to relatedness, severity, and expectedness, as well as their duration and any treatment prescribed. Any medical condition present at the time of randomization, recorded in the patients baseline history at study entry, which remains unchanged or improves (unless the clinician feels it is clinically relevant), will not be recorded as an adverse event at subsequent evaluations. However, worsening of a medical condition that was present at the time of randomization will be considered a new adverse event and reported.

Abnormal laboratory values that are clinically significant will be recorded as adverse events and the site investigator will assess the severity and relationship to the study. Laboratory values that are abnormal at the time of randomization and that do not worsen will not be recorded as adverse events.

Adverse events will be coded using the MedDRA coding vocabulary. Coding will be done centrally at the Data Coordinating Center as this requires specific training.

8.2.5 Unanticipated Problems (UP)

Unanticipated problems (UP) are defined as incidents, experiences, or outcomes that are unexpected, related or possibly to participation in the study, and suggest that the research places subjects at a greater risk of harm than was previously known or recognized. The site investigator will report unanticipated problems to the Data Coordinating Center within 24 hours of becoming aware of the event. A detailed completed report will be required to be sent to the Data Coordinating Center within 3 working days of becoming aware of the event. After receipt of the complete report, the Data Coordinating Center will report these unanticipated problems to the NHLBI Program Official and the NHLBI Executive Secretary for the DSMB in an expedited manner (as close to 24 hours as possible). In accordance with local IRB requirements, the site investigator may be required to report such unanticipated problems to the IRB in addition to notifying the Data Coordinating Center. In the event that the medical monitor believes that such an event warrants emergent suspension of enrollment in the trial, and NHLBI staff cannot be reached expeditiously, the Data Coordinating Center will notify the study investigator (Dr. Morris) and all site investigators to cease enrollment in the trial. Resumption of enrollment will not occur without consent of the NHLBI staff after discussion with the DSMB.

8.2.6 Monitoring Serious Adverse Events

A qualified physician will be designated to fulfill the function of the medical monitor for this study. Site investigators and/or research coordinators will report serious adverse events to the Data Coordinating Center within 24 hours of becoming aware of the event. A detailed completed report will be required to be sent to the Data Coordinating Center within 3 working days of becoming aware of the event, and the medical monitor will assess all serious adverse events reported from site investigators.

For each of these serious adverse events, the site investigator will provide sufficient medical history and clinical details for a safety assessment to be made with regard to continuation of the trial. The medical monitor will sign off on each SAE report after review. All SAE reports will be retained at the Data Coordinating Center, and all SAE reports will be available for review by DSMB members and NHLBI staff. Grade 4–5 SAEs will be reported to NHLBI and the DSMB in real time with a tabular summary of these attached to each report. The SAE reporting process may be incorporated into the Electronic Data Capture (EDC) System in use for the study.

In the unlikely event that the medical monitor believes an unexpected and study-related SAE warrants emergent cessation of enrollment in the trial, NHLBI staff and the DSMB chairperson will be immediately consulted. If these individuals concur with the judgment of the medical monitor, or if the NHLBI staff and the DSMB chairperson cannot be reached expeditiously, the Data Coordinating Center will notify the study investigator (Dr. Morris) and all site investigators to cease enrollment in the trial. Resumption of enrollment will not occur without consent of the NHLBI staff after discussion with the DSMB.

In accordance with local IRB requirements, the site investigator may be required to report such events to the IRB in addition to notifying the Data Coordinating Center.

After notification of the NHLBI Program Official or Project Officer, and the DSMB chairperson, of *serious, unexpected, and study-related* adverse events or unanticipated problems (UP), decisions will be made whether to continue the study without change, and whether to convene the entire DSMB for an emergent meeting. If a decision is made to suspend enrollment in the trial, this will be reported to the study investigator (Dr. Morris) and all clinical investigators, who will be instructed to report this to their local IRB.

The DSMB will review all adverse events (not necessarily serious, unexpected, and study-related) during scheduled DSMB meetings. The Data Coordinating Center will prepare a Summary Report of Adverse Events for the DSMB meetings, classified with the MedDRA coding system.

The IND Sponsor (Dr. Claudia R. Morris MD) will notify the FDA of all study related Suspected, Unexpected, Serious Adverse Reactions (SUSARs) according to FDA guidelines (within 7 days for death and life-threatening SAEs, 15 days for all others) via an IND safety report

8.2.7 Follow-up of Serious Adverse Events

All serious adverse events (expected or unexpected), regardless of relatedness that are unresolved at the time of the patients termination from the study or discharge from the hospital, will be followed by the Clinical Center investigators until the events are resolved, subject is lost to follow-up, the adverse event is otherwise explained or has stabilized, or 12 months have passed from the time of last study dose.

9 Study Training

A formal training program for investigators and research staff will be held prior to the start of enrollment. The training program will cover regulatory topics and Good Clinical Practice. The training will also provide in depth explanations regarding study procedures, clinical care, adverse event reporting, data entry procedures, quality assurance, site monitoring, and the informed consent process. A manual of operations will be provided to each investigator prior to the start of enrollment. The manual will detail specific information about the study procedures, regulatory information, safety reporting, and other necessary information. Updates and revisions to the manual will be made available electronically. The Data Coordinating Center, in collaboration with the study investigator, will be the main contact for study questions.

10 Regulatory Considerations

10.1 Food and Drug Administration

This trial is being conducted under an active Investigational New Drug (IND) application approved by the Food and Drug Administration (IND#66943, to Sponsor – Morris). The clinical investigator at each participating site will complete a Form FDA 1572, "Statement of Investigator.

10.2 Health Insurance Portability and Accountability Act

Data elements collected include the date of birth and date of admission. Prior to statistical analyses, dates will be used to calculate patient age at the time of the study events. The final data sets (used for study analyses and archived at the end of the study) will be de-identified, and will exclude these specific dates.

Data elements for race, ethnicity, and gender are also being collected. These demographic data are required for Federal reporting purposes to delineate subject accrual by race, ethnicity, and gender.

For purposes of the DCC handling potential protected health information (PHI) and producing the de-identified research data sets that will be used for analyses, all study sites have been offered a Business Associate Agreement with the University of Utah. Copies of executed Business Associate Agreements are maintained at the DCC.

10.3 Inclusion of Women and Minorities

There will be no exclusion of patients based on gender, race, or ethnicity.

10.4 ClinicalTrials.gov Requirements

This study will be registered at ClinicalTrials.gov, as it is an interventional trial.

10.5 Retention of Records

For federally funded studies subject to the Common Rule, records relating to the research conducted shall be retained for at least 3 years after completion of the research. Completion of the research for this protocol should be anticipated to include planned primary and secondary analyses, as well as subsequent derivative analyses. Completion of the research also entails completion of all publications relating to the research. All records shall be accessible for inspection and copying by authorized representatives of the regulatory authorities at reasonable times and in a reasonable manner [45 CFR §46.115(b)].

10.6 Public Use Data Set

After subject enrollment and follow up have been completed, the DCC will prepare a final study database for analysis. A releasable database will be produced and completely de-identified in accordance with the definitions provided in the Health insurance Portability and Accountability Act (HIPAA). Namely, all identifiers specified in HIPAA will be recoded in a manner that will make it impossible to deduce or impute the specific identity of any patient. The database will not contain any institutional identifiers.

The DCC will also prepare a data dictionary that provides a concise definition of every data element included in the database. If specific data elements have idiosyncrasies that might affect interpretation or analysis, this will be discussed in the dictionary document. In accordance with policies determined by the investigators and funding sponsors, the releasable database will be provided to users in electronic form.

11 Bibliography

[1] Bunn HF. Pathogenesis and treatment of sickle cell disease. *N Engl J Med.*, 337(11):762–769, 1997.

[2] Lanzkron S, Carroll CP, and Haywood C, Jr. The burden of emergency department use for sickle-cell disease: an analysis of the national emergency department sample database. *American journal of hematology.*, 85(10):797–799., 2010.

[3] Brousseau DC, Owens PL, Mosso AL, Panepinto JA, and Steiner CA. Acute care utilization and rehospitalizations for sickle cell disease. *JAMA.*, 303(13):1288–1294, 2010.

[4] Bundy DG, Strouse JJ, Casella JF, and Miller MR. Urgency of emergency department visits by children with sickle cell disease: a comparison of 3 chronic conditions. *Acad Pediatr.*, 11(4):333–341, 2011.

[5] Frei-Jones MJ, Field JJ, and Debaun MR. Risk factors for hospital readmission within 30 days: A new quality measure for children with sickle cell disease. *Pediatric blood & cancer.*, 2008.

[6] Morris CR, Kuypers FA, Lavrisha L, et al. A randomized, placebo-controlled trial of arginine therapy for the treatment of children with sickle cell disease hospitalized with vaso-occlusive pain episodes. *Haematologica.*, 98 (9):1375–1382, 2013.

[7] Morris CR, Brown LAS, Reynolds M, Dampier CD, Lane PA, Watt A, Kumari P, Harris F, Manoranjithan S, Mendis RD, Figueroa J, and Shiva S. Impact of arginine therapy on mitochondrial function in children with sickle cell disease during vaso-occlusive pain. *Blood*, 136:1402–1406, September 2020.

[8] Yusuf HR, Atrash HK, Grosse SD, Parker CS, and Grant AM. Emergency department visits made by patients with sickle cell disease: a descriptive study, 1999-2007. *Am J Prev Med.*, 38(4 Suppl):S536–541, 2010.

[9] Ballas SK. New era dawns on sickle cell pain. *Blood.*, 116(3):311–312, 2010.

[10] Kauf TL, Coates TD, Huazhi L, Mody-Patel N, and Hartzema AG. The cost of health care for children and adults with sickle cell disease. *American journal of hematology.*, 84(6):323–327, 2009.

[11] Gupta M, Msambichaka L, Ballas SK, and Gupta K. Morphine for the treatment of pain in sickle cell disease. *Scientific World Journal.*, 2015:540154, 2015.

[12] Moncada S and Higgs A. The l-arginine-nitric oxide pathway. *N Engl J Med.*, 329(27):2002–2012, 1993.

[13] Kaul DK, Liu XD, Fabry ME, and Nagel RL. Impaired nitric oxide-mediated vasodilation in transgenic sickle mouse. *Am J Physiol Heart Circ Physiol.*, 278:H1799–1806, 2000.

[14] Aslan M, Ryan T, Adler B, et al. Oxygen radical inhibition of nitric oxide-dependent vascular function in sickle cell disease. *Proc Natl Acad Sci.*, 98:15215–15220, 2001.

[15] Reiter CD and Gladwin MT. An emerging role for nitric oxide in sickle cell disease vascular homeostatis and therapy. *Curr Opin Hematol.*, 10:99–107, 2003.

[16] Gladwin MT and Kato GJ. Cardiopulmonary complications of sickle cell disease: role of nitric oxide and hemolytic anemia. *Hematology (Am Soc Hematol Educ Program).*, pages 51–57, 2005.

[17] Mack AK and Kato GJ. Sickle cell disease and nitric oxide: A paradigm shift? *Int J Biochem Cell Biol.*, 38(8):1237–1243, 2006.

[18] Wood KC, Hsu LL, and Gladwin MT. Sickle cell disease vasculopathy: a state of nitric oxide resistance. *Free Radic Biol Med.*, 44(8):1506–1528, 2008.

[19] Morris CR. Mechanisms of vasculopathy in sickle cell disease and thalassemia. *Hematology / the Education Program of the American Society of Hematology American Society of Hematology Education Program.*, 2008:177–185, 2008.

[20] National Academies of Sciences Engineering and Medicine. Arginine as an example of a conditionally essential nutrient: Sickle cell disease and trauma. paper presented at: Nutrigenomics and the future of nutrition: Proceedings of a workshopin brief 2018; washington, dc. 2018.

[21] Yetik-Anacak G and Catravas JD. Nitric oxide and the endothelium: history and impact on cardiovascular disease. *Vascul Pharmacol.*, 45(5):268–276, 2006.

[22] Reiter C, Wang X, Tanus-Santos J, et al. Cell-free hemoglobin limits nitric oxide bioavailability in sickle cell disease. *Nat Med.*, 8:1383–1389, 2002.

[23] Aslan M and Freeman BA. Oxidant-mediated impairment of nitric oxide signaling in sickle cell disease–mechanisms and consequences. *Cell Mol Biol (Noisy-le-grand).*, 50(1):95–105, 2004.

[24] Morris SM, Jr. Arginases and arginine deficiency syndromes. *Current opinion in clinical nutrition and metabolic care.*, 15(1):64–70, 2012.

[25] Morris CR. Alterations of the arginine metabolome in sickle cell disease: a growing rationale for arginine therapy. *Hematology/oncology clinics of North America.*, 28(2):301–321, 2014.

[26] Morris CR, Hamilton-Reeves J, Martindale RG, Sarav M, and Ochoa Gautier JB. Acquired amino acid deficiencies: A focus on arginine and glutamine. *Nutr Clin Pract.*, 32(1_suppl):30S–47S, 2017.

[27] Morris CR, Kato GJ, Poljakovic M, et al. Dysregulated arginine metabolism, hemolysis-associated pulmonary hypertension and mortality in sickle cell disease. *JAMA.*, 294:81–90, 2005.

[28] Enwonwu CO. Increased metabolic demand for arginine in sickle cell anaemia. *Med Sci Res.*, 17:997–998, 1989.

[29] Morris CR, Kuypers FA, Larkin S, Vichinsky E, and Styles L. Patterns of arginine and nitric oxide in sickle cell disease patients with vaso-occlusive crisis and acute chest syndrome. *Journal of pediatric hematology/oncology.*, 22:515–520, 2000.

[30] Morris CR, Hagar W, van Warmerdam J, et al. Arginine therapy: A new treatment for pulmonary hypertension in sickle cell disease? *Blood.*, 96:485a (abstr 2088, suppl. 2081), 2000.

[31] Morris CR. New strategies for the treatment of pulmonary hypertension in sickle cell disease : The rationale for arginine therapy. *Treat Respir Med.*, 5(1):31–45, 2006.

[32] Waugh W, Daeschner C, Files B, and Gordon D. Evidence that l-arginine is a key amino acid in sickle cell anemia – a preliminary report. *Nutritional Research.*, 19:501–518, 1999.

[33] van de Poll MC, Ligthart-Melis GC, Boelens PG, Deutz NE, van Leeuwen PA, and Dejong CH. Intestinal and hepatic metabolism of glutamine and citrulline in humans. *J Physiol.*, 581(Pt 2):819–827, 2007.

[34] Morris C, Kuypers F, Hagar R, et al. Oral glutamine supplementation improves global arginine bioavailability in patients with sickle cell disease: Preliminary pharmacokinetics results. *Paper presented at: Foundation for Sickle Cell Disease Research 5th Annual Sickle Cell Disease Research and Educational Symposium2011; Hollywood, Florida.*, 2011.

[35] Lopez B, Kreshak A, Morris CR, Davis-Moon L, Ballas S, and Ma X. L-arginine levels are diminished in adult acute vaso-occlusive sickle cell crisis in the emergency department. *British journal of haematology.*, 120:532–534, 2003.

[36] Schnog JB, Teerlink T, van der Dijs FP, Duits AJ, and Muskiet FA. Plasma levels of asymmetric dimethylarginine (adma), an endogenous nitric oxide synthase inhibitor, are elevated in sickle cell disease. *Ann Hematol.*, 84(5):282–286, 2005.

[37] Landburg PP, Teerlink T, Muskiet FA, Duits AJ, and Schnog JJ. Plasma concentrations of asymmetric dimethylarginine, an endogenous nitric oxide synthase inhibitor, are elevated in sickle cell patients but do not increase further during painful crisis. *American journal of hematology.*, 83(7):577–579, 2008.

[38] Kato GJ, Wang Z, Machado RF, Blackwelder WC, Taylor JGt, and Hazen SL. Endogenous nitric oxide synthase inhibitors in sickle cell disease: abnormal levels and correlations with pulmonary hypertension, desaturation, haemolysis, organ dysfunction and death. *British journal of haematology.*, 145(4):506–513, 2009.

[39] Vallance P. The asymmetrical dimethylarginine/dimethylarginine dimethylaminohydrolase pathway in the regulation of nitric oxide generation. *Clin Sci.*, 100:159–160, 2001.

[40] Hsu LL, Champion HC, Campbell-Lee SA, et al. Hemolysis in sickle cell mice causes pulmonary hypertension due to global impairment in nitric oxide bioavailability. *Blood.*, 109:3088–3098, 2007.

[41] Hebbel RP. Auto-oxidation and a membrane-associated 'fenton reagent': a possible explanation for development of membrane lesions in sickle erythrocytes. *Clin Haematol.*, 14(1):129–140, 1985.

[42] Morris CR, Suh JH, Hagar W, et al. Erythrocyte glutamine depletion, altered redox environment, and pulmonary hypertension in sickle cell disease. *Blood.*, 140:104–112, 2008.

[43] Dasgupta T, Hebbel RP, and Kaul DK. Protective effect of arginine on oxidative stress in transgenic sickle mouse models. *Free Radic Biol Med.*, 41(12):1771–1780, 2006.

[44] Kaul DK, Zhang X, Dasgupta T, and Fabry ME. Arginine therapy of transgenic-knockout sickle mice improves microvascular function by reducing non-nitric oxide vasodilators, hemolysis, and oxidative stress. *Am J Physiol Heart Circ Physiol.*, 295(1):H39–47, 2008.

[45] Gornik HL and Creager MA. Arginine and endothelial and vascular health. *J Nutr.*, 134(10 Suppl):2880S–2887S; discussion 2895S, 2004.

[46] Gladwin MT, Kato GJ, Weiner D, et al. Nitric oxide for inhalation in the acute treatment of sickle cell pain crisis: a randomized controlled trial. *JAMA.*, 305(9):893–902, 2011.

[47] Berka V, Yeh HC, Gao D, Kiran F, and Tsai AL. Redox function of tetrahydrobiopterin and effect of l-arginine on oxygen binding in endothelial nitric oxide synthase. *Biochemistry.*, 43(41):13137–13148, 2004.

[48] Wood KC, Hebbel RP, Lefer DJ, and Granger DN. Critical role of endothelial cell-derived nitric oxide synthase in sickle cell disease-induced microvascular dysfunction. *Free Radic Biol Med.*, 40(8):1443–1453, 2006.

[49] Machado RF, Barst RJ, Yovetich NA, et al. Hospitalization for pain in patients with sickle cell disease treated with sildenafil for elevated trv and low exercise capacity. *Blood.*, 118(4):855–864, 2011.

[50] Barbul A, Rettura G, Levenson SM, and Seifter E. Wound healing and thymotropic effects of arginine: A pituitary mechanism of action. *Am J Clin Nutr.*, 37:786–794, 1983.

[51] Barbul A. Arginine: Biochemistry, physiology and therapeutic implications. *J Parent Enter Nutr.*, 10:227–238, 1986.

[52] Romero J, Suzuka S, Nagel R, and Fabry M. Arginine supplementation of sickle transgenic mice reduces red cell density and gardos channel activity. *Blood.*, 99:1103–1108, 2002.

[53] Romero J, Susuka S, Nagel R, and Fabry M. Arginine supplementation of sickle transgenic mice leads to a decrease in red cell density and ca^{2+} – activated k^{+} channel activity. *Paper presented at: 25th Annual Meeting of the National Sickle Cell Disease Program; New York City.*, April14-17 2001.

[54] Sinden RE, Barker GC, Paton MJ, et al. Factors regulating natural transmission of plasmodium berghei to the mosquito vector, and the cloning of a transmission-blocking immunogen. *Parassitologia.*, 35:Suppl:107–112, 1993.

[55] Little JA, Hauser KP, Martyr SE, et al. Hematologic, biochemical, and cardiopulmonary effects of l-arginine supplementation or phosphodiesterase 5 inhibition in patients with sickle cell disease who are on hydroxyurea therapy. *European journal of haematology.*, 82(4):315–321, 2009.

[56] Tang WHW, Wang Z, Cho L, Brennan DM, and Hanzen SL. Diminished global arginine bioavailability and increased arginine catabolism as metabolic profile of increased cardiovascular risk. *Journal of the American College of Cardiology.*, 53(22):2061–2067, 2009.

[57] Omodeo-Sale F, Cortelezzi L, Vommaro Z, Scaccabarozzi D, and Dondorp AM. Dysregulation of l-arginine metabolism and bioavailability associated to free plasma heme. *Am J Physiol Cell Physiol.*, 299(1):C148–154., 2010.

[58] Morris CR, Teehankee C, Kato G, et al. Decreased arginine bioavailability contributes to the pathogenesis of pulmonary artery hypertension. *Paper presented at: American College of Cardiology Annual Meeting; Orlando, Florida.*, March6-9 2005.

[59] Morris CR, Gladwin MT, and Kato G. Nitric oxide and arginine dysregulation: A novel pathway to pulmonary hypertension in hemolytic disorders. *Curr Mol Med.*, 8:81–90, 2008.

[60] Sher GD and Olivieri NG. Rapid healing of leg ulcers during arginine butyrate therapy in patients with sickle cell disease and thalassemia. *Blood.*, 84:2378–2380, 1994.

[61] Morris CR, Morris SM Jr., Hagar W, et al. Arginine therapy: A new treatment for pulmonary hypertension in sickle cell disease? *American journal of respiratory and critical care medicine.*, 168:63–69, 2003.

[62] Morris CR, Kuypers FA, Larkin S, et al. Arginine therapy: A novel strategy to increase nitric oxide production in sickle cell disease. *Brit J Haematol.*, 2000:498–500, 2000.

[63] Morris CR, Vichinsky EP, van Warmerdam J, et al. Hydroxyurea and arginine therapy: impact on nitric oxide production in sickle cell disease. *Journal of pediatric hematology/oncology.*, 25:629–634, 2003.

[64] Maxwell AJ and Cooke JP. Cardiovascular effects of l-arginine. *Current Opinion in Nephrology and Hypertension.*, 7:63–70, 1998.

[65] Merimee TJ, Rabinowitz D, Riggs L, A. BJ, Rimoin DL, and McKusick VA. Plasma growth hormone after arginine infusion. *N Engl J Med.*, 276:434–439, 1967.

[66] Merimee TJ, Rabinowitz D, and Fineberg S. Arginine-initiated release of human growth hormone. *N Engl J Med.*, 28:1434–1438, 1969.

[67] El-Hattab AW, Emrick LT, Craigen WJ, and Scaglia F. Citrulline and arginine utility in treating nitric oxide deficiency in mitochondrial disorders. *Mol Genet Metab.*, 107(3):247–252, 2012.

[68] El-Hattab AW, Hsu JW, Emrick LT, et al. Restoration of impaired nitric oxide production in melas syndrome with citrulline and arginine supplementation. *Mol Genet Metab.*, 105(4):607–614, 2012.

[69] El-Hattab AW, Emrick LT, Williamson KC, Craigen WJ, and Scaglia F. The effect of citrulline and arginine supplementation on lactic acidemia in MELAS syndrome. *Meta Gene.*, 1:8–14, 2013.

[70] El-Hattab AW, Emrick LT, Chanprasert S, Craigen WJ, and Scaglia F. Mitochondria: role of citrulline and arginine supplementation in MELAS syndrome. *Int J Biochem Cell Biol.*, 48:85–91, 2014.

[71] El-Hattab AW, Emrick LT, Hsu JW, et al. Impaired nitric oxide production in children with melas syndrome and the effect of arginine and citrulline supplementation. *Mol Genet Metab.*, 117:407–412, 2016.

[72] El-Hattab AW and Jahoor F. Assessment of nitric oxide production in mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes syndrome with the use of a stable isotope tracer infusion technique. *J Nutr.*, 147(7):1251–1257, 2017.

[73] El-Hattab AW, Almannai M, and Scaglia F. Arginine and citrulline for the treatment of melas syndrome. *J Inborn Errors Metab Screen.*, page 5, 2017.

[74] Morris CR et al. Safety of intravenous arginine therapy in children with sickle cell disease hospitalized for vaso-occlusive pain: A randomized placebo-controlled trial in progress. *Blood*, 134:995, 2019.

[75] Onalo R, Cooper P, Cilliers A, Vorster BC, Uche N, Oluseyi OO, Onalo VD, Zubairu Y, Ayodele-Kehinde AU, Damilare OM, Figueroa J, and Morris CR. A phase 2 randomized placebo-controlled trial of oral l-arginine for the management of children with sickle cell anemia and severe vaso-occlusive pain in nigeria. *Blood*, 134:613, 2019.

[76] Yawn BP, Buchanan GR, Afenyi-Annan AN, et al. Management of sickle cell disease: summary of the 2014 evidence-based report by expert panel members. *JAMA.*, 312(10):1033–1048, 2014.

[77] The Pediatric Emergency Care Applied Research Network (PECARN). The pediatric emergency care applied research network (pecarn): rationale, development, and first steps. *Pediatr Emerg Care.*, 19(3):185–193, 2003.

[78] Peters-Lawrence MH, Bell MC, Hsu LL, et al. Clinical trial implementation and recruitment: lessons learned from the early closure of a randomized clinical trial. *Contemp Clin Trials.*, 33(2):291–297, 2015.

[79] Badaki-Makun O, Scott JP, Panepinto JA, et al. Intravenous magnesium for pediatric sickle cell vaso-occlusive crisis: methodological issues of a randomized controlled trial. *Pediatric blood & cancer.*, 61(6):1049–1054, 2014.

[80] Cardenes N, Corey C, Geary L, et al. Platelet bioenergetic screen in sickle cell patients reveals mitochondrial complex v inhibition, which contributes to platelet activation. *Blood.*, 123(18):2864–2872, 2014.

[81] Panepinto JA, Paul Scott J, Badaki-Makun O, et al. Determining the longitudinal validity and meaningful differences in hrql of the pedsql sickle cell disease module. *Health Qual Life Outcomes.*, 15(1):124, 2017.

[82] Varni JW, Stucky BD, Thissen D, et al. Promis pediatric pain interference scale: an item response theory analysis of the pediatric pain item bank. *The journal of pain : official journal of the American Pain Society.*, 11(11):1109–1119., 2010.

[83] Ameringer S, Elswick RK, Jr., and Smith W. Fatigue in adolescents and young adults with sickle cell disease: biological and behavioral correlates and health-related quality of life. *Journal of pediatric oncology nursing : official journal of the Association of Pediatric Oncology Nurses.*, 31(1):6–17, 2014.

[84] Jacob E, Miaskowski C, Savedra M, Beyer JE, Treadwell M, and Styles L. Quantification of analygesic use in children with sickle cell disease. *Clin J Pain*, 23(1):8–14, 2007.

[85] Holm. A simple sequentially rejective multiple test procedure. *Scndinavian Journal of Statistics*, 6:65–70, 1979.

[86] Ruta NS and Ballas SK. The opioid drug epidemic and sickle cell disease: Guilt by association. *Pain Med.*, 17(10):1793–1798, 2016.

[87] Ballas SK, Gupta K, and Adams-Graves P. Sickle cell pain: a critical reappraisal. *Blood.*, 120(18):3647–3656, 2012.

[88] Farrell. Blood advances. 3(23):3982–4001, 2019.

[89] Shiva S, Rassaf T, Patel RP, and Gladwin MT. The detection of the nitrite reductase and no-generating properties of haemoglobin by mitochondrial inhibition. *Cardiovascular research.*, 3:566–573, 2011.

[90] Brousseau DC, Scott JP, Badaki-Makun O, et al. A multicenter randomized controlled trial of intravenous magnesium for sickle cell pain crisis in children. *Blood.*, 126(14):1651–1657, 2015.

[91] Hertz P and Richardson J. Arginine-induced hyperkalemia in renal failure patients. *Arch Intern Med.*, 130:778–780, 1972.