STUDY TITLE

A Phase IIb Randomized, Double-Blind, Placebo-Controlled Multi-Center Study to Assess the Safety, Tolerability, and Efficacy of Riociguat in Patients with Sickle Cell Diseases (STERIO-SCD)

Test drug:	Riociguat
Study purpose:	Safety, tolerability and efficacy
Clinical study phase:	IIb
Version no.:	6.1 Date: January 7, 2020
IND #	128277
ClinicalTrials.gov registry number:	NCT 02633397
Protocol no.:	PRO15110016
Study Sponsor:	Mark T. Gladwin, MD
Lead Principal Investigator:	Mark T. Gladwin, MD Professor of Medicine Chair, Department of Medicine Director, Heart, Lung, Blood and Vascular Medicine Institute University of Pittsburgh Phone: 412-648-3181

The study will be conducted in compliance with the protocol, ICH-GCP and any applicable regulatory requirements.

PROTOCOL SIGNATURE PAGE

Protocol Number: PRO15110016

Protocol Title: A Phase IIb Randomized, Double-Blind, Placebo-Controlled Multi-Center Study to Assess the Safety, Tolerability, and Efficacy of Riociguat in Patients with Sickle Cell Diseases

Version/Date: Version 6.1 /January 7, 2020

SPONSOR'S APPROVAL

The sponsor signatory agrees to the content of the final clinical study protocol as presented.

Name: Mark T. Gladwin, MD
Title: Lead Co- Principal Investigator, University of Pittsburgh

Signature: _____ Date: _____

INVESTIGATOR AGREEMENT

I confirm agreement to conduct the study in compliance with the protocol.

I acknowledge that I am responsible for overall study conduct. I agree to personally conduct or supervise the described clinical study.

I agree to ensure that all associates, colleagues and employees assisting in the conduct of the study are informed about their obligations. Mechanisms are in place to ensure that site staff receives the appropriate information throughout the study.

Investigator Name: _____ Site #: _____

Investigator Signature: _____ Date: _____

Synopsis

Title	A Phase IIb Randomized, Double-Blind, Placebo-Controlled Multi-Center Study to Assess the Safety, Tolerability, and Efficacy of Riociguat in Patients with Sickle Cell Disease (STERIO-SCD)
Clinical study phase	IIb
Study objective(s)	The purpose of this study is to evaluate the safety and tolerability of 12-weeks of treatment with riociguat versus placebo in high-risk patients with sickle cell disease, with additional evaluation for efficacy signal for improvement of blood pressure, exercise capacity, and/or proteinuria.
Test Drug **Name of active ingredient** **Dose(s)** **Route of administration** **Duration of treatment**	Riociguat Riociguat 0.5 mg, 1.0 mg, 1.5 mg, 2.0 mg, and 2.5 mg three times a day (TID) Oral 12 weeks
Reference Drug **Name of active ingredient** **Dose(s)** **Route of administration** **Duration of treatment**	Placebo Not applicable Matching placebo to riociguat 0.5 mg, 1.0 mg, 1.5 mg, 2.0 mg and 2.5 mg TID Oral 12 weeks
Indication	Prevention or reduction of clinical complications of sickle cell disease
Diagnosis and main criteria for inclusion	• Age 18 years or older • Sickling disorder HbSS, HbSC, HbSbeta-thalassemia, HbSD, HbSO-Arab) documented by past medical history, hemoglobin electrophoresis or hemoglobin HPLC fractionation • At least one of the following findings: a. Systolic blood pressure ≥130 mm Hg b. Urine albumin to creatinine ratio > 300 mg/g c. TRV > 2.9 m/sec d. NT-proBNP level ≥ 160 pg/mL e. Urinalysis protein 1+ or higher • Willingness to provide a blood specimen for DNA analysis

	• Stable therapy for three months if on hydroxyurea, vasodilator therapy, endothelin receptor antagonists, L-glutamine, crizanlizumab, or voxelotor
Study design	Multi-center, randomized, double-blind, placebo-controlled, parallel groups study
Type of control	Placebo controlled
Number of patients	100 completed patients
Study Endpoints	Primary endpoints measure: Proportion of participants experiencing at least 1 treatment emergent SAE in the riociguat arm compared to the placebo arm. Secondary endpoints: • The frequency of SAE adjudicated by a designated committee of protocol investigators to be due to sickle cell related painful crisis in the riociguat group compared to the placebo group. • Overall incidences of treatment-emergent adverse events • Changes in pain intensity from baseline to Week 12 using numerical pain score and the Brief Pain Inventory, and electronic daily pain diary piloted at selected sites. Main efficacy outcomes: • Changes in functional exercise capacity by assessing 6 Minute Walk Distance Test with Borg Dyspnea/Fatigue Scale from baseline to Week 12 • Changes in hemodynamic parameters including blood pressure as the main pharmacodynamic variable, and tricuspid regurgitant velocity, as well as other echocardiography markers of left ventricular diastolic dysfunction after 12 weeks of double blinded treatment with Riociguat compared with placebo. • Changes in the levels of plasma NT-proBNP from baseline to Week 12. • Changes in laboratory measures from baseline to Week 12: o Renal: urine albumin to creatinine ratio, albuminuria stratified as microalbuminuria and

	macroalbuminuria, estimated GFR, CKD stage based on albuminuria and GFR; o Hematologic: Hemoglobin, LDH, reticulocyte count, white blood cell count, fetal hemoglobin • Incidences of Sickle Cell related clinical complications such as acute chest syndrome, priapism, new leg ulcer, new stroke, other new non-CNS thromboembolic event during the 12 week study treatment. Exploratory endpoints: • Maximum and trough plasma concentration (C_{max} and C_{trough}) • Biomarkers of oxidative stress: 3-Nitro-tyrosine, Malondialdehyde and 8-IsoPGF2a. • Biomarkers of NO-Pathway dysregulation: asymmetric dimethylarginine (ADMA), SDMA and cGMP. • Biomarkers of inflammation (C-reactive protein) and erythropoiesis (erythropoietin) • Changes in pain diary scores
Plan for statistical analysis	All variables will be analyzed descriptively with appropriate statistical methods: categorical variables by frequency tables and continuous variables by sample statistics (i.e. mean, standard deviation, minimum, median, quartiles and maximum). A logistic regression is planned for the primary endpoint, and analysis of covariance (ANCOVA) analyses are planned for further endpoints.

Table of Contents

LIST OF ABBREVIATION

Abbreviation	Definition
6MWD	6-minute walking distance
ADMA	asymmetric dimethylarginine
AE(s)	adverse event(s)
ANCOVA	analysis of covariance
ANOVA	analysis of variance
ATS	American Thoracic Society
BNP	brain natriuretic peptide
BP	blood pressure
CBC	complete blood count
cGMP	cyclic guanosine monophosphate
CFR	code of federal regulation
CKD	chronic kidney disease
Cl	clearance
CTCAE	common terminology criteria for adverse events
CTEPH	chronic thromboembolic pulmonary hypertension
DBP	diastolic blood pressure
DCC	Data Coordinating Center
DSMB	data safety monitoring board
EC	Ethics Committee
eCRF	electronic case report form
e.g.	*exempli gratia*, for example
eNOS	endothelial NO synthase
EOT	end of treatment
FDA	Food and Drug Administration
FRP	females of reproductive potential
g	gram
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
HbSS	hemoglobin S homozygote; sickle cell anemia; homozygous sickle cell disease
HbSC	hemoglobin S/hemoglobin C double heterozygote
HbSbeta-thalassemia	hemoglobin S/beta-thalassemia double heterozygote
HbSD	hemoglobin S/hemoglobin D double heterozygote
HbSO-Arab	hemoglobin S/hemoglobin O-Arab double heterozygote
HPLC	high performance liquid chromatography
ICF	informed consent form

Abbreviation	Definition
ICH	International Conference on Harmonization
IEC	independent ethics committee
IIP	idiopathic interstitial pneumonia
ILD	interstitial pneumonia
IPF	idiopathic pulmonary fibrosis
IRB	institutional review board
ITT	intent to treat
IND	investigational new drug
INR	International Normalized Ratio
IxRS	interactive voice response system/ interactive web response system
LDH	lactate dehydrogenase
MAP	mean arterial pressure
MD	medical doctor
Mg	milligram
mL	Milliliter
mmHg	millimeter of mercury
mPAP	mean pulmonary artery pressure
NADPH	nicotinamide adenine dinucleotide phosphate
NCT	National Clinical Trial
NO	nitric oxide
NT-proBNP	N-terminal pro-hormone brain natriuretic peptide
PD	pharmacodynamics
PDE5	phosphodiesterase 5
PDE9	phosphodiesterase 9
PK	pharmacokinetic
PVR	pulmonary vascular resistance
RHC	right heart catheterization
RVF	right ventricular function
RVSp	right ventricular systolic pressure
SAE(s)	serious adverse event(s)
SBP	systolic blood pressure
SDMA	symmetric dimethylarginine
SCD	sickle cell disease
sGC	soluble guanylate cyclase
SNP	sodium nitroprusside
SUSAR	suspected unexpected serious adverse reaction
TEAE(s)	treatment-emergent adverse event(s)

Abbreviation	Definition
TID	*ter in die*; three times a day
TRV	tricuspid regurgitant velocity
US(A)	United States of America
Vd	Volume of distribution
WHO	World Health Organization

1. INTRODUCTION

1.1 Background

1.1.1 Basic pathobiology, incidence and prevalence of sickle cell disease

Sickle cell disease (SCD) is a hereditary disorder of red blood cells that causes a wide variety of acute and chronic complications [1]. A single nucleotide mutation in the gene encoding the beta globin gene results in a single amino acid substitution in beta globin. This seemingly tiny change in the hemoglobin protein results in sickle hemoglobin, which polymerizes, especially in the T conformation favored in deoxygenated sickle hemoglobin. Sickle hemoglobin polymers induce remarkable rigidity and adhesiveness of the red cells that interferes with circulation in the microvasculature, leading to tissue ischemia and infarction, pain and end organ dysfunction. Precise population figures are not available, but the Centers for Disease Control in the United States Federal Government estimates[2]:

- SCD affects 90,000 to 100,000 Americans.
- SCD occurs among about 1 out of every 500 Black or African-American births.
- SCD occurs among about 1 out of every 36,000 Hispanic-American births.

The World Health Organization estimates that 275,000 infants are born globally with SCD each year, 85% of those births in Africa, especially west Africa [3]. The remainder of the SCD births are distributed mainly among the United States, South America, Caribbean and Saudi Arabia-India regions.

1.1.2 Nitric oxide dysfunction in sickle cell disease

Sickle erythrocytes are also prone to both extravascular and intravascular hemolysis. In recent years, work from our group and others has characterized mechanisms by which the products of hemolysis contribute to vascular dysfunction, especially compromising bioavailability of nitric oxide (NO) [4-11].

Nitric Oxide. NO is arguably the most important endogenous regulator of vascular physiology. NO is produced in blood vessels by endothelial NO synthase (eNOS), a tetrahydrobiopterin-dependent enzyme that converts arginine into NO plus citrulline. NO regulates an entire biological program that favors the flow of blood in the vasculature; its deficiency results in vasoconstriction and activation of a pro-thrombotic, inflammatory state in blood vessels, especially arteries. Vasculopathy due to decreased NO bioavailability predisposes to atherosclerosis in the general population, contributing to the risk of heart disease and stroke.

Hemolysis-Endothelial Dysfunction Syndrome. The products of hemolysis found in blood plasma from patients with sickle cell disease contribute to a vasculopathy featuring decreased

NO bioavailability [4-11] [12,13]. In addition, highly active oxidase pathways produce oxygen radicals that can further deplete NO [12-14]. These products in combination decrease NO bioavailability:

Component	Mechanistic Outcome	Citation
• Cell-free plasma hemoglobin	• NO scavenging and oxidant stress	[4]
• Cell-free plasma arginase-1	• L-arginine scavenging, decreased NO production and eNOS uncoupling	[5]
• Asymmetric dimethylarginine (ADMA)	• eNOS inhibition and uncoupling	[6-10]
• Lactate dehydrogenase	• Biomarker of associated plasma hemoglobin, arginase and ADMA	[6,8,15]
• Reactive oxygen species from xanthine oxidase and NADPH oxidase	• NO scavenging and formation of peroxynitrite	[12,14]
• Decreased tetrahydrobiopterin	• eNOS uncoupling	[13,16]

Each of these components serves as a biomarker of risk of pulmonary hypertension in patients with SCD. The same pathobiology has also been implicated in several other complications of SCD, including relative systemic hypertension [17,18], proteinuria [19,20], chronic kidney disease [21-24], stroke [25], leg ulceration [11,26], and priapism [27,28]. Each of these complications is conceivably a consequence of vasculopathy in patients with SCD, much as they are in patients without SCD. Each of these vasculopathic complications has been associated with clinical laboratory biomarkers of hemolysis, such as low total hemoglobin, high reticulocyte count, high serum lactate dehydrogenase and bilirubin [29,30]. This sickle cell vasculopathy has been associated with compromised response to exogenous NO donors in physiological assays of vasodilator function, indicating a state of NO resistance [4,31]. Collectively, our group has termed this constellation of vasculopathic features the Hemolysis-Endothelial Dysfunction Syndrome (Figure 1) [29].

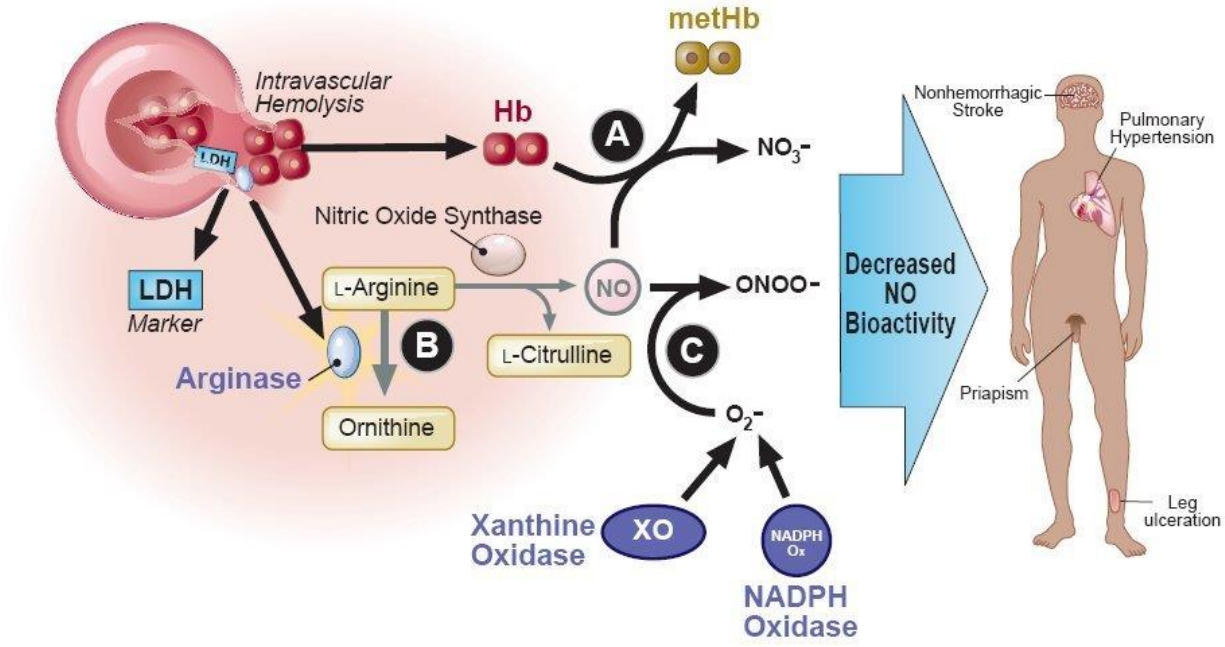

Figure 1. Intravascular hemolysis reduces nitric oxide bioactivity. Nitric oxide is produced by isoforms of nitric oxide (NO) synthase, using the substrate L-arginine. Intravascular hemolysis simultaneously releases hemoglobin, arginase, and lactate dehydrogenase (LDH) from red cells into blood plasma. Cell-free plasma hemoglobin stoichiometrically inactivates NO, generating methemoglobin and inert nitrate (**A**). Plasma arginase consumes plasma L-arginine to ornithine, depleting its availability for NO production by vascular endothelial cells (**B**). LDH also released from the red cell into blood serum serves as a surrogate marker for the magnitude of hemoglobin and arginase release. NO is also consumed by reactions with reactive oxygen species (O_2^-) produced by the high levels of xanthine oxidase activity and NADPH oxidase activity seen in sickle cell disease, producing oxygen radicals like peroxynitrite ($ONOO^-$)(**C**). The resulting decreased NO bioactivity in sickle cell disease is associated with pulmonary hypertension, priapism, leg ulceration, and possibly with non-hemorrhagic stroke. A similar pathobiology is seen in other chronic intravascular hemolytic anemias [29].

The most dramatic complication of this syndrome, pulmonary hypertension, occurs in approximately 10% of adults with SCD [24,32,33], and is associated with high risk of mortality [33,34]. It is associated with three important risk-defining variables that will be considered eligibility criteria for this trial:

- Highly elevated echocardiographic tricuspid regurgitant velocity >2.9 m/s
- Systolic hypertension
- Proteinuria

These have each been associated with poor prognosis in adults with SCD [17,21,35]. Each is hypothetically linked to the hemolysis-endothelial dysfunction syndrome and low NO bioavailability, making them attractive conditions to attempt therapeutic bypass of the NO pathway with an activator of soluble guanylyl cyclase. Furthermore, our own results indicate that sGC stimulator Bay 41-8543 and sGC activator Bay 60-2770 can bypass NO scavenging reactions due to intravascular hemoglobin in rats (Figure 2) [36].

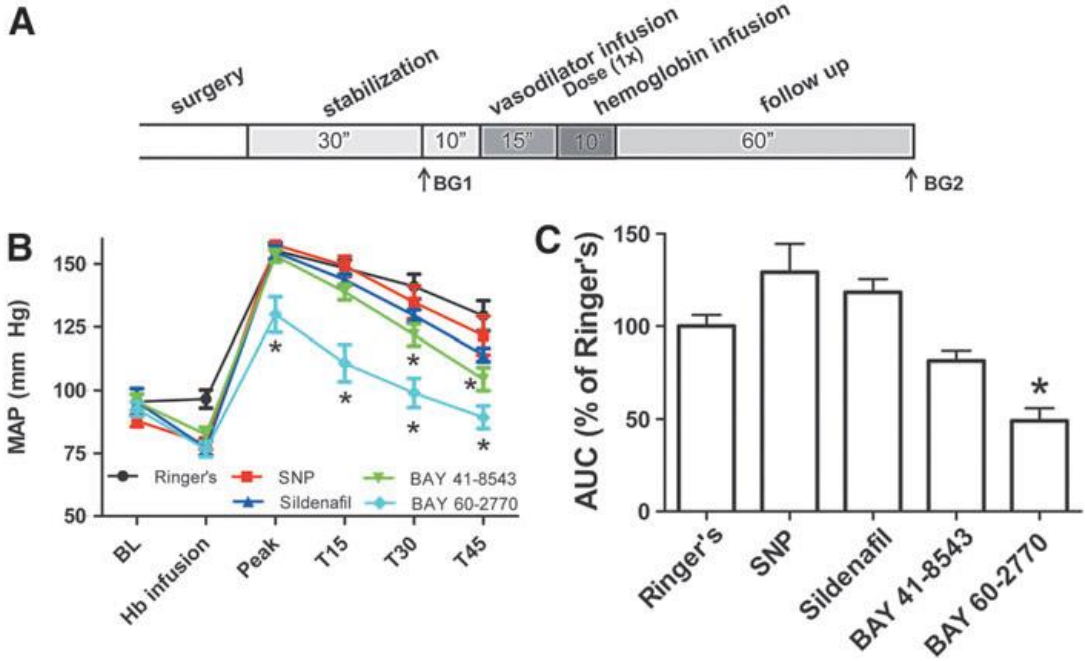

Figure 2. Effect of pre-infusion of vasodilators on hemoglobin-induced vasoconstriction. (A) Experimental timeline. Rats were stabilized for 30min after surgery, and blood gases were drawn as indicated (BG1 and BG2). After determining a baseline (BL), vasodilators were infused, followed by infusion of purified human hemoglobin (8.1mM) until an end concentration of 175mg/kg was reached. MAP at T = 15, 30, and 45min after hemoglobin infusion is displayed. **(B)** Effect of pre-infusion with Ringer's (n = 6) (–), SNP (0.4 μg/kg/min, n = 7) (–), or sildenafil (10 μg/kg/min, n = 8) (–) and bolus administration of BAY 41-8543 (10 μg/kg, n = 8) (–) and BAY 60-2770 (1 μg/kg, n = 9) (–) on basal MAP and peak MAP after infusion of hemoglobin. *Significantly different ($p<0.05$) from Ringer's by two-way ANOVA with Bonferroni correction. **(C)** Area under the curve (AUC) values from the graphs in panel A expressed as percentage from Ringer's values. *Significantly different ($p<0.001$) from Ringer's by one-way ANOVA with Bonferroni correction. SNP, sodium nitroprusside [36].

NO and cell adhesiveness in SCD. A growing theme in SCD research is the role of cell adhesion, and this might be a potential therapeutic target of riociguat. Under steady state conditions in patients and mice with SCD, many adhesion molecules and their counter-ligands are expressed at high levels on endothelium, erythrocytes, leukocytes and platelets, and also in plasma [37,38]. Increases in adhesiveness might be the precipitating cause of the intermittent vaso-occlusive pain crisis episodes that characterize the symptoms of SCD [39], and several pharmaceutical trials are testing approaches to inhibit adhesiveness. Prominent approaches under investigation are to inhibit the adhesion receptor P-selectin with small molecules (rivipansel or pentosan polysulfate sodium)[40,41], or with a monoclonal blocking antibody [42]. Preclinical studies suggest that increasing cGMP via PDE9 inhibition decreases adhesiveness of sickle cell blood leukocytes [43]. All of these promising results suggest a potential additional mechanism of action of riociguat for patients with SCD.

1.1.3 Previous clinical trials of NO pathway drugs in SCD

Sodium nitrite. Nitrite is a prodrug for NO production by enzymatic activity by hemoglobin, myoglobin, xanthine oxidase and other hemoproteins, especially under low oxygen tension [44]. In blood flow physiology studies conducted by our group, sodium nitrite infused in the brachial artery induced dose-dependent vasodilation in healthy volunteers and adults with SCD [45,46]. However, no additional results of systemic sodium nitrite in SCD have been presented.

Inhaled nitric oxide. A small pilot trial of inhaled NO suggested benefit in children with SCD receiving treatment in an emergency department for acute pain crisis [47]. This was perhaps surprising, since NO has a half-life of less than a second, and would not persist in the circulation from the lung to the systemic circulation. In fact, we found no evidence of benefit in a randomized, double blind, placebo-controlled study involving 150 adolescents and adults with SCD hospitalized for acute pain crisis [48].

Oral sildenafil. This phosphodiesterase-5 inhibitor was already approved by the FDA for the treatment of penile erectile dysfunction (Viagra®) and pulmonary arterial hypertension (Revatio®). Its mechanism of action involves inhibition of hydrolysis of cyclic GMP in tissues expressing its target PDE-5, mainly the penis and lung vasculature [49]. In a randomized controlled trial that enrolled adults and adolescents with SCD and elevated TRV and low exercise capacity, the Data Safety and Monitoring Board stopped the study prematurely due to an unexpected toxicity [50]. There was a 2.5-fold higher rate of hospitalization for acute pain in the sildenafil arm (45% for sildenafil, 22% for placebo, P=0.022). In this highly truncated study, there was no evidence of treatment benefit, although only a small minority of study patients completed the trial. Issues concerning the increased pain experienced by sildenafil recipients are addressed below.

1.1.4 Pain and cGMP

A substantial literature has emerged implicating cGMP as a pain sensitizing mechanism in the dorsal root ganglia of the spinal cord [51]. At present, this remains the most coherent hypothesis for the increased pain observed in SCD patients on sildenafil, which also increases cGMP. In further support of this idea, sildenafil also amplified neuropathic pain in a rat model [52], although this is a very complex pathway that is incompletely understood [53,54].

The track record of the cGMP-elevating agent sildenafil in promoting pain in patients with SCD suggests that any future trial of cGMP-elevating therapy in SCD should monitor closely for any evidence of increased pain as an adverse event.

1.2 Rationale of the Study

Riociguat has a dual mode of action: it sensitizes soluble guanylate cyclase (sGC) to the body's own nitric oxide (NO) and can also increase sGC activity in the absence of NO, causing vasorelaxation.

Patients with sickle cell disease have a defect in sGC activation attributed to decreased NO bioavailability [55]. NO deficiency is compounded by several concurrent pathways active in sickle cell disease:

- NO is scavenged by cell-free hemoglobin released from red blood cells as they lyse, and hemolysis is robust in sickle cell disease [4].
- NO scavenging by reactive oxygen species produced by abundant activity of xanthine oxidase and other oxidases [12,14];
- Depletion of the NO synthase substrate, arginine, by high plasma arginase activity, also released from red cells during hemolysis [5,56];

- Inhibition of NO synthase by high plasma levels of its endogenous inhibitor, asymmetric dimethylarginine (ADMA), and by deficiency of its co-factor, tetrahydrobiopterin [6-10]

Reduced sGC activation results in defects in vasodilation and in excessive activation of endothelial, neutrophil and platelet adhesion. These all combine to contribute to (a) vascular occlusion which produces pain and organ injury, and (b) proliferative arteriopathy manifest primarily as pulmonary arterial hypertension, which is life limiting. Adults with sickle cell disease with high systolic blood pressure have a higher risk of pulmonary hypertension, chronic kidney disease, ischemic stroke and death [17,18,57].

In a previous study of cGMP pathway manipulation in sickle cell disease using sildenafil, increased pain was a limiting adverse event, as discussed in the previous section [50]. Back pain is reported in the sildenafil package insert in 3-4% of sildenafil users (erectile dysfunction), and less frequently pain in the abdomen, chest, bone, eye or ear. In pulmonary hypertension patients enrolled in the PATENT-1 and CHEST-1 studies, pain (especially chest pain, a known symptom of pulmonary hypertension patients) was reported more frequently even in the placebo users (5-9%), but was no higher in the riociguat user (4-7%). These results suggest that riociguat may not activate the same pain sensitization pathways that might be activated by sildenafil.

The proposed clinical trial will recruit adults with sickle cell disease with systolic blood pressure (SBP) ≥130 mmHg, and/or macroalbuminuria as manifested by urine albumin to creatinine ratio of >300 mg/g, and/or TRV > 2.9 m/sec and/or urinalysis urine protein 1+ or higher, and/or serum amino-terminal pro-brain natriuretic peptide (NT-proBNP) as a cohort at high risk of sickle cell complications (See Table 1), and test safety and preliminary efficacy in reducing systemic blood pressure, albuminuria, TRV and NT-pro-BNP in an outpatient clinic setting.

Rationale for adjustments to the initial protocol criteria for inclusion and exclusion are discussed below:

* Expand clinical results of macroalbuminuria & TRV acceptable window from 12 months to 24 months.

  - many patients have these results identifying them as higher risk patients in their chart from more than 12 months ago, and loosening the time window is expected to add eligible subjects without the delays of updating their laboratory and echocardiography testing.

* Add inclusion criteria to accept clinical result of NT-proBNP level ≥ 160 pg/mL within the past 24 months.

  - NT-proBNP is a clinically available laboratory marker of cardiac ventricular stress. It has been established in adults with SCD to correlate with elevated TRV (R=0.50, P.001). NT-proBNP level of 160 pg/mL or greater independently predicts increased risk of death (21 deaths at 31 months' median follow-up; risk ratio, 5.1; 95% confidence interval, 2.1-12.5; P=.001; 19.5% absolute increase in risk of death). These results from the NIH SCD cohort study were confirmed in the Multicenter Study of Hydroxyurea. Elevated NT-proBNP correlated with hemodynamic measurements on pulmonary artery catheterization, and with functional capacity indicated by shortened 6-minute walk distance [Machado RF, Anthi A, Steinberg MH, Bonds D, Sachdev V, Kato GJ, Taveira-DaSilva AM, Ballas SK, Blackwelder W, Xu X, Hunter L, Barton B, Waclawiw M, Castro O, Gladwin MT; MSH Investigators. N-terminal pro-brain natriuretic peptide

levels and risk of death in sickle cell disease. JAMA. 2006 Jul 19;296(3):310-8. PubMed PMID: 16849664]. 25% of adults with SCD have NT-proBNP level of 160 pg/mL or greater. Although this population will overlap with other qualifying criteria in this protocol, in the frequent event of incomplete data in the medical record, this criterion will present an additional opportunity to identify high risk SCD patients from existing information in the medical record.

* Add inclusion criteria to accept clinical result of urinalysis protein 1+ or higher within the past 24 months

- Similar to macroalbuminuria, urinalysis protein 1+ or higher identifies an adult SCD subpopulation with early renal disease, associated with elevated TRV. The presence of proteinuria correlated with lower GFR and had a high positive predictive value (0.60) for elevated TRV in subjects with SCA (or HbS-beta-zero-thalassemia). Proteinuria occurs in 20–30% of patients with SCD, although higher incidences have also been reported [19,58,59]. Again, although this population will overlap with other qualifying criteria in this protocol, in the frequent event of incomplete data in the medical record, this criterion will present an additional opportunity to identify high risk SCD patients from existing information in the medical record.

* Exclude patients with PH-IIP to reflect the current riociguat IB

- Patients with pulmonary hypertension due to idiopathic interstitial pneumonia (PH-IIP), a more updated term for pulmonary fibrosis, did not benefit from riociguat in data in the riociguat investigator brochure. Patients on this study with SCD who have the unlikely co-morbidity of PH-IIP will be excluded event, consistent with current updated labeling information [60].

* Remove smoking from exclusion criteria

- Smoking is not a contraindication in current riociguat labeling [60]. Tobacco smoking can increase riociguat elimination, thereby decreasing drug exposure, but is part of real world potential usage of riociguat. Since opening of the study, 40-50% of SCD patients meeting inclusion criteria are smokers. The removal of tobacco smoking exclusion may increase the eligible study pool by nearly double, and add valuable safety and preliminary efficacy information on this large subpopulation.

* Revise exclusion criteria to use Child-Pugh classification to assess the severity of hepatic impairment. This also is in line with current riociguat package insert and IB.

- The current modification proposes to align with the current labeling: “not recommended in patients with severe (Child Pugh C) hepatic impairment [60].” The current exclusion criterion of SCD patients with direct bilirubin level of 1.0 mg/dL or higher is excessively restrictive and will be eliminated in favor of the Child-Pugh C exclusion. This will provide hepatic function standards consistent with the current label.

Study Procedures:

* Add INR as one of the liver function tests

- The INR is used to calculate the Child-Pugh class, as discussed above.

Table 1. Associations of clinical and laboratory characteristics with systemic hypertension (systolic BP ≥130) in SCD patients. Patients in the high SBP group have higher risk features demonstrated to predict higher mortality in sickle cell disease, including higher TRV, NT-proBNP, serum creatinine, and proteinuria. Results are from HbSS patients screened for the walk-PHaSST study. HbSC patients are excluded from this table.

Patient Characteristics	Sys BP < 130 mm Hg		Sys BP≥130 mm Hg		
	n	median (IQR)[2]	n	median (IQR)[2]	p[4]
N (%[1])		400 (80.0)		100 (20.0)	
Demographics and Vital Status					
Age	400	33 (24-44)	100	41 (31-51)	<0.0001
Male gender, N(%)	400	187 (46.8)	100	58 (58.0)	0.04
Deaths, N(%)	376	14 (3.7)	96	4 (4.2)	0.84
Follow-up time, months	376	29.3 (25.3-33.7)	96	28.4 (23.8-33.6)	0.36
BMI	394	22.9 (20.7-25.4)	98	23.6 (21.0-27.0)	0.04
O2 Sat	397	97 (95-99)	100	97 (94-98)	0.07
Respiratory rate	394	18 (16-20)	98	18 (16-20)	0.10
Pulse pressure	400	48 (42-55)	100	61 (54-70)	<0.0001
MAP	400	81 (76-86)	100	95 (91-101)	<0.0001
Clinical Measures					
Hydroxyurea, current use, N(%)	400	175 (43.8)	100	50 (50.0)	0.26
On chronic transfusion therapy, N(%)	399	61 (15.3)	100	11 (11.0)	0.28
20+ lifetime transfusions, N(%)	400	160 (40.0)	100	39 (39.0)	0.86
100+ lifetime transfusions, N(%)	400	52 (13.0)	100	14 (14.0)	0.79
Has chronic pain, N(%)	399	144 (36.1)	100	40 (40.0)	0.47
Acute pain rating	377	9 (7-10)	96	9 (7-10)	0.80
Had mild pain in last year, N(%)	394	251 (63.7)	100	75 (75.0)	0.03
Mild episodes in last year, n[3]	251	10 (3-30)	75	6 (2-36)	0.23
Had moderate pain in last year, N(%)	396	263 (66.4)	100	71 (71.0)	0.38
Moderate episodes in last year, n[3]	263	7 (3-20)	71	5 (2-15)	0.12
Had severe pain in last year, N(%)	400	166 (41.5)	100	53 (53.0)	0.04
Severe episodes in last year, n[3]	166	3 (2-6)	53	3 (1-5)	0.40
Had extremely severe pain in last year, N(%)	400	210 (52.5)	100	57 (57.0)	0.42
Extremely severe episodes in last year, n[3]	210	2 (1-4)	57	2 (1-4)	0.67
Six Minute Walk Distance	395	437 (385-504)	97	445 (378-510)	0.88
Echocardiographic Measures					
TRV, m/sec	361	2.5 (2.3-2.7)	88	2.6 (2.4-2.9)	0.006
TRV ≥ 2.5 m/sec, N(%)	361	220 (60.9)	88	62 (70.5)	0.10
TRV ≥ 2.7 m/sec, N(%)	361	137 (38.0)	88	43 (48.9)	0.06
TRV ≥ 3.0 m/sec, N(%)	361	34 (9.4)	88	18 (20.5)	0.004
LV Lat E/Ea ratio	362	6.33 (5.17-8.11)	90	6.67 (5.36-8.00)	0.32
Abnormal LV function, N(%)	395	20 (5.1)	99	7 (7.1)	0.43
LV ejection fraction	386	61 (60-66)	98	62 (59-67)	0.79
Laboratory Measures					
NT-proBNP (pg/mL)	370	71 (29-147)	94	110 (41-284)	0.003
NT-proBNP ≥ 160, N(%)	370	81 (21.9)	94	38 (40.4)	0.0002
Ferritin	361	273.6 (109.4-684.4)	89	344.4 (154.9-786.2)	0.21
Ferritin ≥ 1000, N(%)	361	66 (18.3)	89	19 (21.3)	0.51
Fetal Hemoglobin	355	6.0 (2.5-11.9)	90	8.6 (3.3-14.1)	0.12
HbF ≥ 5%, N(%)	355	207 (58.3)	90	58 (64.4)	0.29
HbF ≥ 7%, N(%)	355	158 (44.5)	90	49 (54.4)	0.09
Hemolytic Component	333	0.52 (-0.34-1.43)	87	0.68 (-0.54-1.72)	0.50
Absolute Reticulocyte Count (thousands/uL)	368	245 (165-344)	95	249 (160-325)	0.91
Reticulocytes (%)	369	9.0 (6.3-12.9)	92	9.4 (5.9-14.1)	0.78

Patient Characteristics	Sys BP < 130 mm Hg n	Sys BP < 130 mm Hg median (IQR)[2]	Sys BP≥130 mm Hg n	Sys BP≥130 mm Hg median (IQR)[2]	p[4]
Hemoglobin (g/dL)	391	8.8 (7.7-9.8)	98	8.6 (7.6-10.0)	0.67
Hematocrit (%)	392	25.3 (22.0-28.3)	98	25.1 (21.4-29.2)	0.58
MCHC (g/dL)	389	34.6 (33.5-35.6)	98	34.6 (33.7-35.5)	0.77
MCV (fL)	391	91.0 (85.0-99.9)	97	93.8 (87.5-100.4)	0.13
Platelets (thousands/uL)	391	367 (288-459)	98	355 (263-434)	0.15
RBC (millions/uL)	391	2.7 (2.3-3.2)	98	2.7 (2.2-3.1)	0.23
WBC (thousands/uL)	392	9.7 (7.6-12.2)	98	9.3 (6.7-12.5)	0.57
ANC (thousands/uL)	385	4.9 (3.5-6.6)	96	5.3 (3.1-7.1)	0.59
Neutrophils (%)	308	53.4 (44.8-60.1)	81	54.0 (45.5-61.9)	0.47
Albumin (mg/dL)	391	4.3 (3.9-4.5)	98	4.1 (3.8-4.4)	0.01
Alkaline Phosphatase (IU/L)	392	90 (69-120)	97	93 (71-136)	0.41
ALT (IU/L)	393	22 (16-33)	98	25 (18-34)	0.15
AST (IU/L)	382	41 (31-56)	95	48 (29-67)	0.24
AST (standardized unit normal)	382	-0.07 (-0.50-0.62)	95	0.12 (-0.58-0.99)	0.26
CO_2 (mEq/L)	392	24 (22-26)	98	24 (22-26)	0.76
BUN (mg/dL)	393	10 (7-27)	97	14 (8-28)	0.03
Creatinine (mg/dL)	394	0.7 (0.6-0.8)	98	0.9 (0.6-1.2)	<0.0001
LDH (IU/L)	360	416 (306-598)	93	426 (291-636)	0.80
LDH (standardized unit normal)	360	-0.02 (-0.53-0.66)	93	0.14 (-0.45-1.07)	0.21
Total Bilirubin (mg/dL)	392	2.8 (1.9-4.2)	98	2.6 (1.6-3.6)	0.20
Calcium (mEq/L)	384	9 (9-10)	97	9 (9-10)	0.15
Chloride (mEq/L)	394	106 (104-107)	98	105 (104-108)	0.28
Magnesium (mEq/L)	379	0.86 (0.78-0.90)	93	0.86 (0.82-0.93)	0.09
Phosphate/Phosphorus (mEq/L)	369	4.1 (3.6-4.8)	95	4.3 (3.6-5.2)	0.46
Potassium (mEq/L)	391	4.4 (4.1-4.7)	97	4.5 (4.2-4.8)	0.12
Sodium (mEq/L)	394	138 (137-140)	98	139 (137-140)	0.38
Total Protein (mg/dL)	388	7.8 (7.3-8.2)	97	7.5 (7.2-8.0)	0.02
Urine microalbumin (mg/G creatinine)	310	23 (7-120)	85	72 (9-774)	0.012
Urine albumin >30 mg/G creatinine, N (%) micro and macroalbuminuria	310	143 (46.1)	85	47 (55.3)	0.14
Urine albumin >300 mg/G creatinine, N (%) macroalbuminuria	310	44 (14.2)	85	27 (31.8)	0.0002
Protein in Urine, N positive(%)	380	112 (29.5)	94	50 (53.2)	<0.0001
Protein Level in Urine	95	0.0 (0.0-1.0)	42	1.0 (0.0-2.0)	0.02

1.2.1 Implications for clinical trial design

The first priority research question for riociguat in SCD is whether this drug is tolerated in patients with SCD. The primary outcome measure in the trial of riociguat proposed in this protocol will be safety and tolerability, focused on measuring overall incidence of treatment emergent SAE in the riociguat arm compared to the placebo arm. Secondary outcome measures will evaluate the changes in pain intensity and potential signal of efficacy in vasodilator effect on blood pressure, estimated pulmonary artery pressure, and proteinuria. Additional outcome measures will include adverse events attributable to SCD, which hypothetically will be reduced by riociguat, thereby serving simultaneously as a pilot study to identify variables with a signal of improvement with sGC stimulation. All of the selected outcome variables have potential for improvement with sGC stimulation.

1.3 Riociguat

1.3.1 Mechanism of action

Soluble guanylate cyclase (sGC) is an important regulator in the cardiovascular system and is present in vascular cells and platelets. The endogenous stimulus of the sGC is endothelial-cell-derived nitric oxide (NO), which acts in a paracrine fashion. In the underlying smooth muscle cells and in platelets NO activates the sGC, resulting in increased intracellular cyclic guanosine monophosphate (cGMP) concentrations (which induce vasorelaxation), inhibition of cell proliferation and migration as well as inhibition of platelet adhesion and aggregation [55].

Riociguat is a direct stimulator of sGC in vitro and in vivo which is independent from NO, the endogenous activator of the enzyme. Moreover, in the presence of NO, it enhances the effects of NO.

1.3.2 Preclinical

The toxicological program included studies to investigate the systemic toxicity as well as exaggerated pharmacodynamic effects after single and repeated administration up to 26 weeks in rats and up to 52 weeks in dogs, reproductive toxicity studies, genotoxicity studies and carcinogenicity studies as well as studies addressing specific questions (phototoxicity studies, toxicity of impurities, mechanistic investigation and studies in juvenile animals). The toxicological program was designed to support the chronic use of riociguat in humans.

The toxicological profile after repeated administration was characterized by effects secondary to the intracellular cGMP increase. In rats, the toxicological profile of riociguat was characterized by hemodynamic effects and sequelae thereof, as well as by changes on bone metabolism and morphology in juvenile and adolescent rats. The bone findings are considered to be related to the pharmacological mechanism of action, as the NO-sGC-cGMP pathway is involved in bone metabolism. As the findings were restricted to areas undergoing rapid turnover during bone growth, the findings in fast growing animals are not considered relevant for the adult patient population.

In experimental animals, riociguat treatment has revealed no impact on fertility and early embryonic development. Riociguat was negative in a battery of in vitro and in vivo genotoxicity tests and revealed no evidence for a genotoxic risk.

In 2-year lifetime bioassays in rats and mice, no drug-related neoplasms were observed under riociguat treatment, and an absence of a carcinogenic risk for humans is deduced.

In summary, single and repeat-dose toxicity studies in rats, mice and dogs, which fulfill the requirements for non-clinical safety evaluation, revealed no unexpected toxicity of riociguat.

The non-clinical safety profile is characterized by exaggerated pharmacological activity of riociguat subsequent to an increase of intracellular cGMP levels. Studies on genotoxicity, juvenile toxicity and carcinogenicity did not yield any specific concern.

Developmental toxicity revealed an increase of cardiac malformations in rats. This effect is seen to be due to a mechanism of action related to hemodynamic and potential anti-proliferative effect on the developing heart. In rats administered riociguat orally (1, 5, 25 mg/kg/day) throughout organogenesis, an increased rate of cardiac ventricular-septal defect was observed at the highest dose tested. The highest dose produced evidence of maternal toxicity (reduced body weight). Post-implantation loss was statistically significantly increased from the mid-dose of 5 mg/kg/day. Plasma

exposure at the lowest dose is approximately 0.15 times that in humans at the maximally recommended human dose (MRHD) of 2.5 mg three times a day based on area under the time-concentration curve (AUC). Plasma exposure at the highest dose is approximately 3 times that in humans at the MRHD while exposure at the mid-dose is approximately 0.5 times that in humans at the MRHD. In rabbits given doses of 0.5, 1.5 and 5 mg/kg/day, an increase in spontaneous abortions was observed starting at the middle dose of 1.5 mg/kg, and an increase in resorptions was observed at 5 mg/kg/day. Plasma exposures at these doses were 5 times and 15 times the human dose at MRHD respectively. Because riociguat was shown to have teratogenic effects when administered to animals; the drug is only available to females in the US through a restricted REMS (Risk Evaluation and Mitigation Strategies) program.

1.3.3 Clinical experience summary

Phase II studies in patients with pulmonary hypertension demonstrated that sGC stimulation as exerted by riociguat has the expected positive hemodynamic effects in this disease by reducing mean pulmonary artery pressure and pulmonary vascular resistance and increasing cardiac output at doses ≥1 mg in single-dose studies.

- Riociguat administered at doses of 1 to 2.5 mg tid over 12 weeks exerted significant, strong, and favorable effects on pulmonary hemodynamics and functional capacity in patients with PAH or CETPH. This was supported by echocardiographic data, NT-proBNP, and WHO functional class assessment. These improvements were maintained over 4.5 years of follow-up with continuous riociguat treatment.
- Administration of riociguat at doses of 0.5 to 2.0 mg as a titration regimen for 16 weeks did not result in statistically significant improvements in mean pulmonary arterial pressure (PAPmean) as compared to placebo in patients with symptomatic PH associated with left ventricular systolic dysfunction. However, treatment in the 2.0 mg target-dose arm (in comparison to placebo) resulted in consistent improvements in other hemodynamic parameters measured invasively and by echocardiography (left ventricular ejection fraction) (phase II Study 14308 LEPHT).

Phase III studies in patients with PH demonstrated that

- Administration of riociguat at doses of 1.0-2.5 mg (titration regimen) for 16 weeks results in a statistically significant and clinically meaningful improvement in 6MWD as compared to placebo in patients with CTEPH or recurrent or persisting PH after surgical treatment. Between baseline and week 16 an improvement in 6MWD was seen in the riociguat 1.0-2.5 mg group (mean increase of 38.9 m) as compared to a small change in 6MWD in the placebo. The treatment comparison demonstrated a statistically significant improvement in 6MWD for the riociguat 1.0-2.5 mg group compared to the placebo group in the ITT analysis set ($p<0.0001$) [56].
- Administration of riociguat at doses of 1.0-2.5 mg (titration regimen) for 12 weeks results in a statistically significant and clinically meaningful improvement in 6MWD as compared to placebo in patients with symptomatic PAH. The improvement in 6MWD was seen in both riociguat treatment groups: mean increase of 29.6 m in the riociguat 1.0-2.5 mg group and 31.1 m in the riociguat 1.0-1.5 mg group, as compared to a small change in 6MWD in the placebo group (mean –5.6 m, median 8.5 m) The treatment comparison demonstrated a statistically significant improvement in 6MWD for the riociguat 1.0-2.5 mg group as compared to the placebo group in the ITT analysis set ($p<0.001$) [57].
- Administration of riociguat with an individual dose titration regimen (1.0-2.5 mg) to a background treatment of a stable dose of sildenafil 20 mg tid appeared to be generally safe.

However, during the long-term extension phase, 8 of 17 patients discontinued due to AEs (3 deaths and 5 discontinuations due to AEs) during an average treatment period of approximately 10 months. The limited information on efficacy from the small sample, both during the double-blind main phase and the open-label long-term extension, did not indicate a signal of efficacy and clinical benefit. Therefore, the overall benefit-risk balance for the combination is not positive and combined use of riociguat with sildenafil is contraindicated [58].

1.3.4 Safety profile

The safety data described below reflect exposure to riociguat in two, randomized, double blind, placebo-controlled trials in patients with inoperable or recurrent/persistent CTEPH (CHEST-1) and treatment naive or pre-treated PAH patients (PATENT-1). The population (riociguat: n = 490; Placebo: n = 214) was between the age of 18 and 80 years [59].

The safety profile of riociguat in patients with inoperable or recurrent/persistent CTEPH (CHEST 1) and treatment naive or pre-treated PAH (PATENT 1) were similar. Therefore, adverse drug reactions (ADRs) identified from the 12 and 16 week placebo-controlled trials for PAH and CTEPH respectively were pooled, and those occurring more frequently on riociguat than placebo (≥3%) are displayed below.

Table 2. Adverse Reactions Occurring More Frequently (≥3%) on riociguat than Placebo (Pooled from CHEST 1 and PATENT 1)

Adverse Reactions	**Riociguat % (n=490)**	**Placebo % (n=214)**
Headache	27	18
Dyspepsia and Gastritis	21	8
Dizziness	20	13
Nausea	14	11
Diarrhea	12	8
Hypotension	10	4
Vomiting	10	7
Anemia (including laboratory parameters)	7	2
Gastroesophageal reflux disease	5	2
Constipation	5	1

2. STUDY OBJECTIVES

The purpose of this study is to evaluate the safety and tolerability of 12-weeks of treatment with riociguat versus placebo in high-risk patients with sickle cell disease, with additional evaluation for efficacy signal for improvement of blood pressure, exercise capacity and/or proteinuria.

2.1 Primary Hypothesis

We hypothesize that riociguat will be safely tolerated by adults with sickle cell disease.

The alternative hypothesis is that a difference in the proportion of participants experiencing at least 1 treatment-emergent severe adverse event will be seen between study arms. This reflects a superiority trial design, similar to other Phase 2 safety and tolerability studies.

The proposed clinical trial will recruit adults with sickle cell disease with systolic blood pressure (SBP) ≥130 mmHg, and/or macroalbuminuria as manifested by urine albumin to creatinine ratio of >300 mg/g, TRV > 2.9 m/sec, NT-proBNP level ≥ 160 pg/mL, and/or urinalysis protein 1+ or higher as a cohort at high risk of sickle cell complications. The study will enroll 100 adults with sickle cell disease to complete the protocol.

2.2 Secondary Hypothesis

Riociguat will yield a signal of improved outcome in adults with sickle cell disease, with potential improvements in systolic blood pressure, albuminuria, echocardiography TRV, serum NT-proBNP or six minute walk distance, known predictors of early mortality in adults with sickle cell disease.

3. LEAD INVESTIGATORS AND COORDINATING CENTER PERSONNEL

Lead Principal Investigator:	Mark T. Gladwin, MD Professor of Medicine Chair, Department of Medicine Director, Heart, Lung, Blood and Vascular Medicine Institute University of Pittsburgh Phone: 412-648-3181
Director of Regulatory Affairs:	Nydia Chien, MSN, RN, CCRC Heart, Lung, Blood and Vascular Medicine Institute University of Pittsburgh Phone: 412-647-2791
Data Coordinating Center:	Kaleab Z. Abebe, PhD Center for Clinical Trials & Data Coordination University of Pittsburgh Phone: 412-692-2023

Echocardiography Core Lab:	John Gorcsan, MD Washington University, Dept. of Cardiology 660 S. Euclid Ave. Campus Box 8086 St. Louis, MO 63110 Phone (314) 273-2255
Biospecimen Repository Core Lab:	Yingze Zhang, PhD, Director University of Pittsburgh, Dept. of Medicine Division on Pulmonary, Allergy and Critical Care Medicine NW 907 Montefiore 3459 Fifth Avenue Pittsburgh, PA 15213 Phone: (412) 647-2515
External Data Evaluation Bodies:	Institutional Data Safety Review Board (IDSMB) Clinical & Translational Science Institute University of Pittsburgh Phone: 412-864-3472

All other study personnel not included in this section are identified in a separate personnel list (not part of this clinical study protocol) as appropriate. This list will be updated as needed; an abbreviated version with personnel relevant for the participating centers will be available in each center's investigator site file.

A complete list of all participating centers and their investigators, as well as all required signature documents, will be maintained in the Coordinating Center study file.

4. STUDY DESIGN

4.1 Classification and Methodological Design

This is a Phase 2b multi-center, randomized, double-blind, placebo-controlled, parallel groups study aimed to evaluate the safety, tolerability and the efficacy of riociguat compared with placebo in patients with SCD.

4.2 Study Design Overview

The study plans to enroll patients with a clinical diagnosis of SCD for a total of 100 adult patients to complete the protocol.

The study will consist of a screening epoch, a 12 week double blinded treatment epoch, and a follow-up epoch of 30 days post treatment. Following completion of screening and baseline assessment, patients who meet all the inclusion and none of the exclusion criteria will be randomized on a 1:1 basis to receive either placebo or riociguat at the starting dose of 1.0 mg TID (three times a day) for 12 weeks.

The dose will be titrated every 2 weeks (+/-3 days) based on monitoring of subject's systolic blood pressure (SBP) and well-being assessed at that visit (see Figure 3).

PK/PD data will be collected on a subgroup of approximately 25 subjects at select sites. To preserve the subject blind, PK subjects will receive one dose of study medication (1 mg riociguat/placebo) per their randomized assignment.

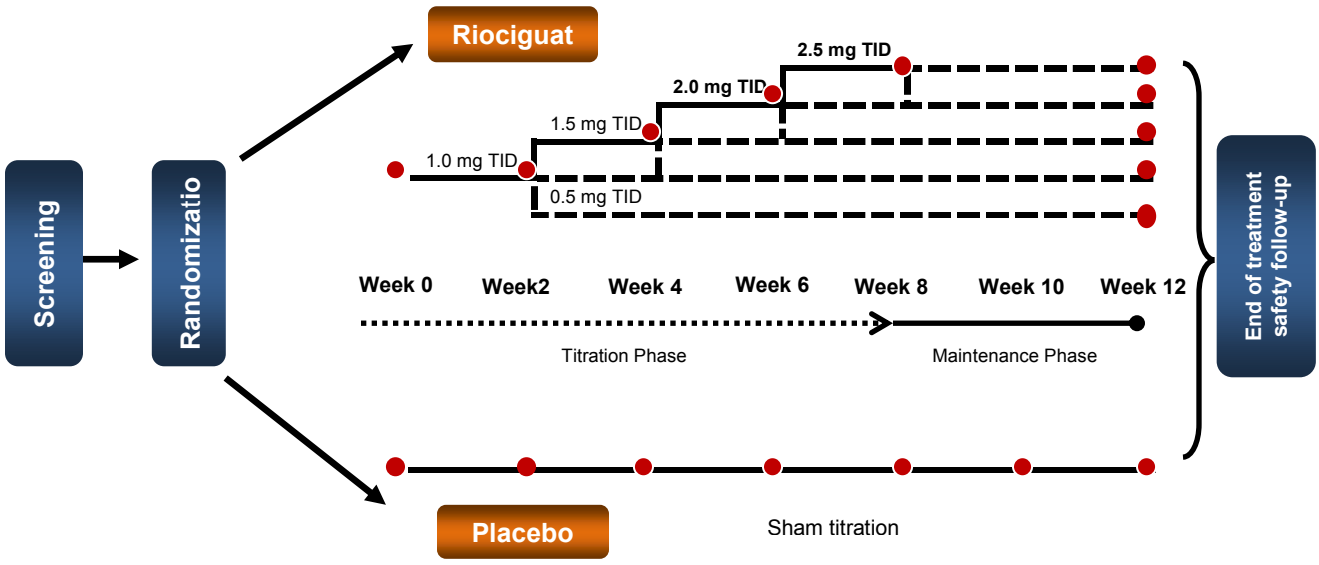

Figure 3. Schematics of Study Design

Following the starting dose of 1.0 mg TID (minimum dose interval - approximately 6 hrs; maximum dose interval - approximately 10 hrs), the subsequent dose modification will not exceed ±0.5 mg TID increments in 2-week intervals (+/- 3 days) to 1.5 mg, 2.0 mg, and 2.5 mg TID, resulting in a maximal total daily dose of 7.5 mg. Patients will be maintained on lower doses if higher doses are not tolerated (minimum lowest possible dose is 0.5 mg TID, total daily dose 1.5 mg). If the patient does not tolerate the lowest possible riociguat dose (0.5 mg TID), he/she must be withdrawn from the study.

At each biweekly titration visit, peripheral SBP will be measured and signs/symptoms of hypotension assessed. The individual patient dose will be assigned based on the following dose titration algorithm (see Figure 4). Date/time of when the subject took their last dose of riociguat will be recorded.

- SBP ≥ 95 mmHg and no signs or symptoms of hypotension, dosage should be increased by 0.5 mg TID
- SBP < 95 mmHg and no signs or symptoms of hypotension, dosage should be maintained
- SBP < 95 mmHg, and any signs or symptoms of hypotension associated with dizziness, lightheadedness or syncope, the current dose should be decreased by 0.5 mg TID

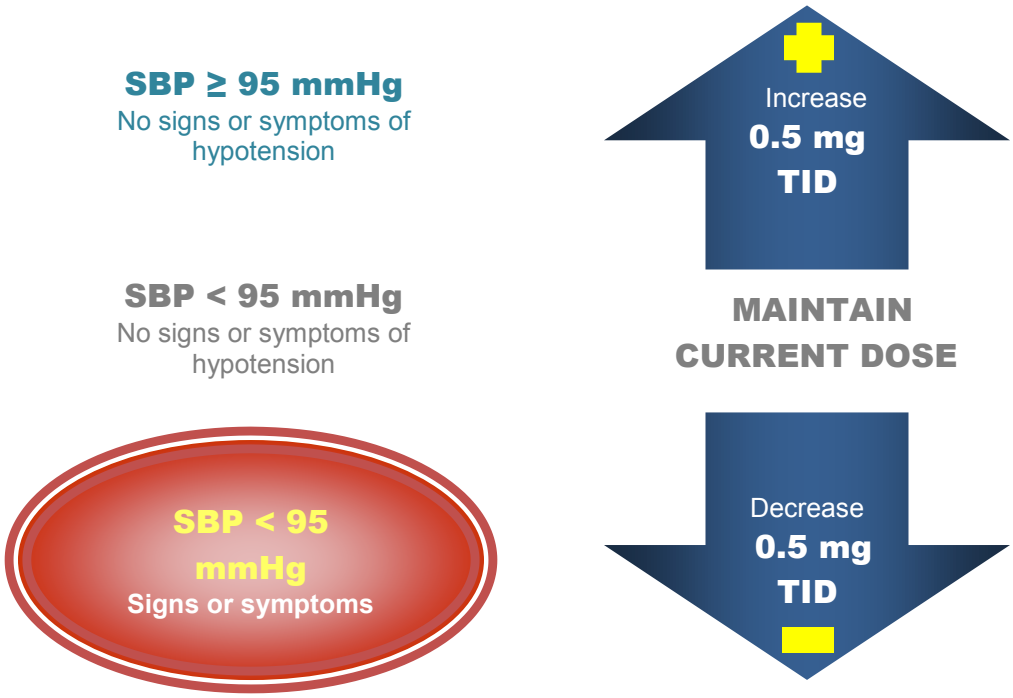

Figure 4. Dose Titration Algorithm

In the SCD population, as acute painful episodes and other associated clinical complications may frequently occur requiring hospitalizations or interruptions of study treatment, dose titration may be delayed, but every effort should be made to keep titrations delays to a maximum of 2 weeks. It is allowed in case of hospitalizations or for safety reasons, to suspend the next up-titration step and to maintain, or reduce, the dose. It is also permissible to up-titrate the dose for a patient if a down-titration has previously taken place. Dose reduction at any time is also permissible at the discretion of the investigator in the event the subject experiences symptomatic hypotension or for other safety reasons.

At the end of the dose titration phase with the target goal of the maximal dose of 2.5 mg TID (total daily dose of 7.5 mg), no further increase in dose will be allowed. The established individual dose will then be taken as the "optimal dose" to be administered for the remainder of the 12-week study period.

To assure blinding of the treatment arms, patients allocated to the placebo group will undergo a sham titration from Baseline Visit onwards following the rules of the individual dose titration scheme.

Safety monitoring including physical examinations, adverse event assessment, vital signs to include sitting blood pressure will be conducted at 2 week intervals during the double blinded study treatment. Safety lab assessment will be done at four week intervals during the treatment phase. Safety and tolerability, especially pain, will be assessed using several specific patient reported outcome instruments. Functional outcomes measuring the changes in Six Minute Walk Distance and Borg Dyspnea/Fatigue Scale will be assessed from baseline to Week 12.

All randomized patients, regardless of whether they completed study treatment through Week 12 or discontinued study treatment prior to Week 12, will be asked to return for a safety follow-up visit (approximately 30 days after stop of study medication intake).

4.3 Dose Interruptions

If a dose is missed, the patient should continue with the next regularly scheduled dose.

If during the trial an interruption takes place that lasts longer than 14 days (42 consecutive missed doses), restarting the study medication again is not allowed (respective patients must be withdrawn from the trial). Up to 14 days of missed doses, the patient can restart study drug at the original starting dose of 1.0 mg and follow the regular dose titration every two weeks as appropriate. In case of interruptions shorter than 3 days (9 doses), it is at the discretion of the investigator if the study medication can be restarted at the same dose or at 0.5 mg less than the last dose, with resumption of dose titration every two weeks as appropriate.

Hospitalization
Study visit data should be collected whenever possible if the subject is hospitalized. The decision to continue the study drug during hospitalization is at the discretion of the PI. If mechanisms are in place at the site to allow patients to receive the study medication while hospitalized, the protocol titration schedule should be followed.

4.4 Randomization

Patients will be randomly allocated to the riociguat arm, or the Placebo arm using a web randomization system. The randomization will be done in a 1:1 ratio in accordance with a computer-generated random code provided by the Data Coordinating Center (DCC) at the Baseline visit. The randomization is planned as a block randomization according to study site and will occur at Baseline. Subjects will begin taking the study medication the morning after the Baseline visit.

To obtain PK/PD data, approximately 25 patients enrolled at select sites will participate in PK/PD testing in order to obtain at least 13 evaluable samples sets. PK/PD subjects will have a trough sample drawn with their baseline labs. After Baseline assessments are completed, subjects will take one dose of riociguat 1 mg/placebo by mouth (based on their randomization assignment) followed by blood sampling at any one time point from 20 minutes to 3 hours post dose. Times of dosing and sampling will be recorded. The DCC will work with the outside laboratory providing the PK/PD analysis to confirm the presence of at least 13 evaluable samples. If data is found to be insufficient, PK/PD sampling may be re-opened until 13 is reached.

5. STUDY POPULATION

The patient population for the clinical trial is adults with sickle cell disease with high systolic blood pressure, and/or macroalbuminuria as manifested by urine albumin to creatinine ratio >300 mg/g, and/or TRV > 2.9 m/sec randomized between two arms of patients on placebo and patients on riociguat. Some patients may meet more than one criterion.

5.1 Eligibility

5.1.1 Inclusion criteria

Patients must fulfill each of the following criteria:

- Age ≥ 18 years.
- Sickling disorder (HbSS, HbSC, HbSbeta-thalassemia, HbSD, HbSO-Arab) documented by medical history, hemoglobin electrophoresis, or hemoglobin HPLC fractionation.
- At least one of the following findings:

  a. Systolic blood pressure ≥130 mm Hg on at least two occasions at least 1 day apart (one of these may be by past medical history and one is a current reading [at screening]),

  b. Macroalbuminuria as manifested by urine albumin to creatinine ratio > 300 mg/g. A clinical result within the past 24 months is acceptable to determine eligibility,

  c. Tricuspid regurgitant velocity (TRV) > 2.9 m/sec measured by echocardiography. A clinical echocardiogram done within the past 24 months while the patient is in his/her stable disease state is acceptable to determine eligibility.

  d. NT-proBNP level ≥ 160 pg/mL. A clinical result within the past 24 months is acceptable to determine eligibility.

  e. Urinalysis protein 1+ or higher. A clinical result within the past 24 months is acceptable to determine eligibility.
- Females of reproductive potential (FRP) must have a negative, pre-treatment pregnancy test. Post-menopausal women (defined as no menses for at least 1 year or post-surgical from bilateral oophorectomy/hysterectomy) are not required to undergo a pregnancy test.
- Females of reproductive potential must use acceptable methods of contraception. Subjects may choose one highly effective form of contraception (intrauterine devices [IUD], contraceptive implants or tubal sterilization) or a combination of methods (hormone method with a barrier method or two barrier methods). If a partner's vasectomy is the chosen method of contraception, a hormone or barrier method must be used along with this method. Adequate contraception is required beginning at the signing of the informed consent form until one month after the last dose of the study medication.
- Patients must be willing to provide a blood sample for DNA analysis.

5.1.2 Exclusion criteria

Patients will be excluded from the study if any of the following criteria are met:

- Pregnant or breastfeeding women
- Patients with severe hepatic impairment defined as Child Pugh C
- End stage renal disease requiring dialysis
- Patients with eGFR <30 mL/min/1.73m, where GFR is estimated based on CKD-epi equation;
- Patients on phosphodiesterase type 5 inhibitors (PDE-5) (such as sildenafil, tadalafil, vardenafil) and nonspecific PDE inhibitors (such as dipyridamole or theophylline) or nitrates.

- Patients on strong cytochrome P450 (CYP) and P-glycoprotein 1(P-gp)/BCRP inhibitors such as systemic azole antimycotics (e.g., ketoconazole, itraconazole), or HIV protease inhibitors (such as ritonavir).
- Patients on St. John's Wort.
- If patients are taking antihypertensive drugs, hydroxyurea, endothelin receptor antagonists, L-glutamine, crizanlizumab, or voxelotor prior to enrollment, they are excluded until the dose level is stable for at least three months. Subjects may return for repeat screening after dose is stable for 3 months. The decision to repeat the Screening labs is at the discretion of the PI.
- Systolic blood pressure < 95 mm Hg at Screening or Baseline Visit before randomization.
- Current enrollment in an investigational new drug trial. Patients are eligible for enrollment 30 days after the last dose of an investigational drug has been received.
- Evidence of positive qualitative urine drug test at screening for cocaine, phencyclidine (PCP), heroin, or amphetamines within three months prior to enrollment. Positive findings in subjects prescribed medications for treatment of pain and/or anxiety should **not** be considered evidence of positive qualitative urine drug test.
- Patients who have recently (last six months) experienced serious bleeding from the lung, or have undergone a bronchial arterial embolization procedure.
- Pulmonary hypertension associated with Idiopathic Interstitial Pneumonias
- Medical disorder, condition, or history that in the investigator's judgment would impair the patient's ability to participate or complete this study or render the patient to be inappropriate for enrollment.

5.2 Withdrawal of Patients from Study

5.2.1 Withdrawal

Patients must be withdrawn from the trial (treatment and procedures) for the following reasons:

- Pregnancy
- Patient withdraws consent from study treatment and study procedures. A patient must be removed from the trial at his/her own request or at the request of his/her legally acceptable representative. At any time during the trial and without giving reasons, a patient may decline to participate further. The patient will not suffer any disadvantage as a result.
- Patient is lost to follow-up.
- Death
- If, in the investigator's opinion, continuation of the trial would be harmful to the patient's well-being.
- At the specific request of the sponsor (e.g., detection of a new significant safety concern related to the study drug).

- Occurrence of AEs or intercurrent diseases which the investigator judges unacceptable for continuation of participation in the study.
- In case no further dose reduction is possible and the patient does not tolerate the lowest possible riociguat dose (0.5 mg TID).
- If treatment is interrupted for more than 42 (14 days) consecutive missed doses during any dose titration phase. (Patient is able to restart study drug at 1.0 mg after being off study drug up for up to 14 days.)

Patients may be withdrawn from the study for the following reasons:

- The patient is non-compliant with study drug, trial procedures, or both
- Development of an intercurrent illness or situation which would, in the judgment of the investigator, significantly affect assessments of clinical status and trial endpoints.
- Use of illicit drugs or other substances that may, in the opinion of the investigator, have a reasonable chance of contributing to toxicity or otherwise skewing trial result.
- Occurrence of adverse drug reactions, which have from the investigator's point of view, a negative impact on the patient's individual risk-benefit ratio. (Investigators are obliged to reassess the patient's individual risk-benefit ratio on a continuous basis. Factors like anticipated treatment effect, progression of underlying disease, occurrence of side effects and alternative treatment options have to be considered.)
- Pertinent non-compliance with the conditions for the trial or instructions by the investigator.
- Participation in another clinical trial requiring study medication

Any patient removed from the trial will remain under medical supervision until discharge or transfer is medically acceptable.

In all cases, the reason for withdrawal must be recorded in the CRF.

5.2.2 Screen failures/dropouts

A patient who discontinues study participation prematurely for any reason is defined as a "dropout" if the patient has already been randomized and received at least one dose of study drug.

A patient who, for any reason (e.g., failure to satisfy the selection criteria), terminates the study before the time point used for the definition of "dropout" (see above) is regarded a "screening failure."

5.2.3 Replacement

Randomized patients who withdraw prematurely will not be replaced.

6. TREATMENT[S]

6.1 Treatments to be Administered

Riociguat immediate release (IR) tablets are available in dose strengths of 0.5 mg, 1.0 mg, 1.5 mg, 2.0 mg, and 2.5 mg. Riociguat IR tablets of all dose strengths are round tablets with 6 mm diameter.

Riociguat and placebo will be self-administered TID (approximately 6 to 10 hours apart), as film-coated tablets with or without food.

Riociguat and placebo will be supplied as film-coated tablets packaged in high-density polyethylene bottles. All tablet formulations will be identical in appearance (size, shape, color) and smell. The packaging and labeling will be designed to maintain blinding to the Investigator's team and to patients.

6.2 Treatment Assignment

A patient number (a unique identification number) will be assigned when a patient is evaluated for inclusion into the study. Patients who complete all screening procedures and meet all eligibility criteria for the study will be allocated using a web-based randomization system in a 1:1 ratio.

6.2.1 Dosing and administration

Riociguat	
Dosage:	0.5, 1 mg, 1.5 mg, 2 mg, 2.5 mg (individual dose titration)
Route of administration:	Oral
Frequency of administration:	TID

Placebo	
Dosage:	0.5 mg, 1 mg, 1.5 mg, 2 mg, 2.5 mg (sham dose titration)
Route of administration:	Oral
Frequency of administration:	TID

All subjects will begin taking study medication the AM after the Baseline visit. The starting dosage is 1.0 mg taken by mouth 3 times a day with or without food. If systolic blood pressure remains greater than 95 mmHg and the patient has no signs or symptoms of hypotension, up-titrate the dose by 0.5 mg taken three times a day. If systolic blood pressure remains less than 95 mmHg and no signs or symptoms of hypotension, dosage should be maintained. Dose increases should be 2 weeks apart (+/-3 days). The dose can be increased to the highest tolerated dosage, up to a maximum of 2.5 mg taken three times a day. SBP < 95 mmHg and no signs or symptoms of hypotension, dosage should be maintained.

Dose reduction at any time is permissible at the discretion of the investigator in the event the participant experiences symptomatic hypotension or for other safety reasons. In the event down titration is required between scheduled study visits, subjects should remain at the lower dose ordered until their next scheduled study visit. Up titration should not be considered until it is determined the subject is tolerating the current dose for at least two weeks (+/- 3 days). The lowest dose permissible is 0.5 mg, and participants who cannot tolerate 0.5 mg should be withdrawn from the trial.

6.3 Blinding and Unblinding

The study will be conducted as a double blind trial. In general, patients and investigators will remain blinded until study database lock and authorization of data release.

Unblinding by drug safety personnel of the sponsor

In compliance with applicable regulations, in the event of a SUSARs, the patient's treatment code will usually be unblinded before reporting to the health authorities, ethic committees and investigators (see Section 7.6.3.4).

Emergency unblinding by the investigator

Unblinding may occur for emergency purposes only. Investigators should note that the occurrence of a serious adverse event or progressive disease should not routinely precipitate the immediate unblinding of the label. The Investigator is required to promptly document and explain to the Sponsor any premature unblinding of the study drug within 24 hours of unblinding.

6.3.1 Accountability

Study drugs will be stored at the investigational site in accordance with Good Clinical Practice (GCP) and Good Manufacturing Practices (GMP) requirements, and will be inaccessible to unauthorized personnel.

The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of the investigational drug products.

6.3.2 Destruction and return

At the end of the study, unused supplies of riociguat and placebo will be destroyed according to site institutional policies. Destruction will be documented in the Drug Accountability Record Form. Written instructions on medication destruction will be made available to affected parties as applicable.

6.4 Treatment Compliance

An adequate record of receipt, distribution, and destruction of all study drugs must be kept in the form of a Drug Accountability Form.

Patient compliance with the treatment and protocol includes willingness to comply with all aspects of the protocol, and to have blood collected for all safety evaluations. At the discretion of the principal investigator, a patient may be discontinued from the trial for non-compliance with follow-up visits or study drug.

Study drug compliance will be calculated by taking the amount of drug ingested divided by the amount the subject should have ingested and multiply by 100. Overall compliance with study drug intake will be assessed during data analysis.

6.5 Prior and Concomitant Therapy

All medication that is considered necessary for the patient's welfare, and which is not expected to interfere with the evaluation of the study treatment, may be given at the discretion of the investigator.

All medications (including contrast media) taken within 30 days prior to the start of the study and during the study must be recorded in the in the eCRF (including start/stop dates, dose frequency, route of administration, and indication).

6.5.1 Permitted medications

- Hydroxyurea: Hydroxyurea is an FDA approved drug for the treatment of sickle cell disease. There is no expected drug interaction or additive side effect between riociguat (mainly vasodilation) and hydroxyurea (principally myelosuppression). We plan to allow enrollment of subjects already on a stable dose of hydroxyurea as well as subjects not taking hydroxyurea. In this way, riociguat will be tested for its effect both independent of hydroxyurea and in combination with hydroxyurea. To a limited extent, hydroxyurea can act as an NO donor, and the safety of its coadministration with riociguat will be an important safety determination of this study.
- L-glutamine: L-glutamine was approved by the FDA on July 7, 2017 to treat sickle cell disease. There is no expected drug interaction or additive side effect between riociguat and L-glutamine. We plan to allow enrollment of subjects already on a stable dose of L-glutamine. It may also be used concurrently with or without hydroxyurea.
- Crizanlizumab: Approved by the FDA on November 15, 2019 to reduce the frequency of VOC in adults with sickle cell disease. There is no expected drug interaction or additive side effect between riociguat and crizanlizumab. We plan to allow enrollment of subjects already on a stable dose of crizanlizumab. It may also be used concurrently with or without hydroxyurea.
- Voxelotor: Approved by the FDA on November 25, 2019 to treat sicke cell disease. There is no expected drug interaction or additive side effect between riociguat and voxelotor. We plan to allow enrollment of subjects already on a stable dose of voxelotor. It may also be used concurrently with or without hydroxyurea.
- Clinically indicated and stably dosed FDA approved medications will be permissible except for PDE5 inhibitors, P450 (CYP) and P-glycoprotein 1(P-gp)/BCRP inhibitors, and oral nitrates.
- Systemic vasodilators, endothelin receptor antagonists, and prostacyclin derivatives will be permissible. However, the dosage of vasodilators, endothelin receptor antagonists, and prostacyclin derivatives need to be stable for at least three months prior to enrollment and preferably their dosage should remain stable throughout the 12-week study treatment period.
- Treatment with non-conventional therapies (e.g., herbs [with the exception of St. John's Wort], acupuncture) and vitamin/mineral supplements is acceptable provided that, in the opinion of the investigator, such treatment will not interfere with the trial endpoints.
- Patients may receive standard of care for any underlying illness.

6.5.2 Restricted medications

Co-administration of riociguat with the following concomitant medications is NOT allowed:

- Nitrates: Nitrates or NO donors (such as amyl nitrite) in any form (e.g., nitrates, molsidomine, sodium nitroprusside) because of hypotension

- PDE inhibitors: PDE-5-inhibitors (such as sildenafil, tadalafil, vardenafil) and nonspecific PDE inhibitors (such as dipyridamole or theophylline) because of hypotension
- Arginine supplements

7. PROCEDURES AND VARIABLES

7.1.1 Study flowchart

See Appendix A for Schedule of Procedures.

7.1.2 Timing of assessments

Outlined below are study procedures that will be performed at screening, during the study treatments, and at follow-up.

Visit 1 (Screening)

- Obtain written informed consent.
- Demographics, and review of medical history for inclusion and exclusion criteria
- Concomitant medications
- Vital Signs
- Clinical laboratory evaluations including CBC with differential, reticulocyte count, hepatic panel to include total bilirubin, INR (International Normalized Ratio), renal chemistry, and LDH (INR may be used from the patient's medical record within 3 months of screening visit)
- Urine or serum pregnancy test for women of child bearing potential
- Electronic pain diary (piloted only at selected sites)

Re-screening: It is permitted to re-screen each subject once. The investigator will use clinical judgment to determine if any of the screening procedures need to be repeated or if re-consenting is required. Additional re-screening may be allowed only upon approval by the Coordinating Center.

Visit 2 Baseline (Week 0)

The Baseline assessments may be done in two visits to accommodate subject/site schedule limitations). The Baseline visit may occur from 0-4 weeks post screening, and may also be combined with the Screening visit procedures.

- Confirm systolic blood pressure eligibility
- Randomization
- Comprehensive Medical History
- Concomitant medications
- Review of Symptoms
- Complete physical examination to include vital signs, body weight, and height.
- Brief Pain Inventory, ASCQ-Me, PROMIS Assessments
- Clinical laboratory evaluations including CBC with differential, reticulocyte count, hepatic chemistry to include total/direct bilirubin, albumin, renal chemistry, and LDH

- Urinalysis
- Urine albumin and urine creatinine concentrations
- Urine or serum pregnancy test for women of child bearing potential
- Research blood sample for hemoglobin HPLC or electrophoresis and ferritin will be processed at the site's clinical lab
- Research blood sample for biomarkers analysis, NT-proBNP, DNA, PK/PD (if appropriate) will be drawn at the clinical site and sent to the Coordinating Center Core Lab for processing
- Research blood sample for genotyping for alpha-thalassemia and BCL11a rs766432
- Baseline research echocardiogram
- 6 Minute Walk Test with Borg Dyspnea/Fatigue Scale
- Electronic pain diary (piloted only at select sites)
- Record results of RHC if performed at standard of care
- PK/PD studies (performed only on approximately 25 patients enrolled at select sites)
- Dispensing study drugs and instructions for use and storage. Study medication should be started the AM after the baseline visit.

Visit 3 (Week 2)

- Treatment compliance
- Physical examination
- Vital signs
- Titration of study drug dose
- Dispensing study drugs
- Concomitant medications
- Review of Symptoms/AE assessment
- Electronic pain diary (piloted only at select sites)

Visit 4 (Week 4)

- Treatment compliance
- Physical examination
- Vital signs
- Clinical laboratory evaluations including CBC with differential, reticulocyte count, hepatic chemistry to include total/direct bilirubin, albumin, renal chemistry, and LDH
- Urine or serum pregnancy test for women of child bearing potential
- Titration of study drug dose
- Dispensing study drugs
- Concomitant medications
- Review of Symptoms/AE assessment
- Electronic pain diary (piloted only at select sites)

Visit 5 (Week 6)

- Treatment compliance
- Physical examination
- Vital signs

- Titration of study drug dose
- Dispensing study drugs
- Concomitant medications
- Review of Symptoms/AE assessment
- Electronic pain diary (piloted only at select sites)

Visit 6 (Week 8)

- Treatment compliance
- Physical examination
- Vital signs
- Clinical laboratory evaluations including CBC with differential, reticulocyte count, hepatic chemistry to include total/direct bilirubin, albumin, renal chemistry, and LDH
- Urine or serum pregnancy test for women of child bearing potential
- Titration of study drug dose
- Dispensing study drugs
- Concomitant medications
- Review of Symptoms/AE assessment
- Electronic pain diary (piloted only at select sites)

Visit 7 (Week 10)

- Treatment compliance
- Physical examination
- Vital signs
- Titration of study drug dose
- Dispensing study drugs
- Concomitant medications
- Review of Symptoms/AE assessment
- Electronic pain diary (piloted only at select sites)

Visit 8 End of Treatment Visit (Week 12)

- Treatment compliance
- Physical examination
- Vital signs and body weight
- Clinical laboratory evaluations including CBC with differential, reticulocyte count, hepatic chemistry to include total/direct bilirubin, albumin, renal chemistry, and LDH
- Research blood sample for hemoglobin HPLC or electrophoresis and ferritin will be processed at the site's clinical lab
- Urinalysis
- Urine albumin and urine creatinine concentrations
- Urine or serum pregnancy test for women of child bearing potential
- Research blood samples for biomarkers analysis and NT-proBNP will be drawn at the clinical site and sent to the Coordinating Center Core Lab for processing
- Research echocardiogram

- 6 minute walk test with Borg Dyspnea/Fatigue Scale
- Brief Pain Inventory, ASCQ-Me, PROMIS Assessments
- Concomitant medications
- Review of Symptoms/AE assessment
- Electronic pain diary (piloted only at select sites)
- Record results of RHC if performed as standard of care after the baseline visit

Visit 9 (Week 13)

- Phone call follow-up
- Concomitant medications
- Review of Symptoms/AE assessment

Final Visit (30 days (+/-3 days) after Week 12 Visit)

- Physical examination
- Vital signs
- Urine or serum pregnancy test for women of child bearing potential
- Concomitant medications
- Review of Symptoms/AE assessment

7.1.3 Early Discontinuation of Study Treatment and/or Early Withdrawal from Study

If for whatever reason a subject was withdrawn from the study drug treatment prior to completing Week 12, the subject should be encouraged to remain in the study and return to the clinic to complete end of treatment assessments as indicated at Visit 8 within 3 days of discontinuing study drug if possible, as well as scheduled telephone follow-up Visit 9 (one week post discontinuation of study drug) and Final Visit 10 (30 days post discontinuation of study drug) in order to collect important safety information.

If a subject cannot or is unwilling to attend any clinic visit(s), at a minimum, the site staff should make every effort to make phone contact with the subject to obtain information concerning the subject's health status including adverse events and concomitant medications for the visit 8, 9 and 10 forms.

If a subject decides to completely withdraw from the study (refuses any further study participation or contact), all efforts should be made to complete and report the observations prior to withdrawal.

7.2 Safety Evaluation and Procedures

7.2.1 Medical history

Medical history findings (i.e. previous diagnoses, diseases or surgeries) meeting all criteria listed below will be collected:

- Indication
- Start before signing of the informed consent

- Considered relevant to the study

Detailed instructions on the differentiation between (i) medical history and (ii) adverse events can be found in Section 7.6.3.1.

7.2.2 Physical examination

A physical examination that includes a review of the following body systems will be performed at each clinic visit beginning at Baseline:

- General Appearance
- Skin
- Head, Ears, Eyes, Nose, Throat
- Spine/Neck/Thyroid
- Respiratory
- Cardiovascular
- Abdomen
- Nervous System
- Musculoskeletal

Any abnormalities or changes in intensity from the baseline assessment noted during the review of body systems should be documented and reported on the appropriate eCRF. If a new clinically significant finding (i.e., not noted at baseline) occurs at subsequent examination, an AE form must be completed. In addition, resolution of any abnormal findings during the study will be noted in the medical record and/or the eCRF if clinically significant.

7.2.3 Vital signs

Blood pressure, heart rate, and respirations will be measured after the subject has been at rest for 10 minutes in a sitting position. Height and weight will be measured at the Baseline Visit. At Visit 8, weight measurement will be repeated.

7.2.4 Pain assessments

Brief Pain Inventory

The Brief Pain Inventory will be used as a tool to measure the intensity of pain and interference of pain as well as to query the patient about pain relief, pain quality, and patient's perception of the cause of pain during the study treatment at Baseline and Week 12.

Adult Sickle Cell Quality of Life Measurement Information System

The Adult Sickle Cell Quality of Life Measure (ASCQ-Me) is an NIH-funded multicenter network that has enrolled over 500 patients in developing a patient reported outcome specific to sickle cell disease. The measure has been adapted to a version that can be administered by computer [61,62]. ASCQ-Me is the first disease-specific partner of the NIH Roadmap Patient-Reported Outcomes Measurement Information System (PROMIS)[63-65]. ASCQ-Me will be accompanied by the ten-item Global Health form from PROMIS. These are not yet validated endpoints accepted by the FDA, but the FDA has encouraged more data to be developed with them to potentially validate future use as endpoints for drug approval.

Patients will report outcomes using the sickle cell-specific patient reported outcome instrument, ASCQ-Me, and PROMIS at Baseline and Week 12. Whenever possible, the site staff will provide study subjects a secure web link that will allow subjects t to enter their responses directly from a computer, tablet computer, or smart phone without additional intervention from the site staff. The ASCQ-Me computer version has been designed and tested for direct user input by sickle cell patients using computers, tablets or smart phones. Subjects may complete paper versions of the assessments if internet access is not feasible.

Electronic Pain Diary

The electronic pain diary will be piloted only at select sites in approximately fifteen patients. This is a secure, password-protected electronic pain diary housed on a secure password-protected server (Fig. 5). The adaptable design allows the application to be accessed using a smartphone, computer, or tablet. The mobile web site has been chosen over an "app" so that the application is platform independent (i.e., users can use an iPhone, Android, or any other platform). The pain diary allows patients to enter medications taken at home. Content validity has been validated and its development and initial end-user reviews have been published in the Clinical Journal of Pain [66].

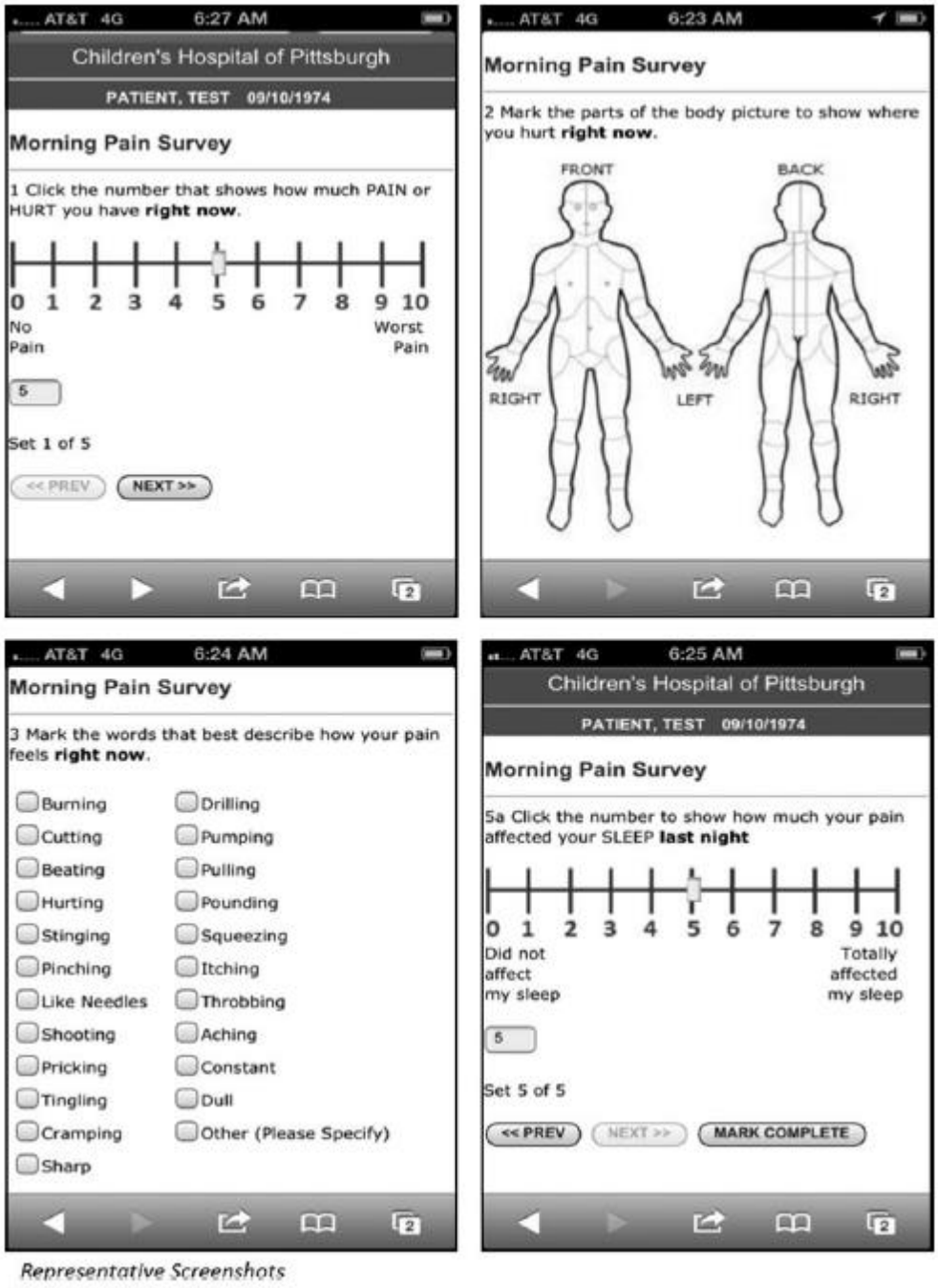

Figure 5. Representative screen shots from the online sickle cell disease electronic pain diary. This pain diary has been developed in an iterative process, customized to the needs of sickle cell disease [66].

7.2.5 Laboratory Parameters

All clinical laboratory assays will be performed locally at each participating site according to the laboratory's normal procedures. Clinical lab results drawn within the +/- 3 day window for the subjects standard care may be accepted for the study labs. Lab assays may be repeated once to rule out lab error, before determining if results are normal or abnormal. It is recognized that in SCD lab values that are out of the reference range may in fact be baseline for that individual. When test results are outside the reference range, the investigator will indicate on the CRF

whether the result is considered to be clinically significant or not. Out of range results not considered to be clinically significant by the investigator will not be considered abnormal for the purposes of this study. The laboratory parameters are outlined below. For details on the timing of blood and urine sampling, refer to Appendix A.

- Hematology: Complete Blood Count (CBC): Hemoglobin, hematocrit, red blood cell (RBC), mean corpuscular hemoglobin concentration (MCHC), mean cell volume (MCV), reticulocyte counts, white blood cell (WBC) count, platelet count, and ANC), reticulocyte counts
- Liver function tests: AST, ALT, albumin, total protein, total bilirubin, direct bilirubin, INR, alkaline phosphatase, LDH
- Renal function tests: potassium, sodium, chloride, bicarbonate, blood urea nitrogen (BUN), and creatinine
- Urinalysis
- Urine albumin and creatinine concentrations
- Pregnancy Test: Urine or serum pregnancy test will be performed only for women of child bearing potential at screening, prior to receiving study treatment (Baseline), and at 4 week intervals. There is site to site variability in preference for urine or serum pregnancy test. Some institutions require a serum pregnancy test, which is equal or superior in sensitivity to the urine pregnancy test. Adding the option of a serum pregnancy test allows for flexibility per site institutional policies.

7.3 Efficacy Evaluation and Procedures

7.3.1 Tricuspid regurgitant velocity by echocardiography

Increased tricuspid regurgitant velocity (TRV) is shown to link to high incidences of unexplained deaths in adult SCD patients with cardiopulmonary complications. TRV and echocardiography markers of left ventricular diastolic dysfunction will be assessed using a non-invasive transthoracic echocardiography at baseline and the end of double blinded study treatment at Week 12 according to the Echo Manual.

Other routine echocardiographic measures will include LV volumes and ejection fraction, LV mass and LV mass index, and left atrial volume. Mitral inflow recordings will be obtained with the pulsed Doppler sample volume at the level of the mitral leaflet tips during maximal opening in diastole. Tissue Doppler recordings will also be measured with sample volumes at both the septal mitral annulus and lateral mitral annulus. The ratio of early diastolic LV inflow velocity to lateral mitral annular velocity will be calculated as an estimate of LV filling pressure. TRV will be used to estimate pulmonary artery systolic pressures.

The de-identified echocardiograms will be sent to the Echo Core Lab at Washington University in St. Louis, MO for central reading. Echocardiography will be performed at baseline and Week 12 on all randomized subjects. Readers will be blinded to randomization assignment.

7.3.2 Optional right heart catheterization

Right heart catheterization is <u>not required</u> but may be considered as standard of care at discretion of the local investigator. It could be considered for patients who have both TRV >2.7 m/s and 6 MWD <500 m. The parameters described in Table 3 below will be recorded if available at

Baseline and Week 12. If the patient has had right heart catheterization up to 12 months prior to enrollment, these results could be collected at time of enrollment.

Table 3. Right Heart Catheterization Parameters

Directly measured parameters	Value	Unit
Mean right atrial pressure (RAPmean)		mmHg
Right Ventricular Systolic Pressure (RVSP)		mmHg
Right Ventricular Diastolic Pressure (RVDP)		mmHg
Systolic, diastolic, and mean pulmonary arterial pressure (PAPsyst, PAPdiast, mPAP)		mmHg
Pulmonary capillary wedge pressure (PCWP)		mmHg
Heart rate (HR)		beats per minute
Systolic, diastolic, and mean systemic arterial pressure (SBP, DBP, SAPmean)		mmHg
Cardiac output (CO); average of 3 measurements, performed and calculated by CO device/thermodilution methodology; 5 measurements in case of atrial fibrillation		L/min
Mixed venous oxygen saturation rate (SvO2)		%

7.3.3 Six (6) Minute Walk Distance (6MWD) Test

The 6MWD Test must be performed at Baseline and Week 12. Per the American Thoracic Society (ATS) Guideline, the 6MWD Test will be carried out indoors, along a long, flat, straight, enclosed corridor with hard surface that is seldom traveled. The walking course must be 30 meters in length, but not less than 25 meters. The length of the corridor and turnaround points should be marked. Patients will be instructed to walk alone, not run, from one end to the other end of the walking course, at their own pace, while attempting to cover as much ground as possible in 6 min.

7.3.4 The Borg Dyspnea/Fatigue Scale

The Borg Scale will be measured in conjunction with the 6MWD Test at Baseline and Week 12. The scale will be used to measure the level of severity of breathlessness perceived by the patient before and after 6MWD test. The severity is measured on a 10 point scale with 0 = nothing at all and 10 = maximum severity of breathlessness. The Borg Scale will be explained to the patients before starting the 6MWD Test. Patients will be asked to rank their exertion at the end of the 6MWD Test. The rate of exertion will be given according to the following scale ranging from 0 to 10.

7.3.5 Biomarkers and Repository

At Baseline and Week 12, blood will be collected in specific tubes provided by the Coordinating Center. The details of tube type and sample processing will be provided in the lab manual for this protocol.

N-terminal prohormone of brain natriuretic peptide (NT-proBNP)

NT-proBNP, a protein generated by the heart, is elevated in SCD patients suffering from congestive heart failure and pulmonary hypertension. NT-proBNP will be evaluated at Baseline and Week 12. NT-proBNP will be quantified in plasma. Samples will be drawn before intake of study drugs. Further details on collection, labeling, storage, and shipping of samples are provided in a separate laboratory manual.

Additional exploratory biomarkers

The following additional exploratory plasma biomarkers known to play important roles in prognosis of sickle cell disease will be quantified at Baseline and Week 12.

- Biomarkers of oxidative stress: 3-Nitro-tyrosine, Malondialdehyde and 8-IsoPGF2a.
- Biomarkers of NO-Pathway dysregulation: asymmetric dimethylarginine (ADMA), SDMA and cGMP.
- Biomarkers of inflammation (C-reactive protein) and erythropoiesis (erythropoietin)

Biospecimen Repository

Any remaining blood will be stored indefinitely at the University of Pittsburgh's Core Biospecimen Repository for future research. Samples will be stored without identifiers.

7.4 Pharmacokinetics / Pharmacodynamics Studies

A small subgroup of patients will be used to obtain at least 13 evaluable sample sets for PK/PD of riociguat in SCD. We will look at drug concentrations at two timepoints for each patient – approximately at Cmax and at expected time of trough. Data analysis will be conducted using non-compartmental methods.

Pharmacokinetics studies, coupled with the individual patient's systolic blood pressure as the pharmacodynamic parameter, will be performed in a subset of approximately 25 patients at select sites. PK/PD subjects will be given one dose of riociguat/placebo (based on randomization assignment) by mouth at the Baseline Visit. The plasma concentrations of riociguat will be determined using a sparse sampling approach. Blood samples of 4 mL each for PK analysis will be collected at two time points: (1) at trough (before a study medication tablet is taken) and (2) at any time between 20 minutes and 3 hours post dose. Time of dose administration and sample collection will be recorded. Vital signs and blood pressure will be measured and monitored closely at each time points.

Details on collection, labeling, storage, and shipping of samples are provided in a separate laboratory manual. Riociguat samples will be sent to the outside lab for PK/PD analysis. Should

data be insufficient, PK/PD sampling may be re-opened in order to obtain the minimal samples sets required for adequate data.

7.5 Genotyping for α-Thalassemia and BCL11a

Exploratory genotyping for α-Thalassemia and BCL11a is planned to better understand the relationships between the pathophysiology and specific genetic polymorphisms, and its association with fetal hemoglobin levels and pain crises in adults with SCD.

A one-time 5 mL of blood sample will be obtained from each patient at Baseline. De-identified blood samples will be shipped to and stored at University of Pittsburgh Sickle Cell Disease biospecimen repository for analysis. Alpha globin gene sequence analysis will be performed to identify nondeletional point mutations. SNaPshot PCR, capillary electrophoresis and cycle sequencing will be used for the genotyping of common single nucleotide polymorphisms (SNPs) at the BCL11a gene known to influence HbF levels and associate with pain crisis rate in SCD patients.

7.6 Data Safety Monitoring Plan

7.6.1 Data Safety and Monitoring Board

An independent Data Safety and Monitoring Board (DSMB) will be created to review this study. The DSMB will comprise a panel of experts with relevant experience and will periodically review safety subject enrollment data and the supporting documentation. None of the DSMB members will be affiliated with this study or have a conflict of interest.

In order to allow ongoing safety monitoring during the conduct of the study, DSMB will receive unblinded safety data. If significant safety issues arise in between scheduled meetings, ad hoc meetings will be scheduled to review the data. The DSMB will also review the interim analysis after 50 subjects have been enrolled and received study drugs; however, enrollment will not be suspended pending DSMB review. Based on the safety implications of the data, the DSMB may recommend modification or termination of the study.

7.6.2 Data Safety and Monitoring Plan

The DSMB will meet at periodic intervals during the course of the study. The DSMB will be expected to convene as needed, but not less than every 6 months to review the progression of the study including subject enrollment, protocol compliance, and adverse event reports. The DSMB will conduct interim monitoring of accumulating data from research activities to assure the continued safety of human subjects, relevance and appropriateness of the study, and the integrity of research data.

Apart from the DSMB mentioned above, the study investigator has primary responsibility for the oversight of the data and safety monitoring at each participating site. An independent medical monitor will review related adverse events and serious adverse events reported from all sites. Subject recruitment and retention as well as adverse events and unanticipated problems will be monitored locally at each site as well as centrally by the Data Coordinating Center. In addition to DSMB meetings, the DCC will report adverse events on a monthly basis to the Steering Committee for this study.

The IND Sponsor is responsible to comply with the local regulation and legislation for adverse events reporting.

All patients who receive at least one dose of study treatment will be valid for the safety analysis.

All observations pertinent to the safety of the study treatment will be recorded and included in the final report.

All AEs whether considered drug-related or not, will be reported with a diagnosis, start/stop dates, action taken, whether treatment was discontinued, any corrective measures taken, outcome, and other possible causes. For all events, the relationship to treatment and the intensity of the event will be determined by the investigator.

Safety variables may include but are not limited to the following: laboratory changes in vital signs (blood pressure, heart rate, respiratory rate, and temperature) and, in some instances, changes in chest x-ray images, as produced at the investigator's discretion (e.g., for evaluation for pneumonia).

7.6.3 Adverse events

Patients should be monitored for side effects of all concomitant medications regardless of the path of drug elimination. Investigators should refer to the Safety Information section of the current IB for riociguat, including the DCSI (development core safety information), for the expected side effects of riociguat. As with any agent, there is always the potential for unexpected AEs, including hypersensitivity reactions. The IB will be updated if any new relevant safety data are obtained.

Therapeutic monitoring should be performed following dose selection of riociguat in a manner consistent with the local clinical standard of care.

All concomitant medications must be recorded.

Patients must be carefully monitored for AEs. This monitoring also includes clinical laboratory tests. Adverse events should be assessed in terms of their seriousness, intensity, and relationship to the study drug, AEs will be collected from the time of enrollment until the study visit at 30±2 days following discontinuation of the study drug.

7.6.3.1 Definitions

Definition of adverse event (AE)

In a clinical study, an AE is any untoward medical occurrence (i.e. any unfavorable and unintended sign [including abnormal laboratory findings], symptom or disease) in a patient or clinical investigation patient after providing written informed consent for participation in the study. Therefore, an AE may or may not be temporally or causally associated with the use of a medicinal (investigational) product.

A surgical procedure that was planned prior to the start of the study by any physician treating the patient should not be recorded as an AE (however, the condition for which the surgery is required may be an AE if the condition worsens compared to baseline).

- Conditions that started before signing of informed consent and for which no symptoms or treatment are present until signing of informed consent are recorded as medical history (e.g., seasonal allergy without acute complaints).

- Conditions that started before signing of informed consent and for which symptoms or treatment are present after signing of informed consent, at *unchanged intensity*, are recorded as medical history (e.g., allergic pollinosis).
- Conditions that started or deteriorated after signing of informed consent will be documented as adverse events.

Definition of serious adverse event (SAE)

An SAE is classified as any untoward medical occurrence that, at any dose, meets any of the following criteria (a – f):

a. Results in death.

b. Is life-threatening.

   The term 'life-threatening' in the definition refers to an event in which the patient was at risk of death at the time of the event, it does not refer to an event which hypothetically might have caused death if it were more severe.

c. Requires inpatient hospitalization or prolongation of existing hospitalization.

   A hospitalization or prolongation of hospitalization will not be regarded as an SAE if at least one of the following exceptions is met:

   - The admission results in a hospital stay of less than 12 hours.
   - The admission is pre-planned (i.e., elective or scheduled surgery arranged prior to the start of the study).
   - The admission is not associated with an AE (e.g., social hospitalization for purposes of respite care).

   However, it should be noted that invasive treatment during any hospitalization may fulfill the criterion of 'medically important' and as such may be reportable as an SAE dependent on clinical judgment. In addition, where local regulatory authorities specifically require a more stringent definition, the local regulation takes precedence.

d. Results in persistent or significant disability / incapacity.

   Disability means a substantial disruption of a person's ability to conduct normal life's functions.

e. Is a congenital anomaly / birth defect.

f. Is another medically important serious event as judged by the investigator.

7.6.3.2 Classifications for adverse event assessment

The following classifications will be used:

- Seriousness
- Intensity
  As an alternative to the grading system described in the standard text below (mild, moderate, severe), other systems for intensity may be used (e.g., CTCAE, Grade 1 to Grade 5). If used, this needs to be stated and definitions of the grades should be provided. If applicable, a "translation" between the CTCAE system and the standard system of intensity grading may have to be provided.

- Attribution
- Study treatment action
- Other specific treatment of AE
- Outcome

All AEs will be assessed and documented by the investigator according to the categories detailed below.

7.6.3.2.1 Seriousness

For each AE, the seriousness must be determined according to the criteria given in Section 7.6.3.1.

7.6.3.2.2 Intensity

The severity of the AE is assessed by the investigator and classified as:

Mild

Moderate

Severe

7.6.3.2.3 Attribution

Attribution is an assessment of the relationship between the AE and the medical intervention. After naming and grading the event, the clinical investigator must assign an attribution to the AE using the following attribution categories:

RELATIONSHIP	ATTRIBUTION	DESCRIPTION
Unrelated to investigational agent/intervention	Unrelated	The AE is clearly **NOT** related to the intervention
	Unlikely	The AE is **doubtfully related** to the intervention
Related to investigational agent/intervention	Possible	The AE **may be related** to the intervention
	Probable	The AE **is likely related** to the intervention
	Definite	The AE **is clearly related** to the intervention

Factors to be considered in assessing the relationship of the AE to study treatment include:

- The temporal sequence from drug administration: The event should occur after the drug is given. The length of time from drug exposure to event should be evaluated in the clinical context of the event.
- Recovery on drug discontinuation (de-challenge), recurrence on drug re-introduction (re-challenge):
- Patient's response after de-challenge or patients response after re-challenge should be considered in the view of the usual clinical course of the event in question.
- Underlying, concomitant, intercurrent diseases:
  Each event should be evaluated in the context of the natural history and course of the disease being treated and any other disease the patient may have.
- Concomitant medication or treatment:
  The other drugs the patient is taking or the treatment the patient receives should be examined to determine whether any of them may be suspected to cause the event in question.
- The pharmacology and pharmacokinetics of the study treatment:
  The pharmacokinetic properties (absorption, distribution, metabolism and excretion) of the study treatment, coupled with the individual patient's pharmacodynamics should be considered.

7.6.3.2.4 Action taken with study treatment

Any action on study treatment to resolve the AE is to be documented using the categories listed below.

The study treatment action should be recorded separately for each study treatment.

- Drug withdrawn
- Drug interrupted
- Dose not changed
- Dose increased
- Not applicable
- Unknown

7.6.3.2.5 Other specific treatment(s) of adverse events

- None
- Other

7.6.3.2.6 Outcome

The outcome of the AE is to be documented as follows:

- Recovered/resolved
- Recovering/resolving
- Recovered/resolved with sequelae
- Not recovered/not resolved
- Fatal
- Unknown

7.6.3.3 Assessments and documentation of adverse events

Each serious adverse event must be followed up until resolution or stabilization, by submission of updated reports to the designated person. An isolated laboratory abnormality that is assigned grade 4, according to CTCAE definition, is not reportable as an SAE; unless the investigator assesses that the event meets standard ICH criteria for an SAE. CTCAE grade 4 baseline laboratory abnormalities that are part of the disease profile should not be reported as an SAE, specifically when they are allowed or not excluded by the protocol inclusion/exclusion criteria.

7.6.3.4 Reporting of serious adverse events and unanticipated problems

Each serious adverse event must be followed up until resolution or stabilization, by submission of updated reports to the designated person. An isolated laboratory abnormality that is assigned grade 4, according to CTCAE definition, is not reportable as an SAE; unless the investigator assesses that the event meets standard ICH criteria for an SAE. CTCAE grade 4 baseline laboratory abnormalities that are part of the disease profile should not be reported as an SAE, specifically when they are allowed or not excluded by the protocol inclusion/exclusion criteria.

When required, and according to local law and regulations, serious adverse events must be reported to the Ethics Committee and Regulatory Authorities.

All serious adverse events should be reported to the Coordinating Center. The investigator will then submit a detailed written report to the Coordinating Center. The DSMB, all investigators, and IRB will also be promptly notified in accordance with the respective policies and procedures.

7.6.3.5 Expected adverse events

For this study, the applicable reference document is the most current version of the investigator's brochure (IB) / summary of product characteristics.

Overview listings of frequent events that have occurred so far in the clinical development are shown in the current IB. If relevant new safety information is identified, the information will be integrated into an update of the IB and distributed to all participating sites.

The expectedness of AEs will be determined by Bayer according to the applicable reference document and according to all local regulations.

7.6.4 Adverse event of special safety interest: Pregnancy

Pregnancy has been defined as an event of special interest for riociguat; therefore the investigator must report to the Coordinating Center any pregnancy occurring in a study patient, during the patient's participation in this study or within one month of discontinuing riociguat. The report should be submitted within the same timelines as an SAE, although a pregnancy per se is not considered an SAE.

The outcome of the pregnancy should be followed up whenever possible, and any abnormal outcome of the mother or the child should be reported. In the event that study drug exposure occurs in a pregnancy via a male study participant, efforts should be made to obtain information on any abnormality of pregnancy course or outcome.

8. STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

8.1 Analysis Sets

A randomized patient will be valid for safety and efficacy analyses regardless of whether study drug was administered. Results will be analyzed according to intention-to-treat (ITT).

8.2 Variables/Endpoints

8.2.1 Primary variables/endpoints

The primary endpoints measure: The proportion of participants who experience at least 1 treatment-emergent SAE, which is defined as those occurring after the baseline visit (i.e. after initiation of study drug), adjudicated by a designated committee of protocol investigators and outside experts. This endpoint will monitor the safety and tolerability of riociguat in patients with sickle cell disease.

8.2.2 Secondary variables endpoints

Secondary outcomes will include more detailed safety measures that evaluate whether riociguat increases pain signals:

- The frequency of SAE adjudicated by a designated committee of protocol investigators and outside experts to be due to sickle cell related painful crisis in the riociguat group compared to the placebo group, excluding study drug or disease independent SAE (e.g., accidents)
- Overall incidences of treatment-emergent adverse events
- Changes in pain intensity from baseline to Week 12 using numerical pain score and the Brief Pain Inventory

Efficacy measures will be among the secondary variables to evaluate signals related to the pulmonary and systemic vascular tone:

- Changes in functional exercise capacity by assessing 6 Minute Walk Distance test from baseline to Week 12 (primary efficacy outcome)
- Changes in hemodynamic parameters including blood pressure as the main pharmacodynamic variable, and tricuspid regurgitant velocity, echocardiography markers of left ventricular diastolic dysfunction after 12 weeks of double blinded treatment with riociguat compared with placebo

- Changes in the levels of plasma NT-proBNP from baseline to Week 12
- Changes in the Modified Borg Dyspnea/Fatigue Scale from baseline to Week 12
- Changes in laboratory measures from baseline to Week 12 (1) Renal: urine albumin to creatinine ratio, albuminuria stratified as microalbuminuria and macroalbuminuria, estimated GFR, CKD stage based on albuminuria and GFR; (2) Hematologic: Hemoglobin, LDH, reticulocyte count, white blood cell count, fetal hemoglobin
- Incidences of sickle cell disease-related clinical complications such as acute chest syndrome, priapism, new leg ulcer, new stroke, other new non-CNS thromboembolic event during the 12 week study treatment.

8.2.3 Exploratory variables/endpoints

Exploratory endpoints:

- Maximum and trough plasma concentration (Cmax and Ctrough)
- Changes from baseline to week 12 in biomarkers of oxidative stress: 3-Nitro-tyrosine, Malondialdehyde and 8-IsoPGF2a
- Changes from baseline to week 12 in biomarkers of NO-Pathway dysregulation: asymmetric dimethylarginine (ADMA), SDMA and cGMP. Changes in pain diary scores
- Changes from baseline to week 12 in biomarkers of inflammation (C-reactive protein) and erythropoiesis (erythropoietin)
- Changes from baseline to week 12 in electronic daily pain diary

8.3 Statistical and Analytical Plans

All primary and secondary outcomes will be described using sample means or proportions along with 90% confidence intervals within and between study arms. Suitable transformations will be considered for continuous variables if there are substantial departures from normality. Demographic and baseline characteristics will be compared between the study arms using two-sample t-tests or chi-square tests, as appropriate. Any covariates that are significantly associated with study arm will be included as relevant covariates in the primary safety and efficacy analyses only if they are highly prognostic of the outcome measures. All hypothesis testing will be conducted with two-tailed, 10% type I error.

Attrition & Missing Data: We will make every effort to minimize attrition and missing data in the study. We will record reasons for drop-out and investigate any relationship with study arm. We will also compare baseline characteristics between participants who do and do not complete the study. With regard to missing data, we will investigate the mechanism and degree of missingness using established techniques.[67] If the amount of missingness is great, we will compare various imputation techniques to see how robust the overall inferences are.

8.3.1 Safety analysis

The safety analysis will be performed in the population of patients valid for ITT.

<u>SAEs</u>
The null hypothesis, as it relates to the safety analysis, is that there will be no difference in the proportion of participants who experience at least 1 treatment-emergent SAE between study

arms. Logistic regression will be used to compare the proportion of participants experiencing at least 1 treatment-emergent SAE by study arm, adjusting for clinical site. We will also investigate the incidence of treatment-emergent SAEs by treatment group using a stratified log-rank test. SAEs are considered to be treatment-emergent if they have started or worsened after first dose of study medication up to 7 days after end of study treatment. The incidence of AEs during follow-up (i.e. AEs occurring more than 7 days after end of study medication) will be tabulated separately.

Mortality during the 12 week study treatment period will be summarized descriptively. Any deaths in the study treatment period will be listed, with day of death relative to start and stop of study drug and cause of death.

The eligibility criteria of this clinical trial will enrich for a population of adults with sickle cell disease at high risk for death. Observational studies indicate that for adult SCD patients with elevated TRV, mortality can be as high as 40% at 45 months [21]. This translates to a crude mortality rate of 0.009 per patient-month. On this study, we will have approximately 100 patients each enrolled for approximately 3.5 months, or 350 patient-months in aggregate. Even with no treatment, we could anticipate up to 3 deaths for this cohort while on study. This baseline mortality rate will need to be considered by the regulatory bodies for appropriate perspective. SCD adults with systemic hypertension or proteinuria are similarly at risk for mortality [17,18].

Pain intensity
A secondary safety measure will be the Brief Pain Inventory (BPI) and numerical pain score measured at 12 weeks. We will utilize an analysis of covariance (ANCOVA) for each outcome adjusting for the baseline measure and including predictors for study arm and clinical site. The utilization rate of acute care facilities will be compared between study arms using Poisson regression as a function of study arm and clinical site.

Laboratory data
The safety evaluation of laboratory data will include:

- listings of laboratory data out of normal range
- incidence rates of treatment-emergent laboratory values outside of normal range by treatment group
- incidence rates of pre-specified laboratory data abnormalities by treatment group
- descriptive analysis of continuous laboratory parameters, and their changes from baseline by visit and treatment group

Vital signs
Vital signs (systolic BP, diastolic BP, and heart rate) analysis will be summarized by visit and treatment group.

8.3.2 Efficacy analysis

6MWD
The evaluation of the primary efficacy outcome will be based on change from baseline in 6MWD using analysis of covariance (ANCOVA) with baseline 6MWD, treatment arm and clinical site as covariates. The main comparison in this analysis will be the difference in 6MWD

between the riociguat arm and placebo using a two-sided test of the relevant beta coefficient at the 5% level of significance. Effect sizes and 90% confidence intervals of treatment difference will also be calculated. The above approaches assume 6MWD will be approximately normally distributed. The residuals from the models will be tested for normality using the Shapiro-Wilk test. Should this be significant at the 5% level, suitable transformations will be investigated (i.e. natural log) for the outcome or comparisons on 6MWD will be made using the nonparametric Wilcoxon test.

Other Efficacy measures

Changes from baseline to Week 12 will also be assessed between study arms on hemodynamic parameters, levels of plasma NT-proBNP, the Borg Dyspnea/Fatigue Scale, laboratory measures, and Sickle Cell related complications. Continuous outcomes will be analyzed as above, using ANCOVA with the baseline outcome, study arm, and clinical site as covariates. Logistic regression with similar covariates will be conducted for binary outcomes. Finally, incidence of clinical complications will be compared between study arms using Poisson regression.

8.3.3 Exploratory analysis

The exploratory variables will be summarized using appropriate statistical methods: categorical variables by frequency tables and continuous variables by sample statistics. The 95% confidence intervals will be provided based on either, ANCOVA or stratified Wilcoxon test or Mantel-Haenszel weights, stratified by region, depending on the nature and distribution of the data.

Pharmacokinetic analysis

Due to the limited number of plasma concentrations, PK parameters will be estimated by fitting a compartmental model derived from richer datasets in healthy volunteers.
PK parameters to be estimated will include:

	concentration time curve (AUC)
CL	Total plasma clearance
Vd	Volume of distribution

PK parameters will be summarized using descriptive statistics (geometric means, standard deviations, coefficients of variation, sample size [N], minimum, maximum and median). Figures will be created to display mean and individual (observed and model-predicted) analyte concentration-time curves.

Additional analyses will be performed as deemed necessary upon review of the data.

8.4 Planned Interim Analyses

In addition to periodic safety monitoring, an interim analysis will be performed on the primary outcome of treatment-emergent SAEs after the first 50 patients have completed study follow up. A statistician who is independent of the study will conduct the interim analysis and report the unblinded results to the independent data and safety monitoring board (DSMB). Assuming an overall two-sided, type I error of 10%, an O'Brien-Fleming (O-F) spending function will be used to determine the stopping rule for the primary outcome. If the stopping criteria is met (absolute

value of O-F test statistic > 2.538) during the interim analysis, follow up may be halted. Otherwise, the remaining 50 patients will be recruited and followed. Use of the O'Brien-Fleming spending function allows the overall type I error to remain at 10% while still accounting for the interim peek at the data.

8.5 Determination of Sample Size

Sample size and power considerations were based on detectable effects in the previous walk-PHASST trial, mainly an unanticipated safety signal that showed an increased frequency of pain events in the sickle cell patients on sildenafil compared to the placebo group. This will be a group-sequential design, utilizing an O'Brien-Fleming alpha-spending function with an overall potential sample size of 100 and a planned interim analysis using available data for the first 50 patients. If an O-F stopping boundary of ±2.538 (alpha=0.011) is not exceeded, enrollment will continue for the remaining 50 patients. The primary analysis will be conducted with an 8.9% type I error rate.

We present detectable effect sizes for a group-sequential parallel arm design with 1:1 allocation ratio of 100 participants.

For the primary safety outcome of treatment-emergent SAEs, an absolute difference of 24% (95% CI: 3.1%, 44.9%) was seen in the walk-PHASST trial, favoring the placebo arm in a superiority trial design typical of other safety and tolerability studies. With an overall sample size of 100 patients completing the 12 week visit, we will have approximately 82% power to detect differences in the safety outcome similar to that of the walk-PHASST trial. In fact, the study would have sufficient power (at least 80%) to detect absolute differences as small as 0.23, assuming 22% of placebo participants experience at least 1 SAE. If only 70% of randomized participants complete the 12 week visit, our power to detect differences seen in walk-PHASST reduces to approximately 68%; however we would have at least 80% to detect absolute differences as small as .28.

We have performed additional power analyses based upon the results of the PATENT-1 trial of riociguat to evaluate the likelihood, as part of the secondary outcomes, of detecting efficacy signals in this riociguat trial. The following analyses present only a frame of reference, and are not used to determine the study size of this trial. In terms of detecting an efficacy signal such as 6MWD and MAP, 100 total patients will provide at least 80% power to detect changes of 42.6 m and 6.5 mmHg, respectively. These scenarios assume an overall type I error rate of 10%.

We will have approximately 50 participants randomized to the riociguat arm. As a result, we will have 93% and > 99% power to detect within-arm changes of 30 m and 9 mmHg for 6MWD and MAP, respectively. These meaningful differences were seen in the PATENT-1 trial of riociguat.

9. DATA HANDLING AND QUALITY ASSURANCE

9.1 Data Recording

Data may be entered directly into the eCRF. However, if direct data entry is not possible, sites may print and record data on the paper CRF for later data entry into the web-based system.

9.2 Monitoring

In accordance with applicable regulations, GCP, Coordinating Center personnel will contact the site prior to the start of the study to review with the site staff the protocol, study requirements, and their responsibilities to satisfy regulatory, ethical, and sponsor's requirements. When reviewing data collection procedures, the discussion will also include identification and documentation of source data items.

The sponsor/designee will monitor the site activity to verify that the:

- Data are authentic, accurate and complete
- Safety and rights of patients are being protected
- Study is conducted in accordance with the currently approved protocol (including study treatment being used in accordance with the protocol)
- Any other study agreements, GCP, and all applicable regulatory requirements are met.

9.3 Data Management

The Data Coordinating Center will oversee all aspects of data management. With the consultation of the DCC, the PI and coordinator will develop an operations manual to standardize all procedures and staff training in areas such as patient recruitment, measurement, assessment, and data entry, management, and security.

The DCC will create an electronic System for Data Management (eSYSDM), based on detailed study protocols and requirements that includes an electronic case report form and a tracking system, with the capability to incorporate EHR. The eSYSDM is developed using .NET 2.0 to create the interface and SQL for the database. The DCC will work closely with investigative team and other study personnel to ensure that protocols are being followed, data integrity and confidentiality are maintained, and that the data contains a minimum amount of missing data. All study files residing in designated network folders will be backed-up daily and archived weekly. The weekly archived files are maintained for 1 year until the data are erased. All study subjects will be assigned unique study identifiers that will appear on all data collection instruments, electronic media, documents, and files used in the statistical analysis and manuscript preparation. Only authorized team members will have access to personal information needed for tracking and informed consent. Other data quality assurance measures will include detailed documentation of computer operations and data editing procedures and regular meetings with project staff to review any changes in procedure. The DCC also has specific data quality measures that will be implemented. These include data verification, built in data validation mechanisms such as logic and out of range data checks, and repeated evaluation of the data collection and entry process.

9.4 Audit and Inspection

To ensure compliance with GCP and regulatory requirements, a member of the Coordinating Center may arrange to conduct an audit to assess the performance of the study at the study site and of the study documents originating there. The investigator/institution will be informed of the audit outcome.

In addition, inspections by regulatory health authority representatives and IEC(s)/IRB(s) are possible. The investigator should notify the Coordinating Center immediately of any such inspection. The investigator/institution agrees to allow the auditor or inspector direct access to all relevant documents and allocate his/her time and the time of his/her staff to the auditor/inspector

to discuss findings and any issues. Audits and inspections may occur at any time during or after completion of the study.

9.5 Archiving

Essential documents shall be archived safely and securely in such a way that ensures that they are readily available upon authorities' request.

Patient files will be archived according to local regulations and in accordance with the maximum period of time permitted by the hospital, institution or private practice. Where the archiving procedures do not meet the minimum timelines required by the Coordinating Center, alternative arrangements must be made to ensure the availability of the source documents for the required period.

The investigator/institution notifies the Coordinating Center if the archival arrangements change (e.g., relocation or transfer of ownership). The investigator site file is not to be destroyed without the sponsor's approval. The contract with the investigator/institution will contain all regulations relevant for the study center.

10. PREMATURE TERMINATION OF THE STUDY

- If risk-benefit ratio becomes unacceptable owing to, for example,
  - Safety findings from this study (e.g., SAEs)
  - Results of any interim analysis
  - Results of parallel clinical studies
  - Results of parallel animal studies (on e.g., toxicity, teratogenicity, carcinogenicity or reproduction toxicity).
- If the study conduct (e.g., recruitment rate; drop-out rate; data quality; protocol compliance) does not suggest a proper completion of the trial within a reasonable time frame.

The investigator has the right to close his/her center at any time.

For any of the above closures, the following applies:

- Closures should occur only after consultation between involved parties.
- All affected institutions (e.g., IEC(s)/IRB(s); competent authority(ies); study center; head of study center) must be informed as applicable according to local law.
- In case of a partial study closure, ongoing patients, including those in post study follow-up, must be taken care of in an ethical manner.

Details for individual patient's withdrawal can be found in Section 5.2.1.

11. ETHICAL AND LEGAL ASPECTS

11.1 Ethical and Legal Conduct of the Study

The procedures set out in this protocol, pertaining to the conduct, evaluation, and documentation of this study, are designed to ensure that the investigator abide by Good Clinical Practice (GCP)

guidelines and under the guiding principles detailed in the Declaration of Helsinki. The study will also be carried out in keeping with applicable local law(s) and regulation(s).

Documented approval from appropriate IEC(s)/IRBs will be obtained for all participating centers before start of the study, according to GCP, local laws, regulations and organizations. When necessary, an extension, amendment or renewal of the EC/IRB approval must be obtained and also forwarded to the Coordinating Center.

Strict adherence to all specifications laid down in this protocol is required for all aspects of study conduct; the investigator may not modify or alter the procedures described in this protocol.

Modifications to the study protocol will not be implemented by the investigator without discussion and agreement by the Coordinating Center. However, the investigator may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard(s) to the trial patients without prior IEC/IRB/Bayer approval/favorable opinion. As soon as possible, the implemented deviation or change, the reasons for it and if appropriate the proposed protocol amendment should be submitted to the IEC/IRB/head of medical institution. Any deviations from the protocol must be explained and documented by the investigator.

The Principal Investigator is responsible for the conduct of the clinical trial at the site in accordance with Title 21 of the Code of Federal Regulations and/or the Declaration of Helsinki. The Principal Investigator is responsible for personally overseeing the treatment of all study patients. The Principal Investigator must assure that all study site personnel, including sub-investigators and other study staff members, adhere to the study protocol and all FDA/GCP regulations and guidelines regarding clinical trials both during and after study completion.

The Principal Investigator at each institution or site will be responsible for assuring that all the required data will be collected and properly documented.

11.2 Patient Information and Consent

Each patient will have ample time and opportunity to ask questions and will be informed about the right to withdraw from the study at any time without any disadvantage and without having to provide reasons for this decision.

Only if the patient voluntarily agrees to sign the informed consent form and has done so, may he/she enter the study. Additionally, the investigator and other information provider (if any) will personally sign, date and time the form. The patient will receive a copy of the informed consent form.

The signed informed consent statement is to remain in the investigator site file or, if locally required, in the patient's note/file of the medical institution.

In the event that informed consent is obtained on the date that baseline study procedures are performed, the study record or patient's clinical record must clearly show that informed consent was obtained prior to these procedures.

The informed consent form and any other written information provided to patients will be revised whenever important new information becomes available that may be relevant to the patient's consent, or there is an amendment to the protocol that necessitates a change to the content of the patient information and / or the written informed consent form. The investigator will inform the patient consenter of changes in a timely manner and will ask the patient to confirm his/her participation in the study by signing the revised informed consent form. Any

revised written informed consent form and written information must receive the IEC/IRB's approval in advance of use.

11.3 Publication Policy

The details of publication policy between Bayer and the sponsor are provided in the contract. The Steering Committee will help guide publications from this study. The Principal Investigators at the Coordinating Center should ensure that the information regarding the study be publicly available on the internet at www.clinicaltrials.gov.

11.4 Confidentiality

All records identifying the patient will be kept confidential and, to the extent permitted by the applicable laws and/or regulations, will not be made publicly available.

Should direct access to medical records require a waiver or authorization separate from the patient's statement of informed consent, it is the responsibility of the Investigator to obtain such permission in writing from the appropriate individual.

12. REFERENCE LIST

1. Stuart MJ, Nagel RL. Sickle-cell disease. *Lancet* 2004:364(9442):1343-1360.
2. 10/01/2015. Sickle Cell Disease Data & Statistics. Centers for Disease Control and Prevention <http://www.cdc.gov/ncbddd/sicklecell/data.html>. 10/01/2015.
3. Modell B, Darlison M. Global epidemiology of haemoglobin disorders and derived service indicators. *Bulletin of the World Health Organization* 2008:86(6):417-496.
4. Reiter CD, Wang X, Tanus-Santos JE, et al. Cell-free hemoglobin limits nitric oxide bioavailability in sickle-cell disease. *Nat Med* 2002:8(12):1383-1389.
5. Morris CR, Kato GJ, Poljakovic M, et al. Dysregulated arginine metabolism, hemolysis-associated pulmonary hypertension, and mortality in sickle cell disease. *JAMA* 2005:294(1):81-90.
6. Kato GJ, Wang Z, Machado RF, et al. Endogenous nitric oxide synthase inhibitors in sickle cell disease: abnormal levels and correlations with pulmonary hypertension, desaturation, haemolysis, organ dysfunction and death. *Br J Haematol* 2009:145(4):506-513.
7. El-Shanshory M, Badraia I, Donia A, et al. Asymmetric dimethylarginine levels in children with sickle cell disease and its correlation to tricuspid regurgitant jet velocity. *Eur J Haematol* 2013:91(1):55-61.
8. Landburg PP, Teerlink T, Biemond BJ, et al. Plasma asymmetric dimethylarginine concentrations in sickle cell disease are related to the hemolytic phenotype. *Blood Cells Mol Dis* 2010:44(4):229-232.
9. Landburg PP, Teerlink T, Muskiet FA, et al. 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. *Am J Hematol* 2008:83(7):577-579.
10. Schnog JB, Teerlink T, van der Dijs FP, et al. Plasma levels of asymmetric dimethylarginine (ADMA), an endogenous nitric oxide synthase inhibitor, are elevated in sickle cell disease. *Ann Hematol* 2005:84(5):282-286.
11. Kato GJ, McGowan V, Machado RF, et al. Lactate dehydrogenase as a biomarker of hemolysis-associated nitric oxide resistance, priapism, leg ulceration, pulmonary hypertension, and death in patients with sickle cell disease. *Blood* 2006:107(6):2279-2285.
12. Wood KC, Hebbel RP, Granger DN. Endothelial cell NADPH oxidase mediates the cerebral microvascular dysfunction in sickle cell transgenic mice. *FASEB J* 2005:19(8):989-991.
13. Wood KC, Hebbel RP, Lefer DJ, et al. Critical role of endothelial cell-derived nitric oxide synthase in sickle cell disease-induced microvascular dysfunction. *Free Radical Biology and Medicine* 2006:40(8):1443-1453.
14. Aslan M, Ryan TM, Adler B, et al. Oxygen radical inhibition of nitric oxide-dependent vascular function in sickle cell disease. *ProcNatlAcadSciUSA* 2001:98(26):15215-15220.
15. Kato GJ, McGowan VR, Machado RF, et al. Lactate dehydrogenase as a biomarker of hemolysis-associated nitric oxide resistance, priapism, leg ulceration, pulmonary hypertension and death in patients with sickle cell disease. *Blood* 2006:107(6):2279-2285.
16. Hsu L, Ataga KI, Gordeuk VR, et al. Tetrahydrobiopterin (6R-BH4): Novel Therapy for Endothelial Dysfunction in Sickle Cell Disease. *ASH Annual Meeting Abstracts* 2008:112(11):lba-5-.
17. Pegelow CH, Colangelo L, Steinberg M, et al. Natural history of blood pressure in sickle cell disease: risks for stroke and death associated with relative hypertension in sickle cell anemia. *Am J Med* 1997:102(2):171-177.
18. Gordeuk VR, Sachdev V, Taylor JG, et al. Relative systemic hypertension in patients with sickle cell disease is associated with risk of pulmonary hypertension and renal insufficiency. *Am J Hematol* 2008:83(1):15-18.

19. De Castro LM, Jonassaint JC, Graham FL, et al. Pulmonary hypertension associated with sickle cell disease: clinical and laboratory endpoints and disease outcomes. *Am J Hematol* 2008:83(1):19-25.
20. Forrest S, Kim A, Carbonella J, et al. Proteinuria is associated with elevated tricuspid regurgitant jet velocity in children with sickle cell disease. *Pediatr Blood Cancer* 2012:58(6):937-940.
21. Gladwin MT, Sachdev V, Jison ML, et al. Pulmonary hypertension as a risk factor for death in patients with sickle cell disease. *N Engl J Med* 2004:350(9):886-895.
22. Saraf SL, Zhang X, Kanias T, et al. Haemoglobinuria is associated with chronic kidney disease and its progression in patients with sickle cell anaemia. *Br J Haematol* 2014:164(5):729-739.
23. Ataga KI, Brittain JE, Moore D, et al. Urinary albumin excretion is associated with pulmonary hypertension in sickle cell disease: potential role of soluble fms-like tyrosine kinase-1. *Eur J Haematol* 2010:85(3):257-263.
24. Fonseca GH, Souza R, Salemi VM, et al. Pulmonary hypertension diagnosed by right heart catheterisation in sickle cell disease. *Eur Respir J* 2012:39(1):112-118.
25. Bernaudin F, Verlhac S, Chevret S, et al. G6PD deficiency, absence of alpha-thalassemia, and hemolytic rate at baseline are significant independent risk factors for abnormally high cerebral velocities in patients with sickle cell anemia. *Blood* 2008:112(10):4314-4317.
26. Nolan VG, Adewoye A, Baldwin C, et al. Sickle cell leg ulcers: associations with haemolysis and SNPs in Klotho, TEK and genes of the TGF-beta/BMP pathway. *Br J Haematol* 2006:133(5):570-578.
27. Kato GJ, McGowan VR, Machado RF, et al. Lactate dehydrogenase as a biomarker of hemolysis-associated nitric oxide resistance, priapism, leg ulceration, pulmonary hypertension and death in patients with sickle cell disease. *Blood* 2005.
28. Nolan VG, Wyszynski DF, Farrer LA, et al. Hemolysis associated priapism in sickle cell disease. *Blood* 2005.
29. Kato GJ, Gladwin MT, Steinberg MH. Deconstructing sickle cell disease: reappraisal of the role of hemolysis in the development of clinical subphenotypes. *Blood Rev* 2007:21(1):37-47.
30. Kato GJ, Hebbel RP, Steinberg MH, et al. Vasculopathy in sickle cell disease: Biology, pathophysiology, genetics, translational medicine, and new research directions. *Am J Hematol* 2009:84(9):618-625.
31. Wood KC, Hsu LL, Gladwin MT. Sickle cell disease vasculopathy: a state of nitric oxide resistance. *Free Radic Biol Med* 2008:44(8):1506-1528.
32. Mehari A, Alam S, Tian X, et al. Hemodynamic predictors of mortality in adults with sickle cell disease. *Am J Respir Crit Care Med* 2013:187(8):840-847.
33. Parent F, Bachir D, Inamo J, et al. A hemodynamic study of pulmonary hypertension in sickle cell disease. *N Engl J Med* 2011:365(1):44-53.
34. Mehari A, Gladwin MT, Tian X, et al. Mortality in adults with sickle cell disease and pulmonary hypertension. *JAMA* 2012:307(12):1254-1256.
35. Elmariah H, Garrett ME, De Castro LM, et al. Factors associated with survival in a contemporary adult sickle cell disease cohort. *Am J Hematol* 2014:89(5):530-535.
36. Raat NJ, Tabima DM, Specht PA, et al. Direct sGC activation bypasses NO scavenging reactions of intravascular free oxy-hemoglobin and limits vasoconstriction. *Antioxid Redox Signal* 2013:19(18):2232-2243.
37. Parise LV, Telen MJ. Erythrocyte adhesion in sickle cell disease. *Curr Hematol Rep* 2003:2(2):102-108.
38. Kutlar A, Embury SH. Cellular adhesion and the endothelium: P-selectin. *Hematol Oncol Clin North Am* 2014:28(2):323-339.
39. Manwani D, Frenette PS. Vaso-occlusion in sickle cell disease: pathophysiology and novel targeted therapies. *Hematology Am Soc Hematol Educ Program* 2013:2013:362-369.
40. Kutlar A, Ataga KI, McMahon L, et al. A potent oral P-selectin blocking agent improves microcirculatory blood flow and a marker of endothelial cell injury in patients with sickle cell disease. *Am J Hematol* 2012:87(5):536-539.

41. Okpala I. Investigational selectin-targeted therapy of sickle cell disease. *Expert Opin Investig Drugs* 2015:24(2):229-238.
42. Mandarino D, Kawar Z, Alvarez R, et al. *Placebo-Controlled, Double-Blind, First-In-Human, Ascending Single Dose and Multiple Dose, Healthy Subject Study Of Intravenous-Administered SelG1, a Humanized Anti-P-Selectin Antibody In Development For Sickle Cell Disease*2013. 970-970 p.
43. Almeida CB, Scheiermann C, Jang JE, et al. Hydroxyurea and a cGMP-amplifying agent have immediate benefits on acute vaso-occlusive events in sickle cell disease mice. *Blood* 2012:120(14):2879-2888.
44. Kim-Shapiro DB, Gladwin MT. Mechanisms of nitrite bioactivation. *Nitric Oxide* 2013.
45. Cosby K, Partovi KS, Crawford JH, et al. Nitrite reduction to nitric oxide by deoxyhemoglobin vasodilates the human circulation. *Nature Medicine* 2003:9(12):1498-1505.
46. Mack AK, McGowan Ii VR, Tremonti CK, et al. Sodium nitrite promotes regional blood flow in patients with sickle cell disease: a phase I/II study. *Br J Haematol* 2008:142(6):971-978.
47. Weiner DL, Hibberd PL, Betit P, et al. Preliminary assessment of inhaled nitric oxide for acute vaso-occlusive crisis in pediatric patients with sickle cell disease. *JAMA* 2003:289(9):1136-1142.
48. 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* 2011:305(9):893-902.
49. Corbin JD, Francis SH. Cyclic GMP phosphodiesterase-5: target of sildenafil. *J Biol Chem* 1999:274(20):13729-13732.
50. 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* 2011:118(4):855-864.
51. Schmidtko A, Tegeder I, Geisslinger G. No NO, no pain? The role of nitric oxide and cGMP in spinal pain processing. *Trends Neurosci* 2009:32(6):339-346.
52. Patil CS, Padi SV, Singh VP, et al. Sildenafil induces hyperalgesia via activation of the NO-cGMP pathway in the rat neuropathic pain model. *Inflammopharmacology* 2006:14(1-2):22-27.
53. Hannig G, Tchernychev B, Kurtz CB, et al. Guanylate cyclase-C/cGMP: an emerging pathway in the regulation of visceral pain. *Front Mol Neurosci* 2014:7:31.
54. Tegeder I, Scheving R, Wittig I, et al. SNO-ing at the nociceptive synapse? *Pharmacol Rev* 2011:63(2):366-389.
55. Potoka KP, Gladwin MT. Vasculopathy and pulmonary hypertension in sickle cell disease. *Am J Physiol Lung Cell Mol Physiol* 2015:308(4):L314-324.
56. Morris CR. Alterations of the arginine metabolome in sickle cell disease: a growing rationale for arginine therapy. *Hematol Oncol Clin North Am* 2014:28(2):301-321.
57. Rodgers GP, Walker EC, Podgor MJ. Is "relative" hypertension a risk factor for vaso-occlusive complications in sickle cell disease? *American Journal of the Medical Sciences* 1993:305(3):150-156.
58. Falk RJ, Jennette JC. Sickle cell nephropathy. *Adv Nephrol Necker Hosp* 1994:23:133-147.
59. Falk RJ, J.C. Renal disease. In: Embury SHH, R.P.; Mohandas, N.; Steinberg, M.H., editor. Sickle Cell Disease: Basic Principles and Clinical Practice. New York: Raven Press; 1994. p 673–680.
60. Bayer HealthCare Pharmaceuticals I. 07/19/2017. NDA 204819 FDA Approved 23 Feb 2017. <http://labeling.bayerhealthcare.com/html/products/pi/Adempas_PI.pdf>. Accessed 2017 07/19/2017.
61. Keller SD, Yang M, Treadwell MJ, et al. Patient reports of health outcome for adults living with sickle cell disease: development and testing of the ASCQ-Me item banks. *Health and Quality of Life Outcomes* 2014:12:125.
62. Treadwell MJ, Hassell K, Levine R, et al. Adult sickle cell quality-of-life measurement information system (ASCQ-Me): conceptual model based on review of the literature and formative research. *Clinical Journal of Pain* 2014:30(10):902-914.

63. Gershon RC, Rothrock N, Hanrahan R, et al. The use of PROMIS and assessment center to deliver patient-reported outcome measures in clinical research. *Journal of Applied Measurement* 2010:11(3):304-314.
64. Cella D, Riley W, Stone A, et al. The Patient-Reported Outcomes Measurement Information System (PROMIS) developed and tested its first wave of adult self-reported health outcome item banks: 2005-2008. *Journal of Clinical Epidemiology* 2010:63(11):1179-1194.
65. Liu H, Cella D, Gershon R, et al. Representativeness of the Patient-Reported Outcomes Measurement Information System Internet panel. *Journal of Clinical Epidemiology* 2010:63(11):1169-1178.
66. Bakshi N, Stinson JN, Ross D, et al. Development, Content Validity, and User Review of a Web-based Multidimensional Pain Diary for Adolescent and Young Adults With Sickle Cell Disease. *Clin J Pain* 2015:31(6):580-590.
67. Little RJA, Rubin DB. *Statistical Analysis with Missing Data* New York: John Wiley & Sons; 2002.

Appendix A: Schedule of Procedures

Study Epoch	Screening	Double-Blinded Study Treatment Phase							Telephone Follow-up	Final Visit
Visit Number	1	2	3	4	5	6	7	8	9	10
Week on Study Intervention	Week -0 ~ -4	Baseline Week 0	Week 2	Week 4	Week 6	Week 8	Week 10	Week 12 or ED	Week 13 or ED	Week 16 or 30 days post treatment
Assessment Window / Study Procedure		-	± 3d	± 3d	± 3d	± 3d	± 3d	± 3d	± 3 d	± 3 d
Informed consent	x									
Demographics and Review of Eligibility	x									
Comprehensive Medical history		x								
Review of Inclusion & Exclusion criteria	x	x								
Physical exam		x	x	x	x	x	x	x		x
Height and weight #		x						x		
Vital signs	x	x	x	x	x	x	x	x		x
Randomization		x								
Dispense study drug		x	x	x	x	x	x			
Titration of study drug dose			x	x	x	x	x			
Urine or serum pregnancy test ¥	x	x		x		x		x		x
Child Pugh with INR±	x									
Lab evaluation±	x	x		x		x		x		
NT-proBNP		x						x		
Ferritin ±		x						x		
Hemoglobin HPLC±		x						x		
U/A & urine albumin & creatinine	£	x						x		
Blood samples for biomarkers		x						x		
Blood sample for genotyping		x								
PK Sampling/PD *		x								
Echocardiogram	≠	x						x		
Record Results of RHC Ω		A						A		
6MWD Test		x						x		
Borg Dyspnea/Fatigue Scale		x						x		
Brief Pain Inventory		x						x		
ASCQ-Me/PROMIS		x						x		
Electronic Pain diary §	S	R	S	R	S	R	S	R		
Treatment compliance			x	x	x	x	x	x		
Concomitant medications	x	x	x	x	x	x	x	x	x	x
ROS/AE assessment		x	x	x	x	x	x	x	x	x

X=Required A=When available § Piloted only at select sites (S= set up diary & R= review diary) ED=Early Discontinuation

\# Only weight measurement will be repeated at Visit 8

¥ For women of childbearing potential only

* Only in approximately 25 subjects enrolled at select sites

£ A clinical U/A protein result or urine albumin to creatinine ratio result obtained within the past 24 months is acceptable at screening to determine macroalbuminuria eligibility.
UA, urine albumin and urine creatinine will be done on all subjects at Baseline and Week 12.

≠ A clinical echocardiogram performed within the past 24 months when the patient is in his/her stable disease state is acceptable at screening to determine TRV eligibility. A research echocardiogram will be performed on all randomized subjects at Baseline and at Week 12.

Ω RHC is not required but record results if performed as standard of care

± Labs that will be drawn and processed at the site's clinical lab