Study Protocol Title: Assessing Combination Hydroxyurea and Exogenous Erythropoietin in Sickle Cell Disease (ACHiEvE-SCD)

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1. Introduction

1.1 Background

Chronic anemia is a hallmark complication of sickle cell disease (SCD). SCD is a major global health problem, with over 300,000 SCD births annually.[1] Polymerization of deoxygenated hemoglobin (Hb) S and consequent sickling of erythrocytes lead to the hallmark manifestations of chronic hemolytic anemia and acute vaso-occlusive crises (VOCs) that result in multiorgan damage and decreased life expectancy.[2,3] The pathophysiology of anemia in SCD is multifactorial, with the predominant mechanisms of red blood cell (RBC) destruction being intravascular and extravascular hemolysis resulting from damage to RBC membrane and contents through repeated cycles of HbS polymerization and depolymerization, phosphatidylserine exposure (PS) facilitating complement-mediated lysis, loss of RBC deformability, depletion of adenosine triphosphate (ATP) and RBC dehydration, oxidative stress, and clearance of deformed sickle erythrocytes by the reticuloendothelial system.[4]

Anemia plays a key role in the pathogenesis of the chronic multi-organ damage in SCD. Cardiopulmonary disease, SCD nephropathy, and cerebrovascular disease are the leading causes of morbidity and mortality in SCD and are all exacerbated by the chronic anemic state and hypercirculatory physiology in SCD.[2,3,5] Compensatory increased cardiac output, lowered systemic vascular resistance, and neovascularization result in cardiomegaly, left ventricular hypertrophy, high output heart failure, pulmonary hypertension, proliferative retinopathy, and cerebral vasculopathy, among other complications.[5] Anemia activates the renin-angiotensin-aldosterone system and sympathetic nervous system, contributing to renal and vascular dysfunction, chronic inflammation, and increased oxidative stress.[6,7] Furthermore, chronic anemia affects the quality of life and physical functioning of individuals with SCD. Fatigue and weakness associated with anemia may greatly limit patients' physical activity, daily functioning, level of disability, and overall health-related quality of life.[8-11] Additionally, neurocognitive impairment has been reported in a large proportion of children and adults with SCD, even in the absence of overt or silent strokes suggesting that other factors may contribute to its pathogenesis.[12] Two cross-sectional studies have shown better cognitive performance of individuals taking therapies that improve anemia when compared to participants not on these therapies.[13] As oxygen delivery is crucial for brain function, it is feasible that an increase in hemoglobin level may improve neurocognitive outcomes [6].

There are limited therapeutic options for anemia in SCD. Blood transfusions are the mainstay therapy for acute anemic episodes but carry risks of hemolytic transfusion reactions, alloimmunization, and iron overload. Additionally, anecdotal case reports suggest that rapid correction of anemia through simple transfusions may increase the risk of viscosity-related complications, likely related to the decreased deformability and increased adhesiveness of sickle RBCs.[14] As such, the risk of increasing blood viscosity must be weighed against the benefits of correcting anemia. Hydroxyurea has a salutary anti-sickling effect, but it has only a modest effect on Hb levels, particularly in the adult population.[15,16] Chronic anemia is not an indication for initiating hydroxyurea therapy, and patients who remain anemic despite hydroxyurea need additional therapy. Voxelotor (Oxbryta®, GBT-440) received recent FDA approval on the basis of raising hemoglobin levels in SCD,[17] though its mechanism of action of increasing Hb oxygen affinity has raised concerns about its effects on tissue oxygen delivery.[18-21] Indeed, the effects of increasing Hb level and blood viscosity may be tissue-specific, demonstrating the need for better laboratory biomarkers and tissue-specific measures of blood flow, oxygen saturation, and vascular tone.

Erythropoiesis-stimulating agents (ESAs) may be an effective therapy for anemia in SCD patients who remain anemic despite hydroxyurea. ESAs are the standard of care for anemia in chronic kidney disease (CKD) and may be ideal for use in SCD due to the high prevalence of subclinical renal dysfunction in SCD. In fact, ESAs have long been used in clinical practice for SCD patients who have severe or symptomatic anemia and cannot tolerate or accept blood transfusions, have severe renal dysfunction, or have an acute aplastic or hemolytic crisis (e.g., acute parvovirus B19 infection or hyperhemolysis associated with delayed hemolytic transfusion reactions). Furthermore, ESAs have been shown to have potential neuroprotective and neurogenerative effects[22] and may improve neurocognitive functioning in animal models[23] and in patients with neuropsychiatric disorders.[24,25] However, the use of ESA therapy in combination with hydroxyurea is still not considered standard-of-care for the treatment of anemia in SCD. Older case reports and small studies,[26-37] as well as recent retrospective studies have shown that ESAs may increase Hb level and Hb F %,[33] as well as decrease transfusion burden in patients with SCD.[30,32] Moreover, these studies have not identified an increased risk of VOC or venous thromboembolism (VTE) with ESA therapy. Additionally, patients stabilized on hydroxyurea are potentially protected from developing vaso-occlusive and viscosity-related complications due to the inhibitory effect of HbF on HbS polymerization, and ESAs may synergistically potentiate the Hb F-inducing effect of hydroxyurea by increasing stress erythropoiesis.[35] Despite the abundance of clinical reports of ESA utilization in SCD in the literature,[26-37] anecdotal evidence of increased pain or VOCs after ESA treatment in SCD patients[31,33] and a lack of practice guidelines for how to manage ESA therapy in combination with hydroxyurea have led ESAs to be prescribed ad hoc by SCD providers and to be potentially underutilized as a therapeutic option. Therefore, there is a need for prospective evaluation of the efficacy and safety of ESAs in combination with hydroxyurea as a therapeutic strategy for anemia in the SCD population.

1.2 Rationale

Recombinant human erythropoietin (rHuEPO) is commonly used for the treatment of anemia of CKD. Epoetins (and its biosimilars) are short-acting rHuEPO drugs that are widely available globally. The use of EPO (which henceforth refers to epoetins) in combination with hydroxyurea may alleviate the detrimental effects of chronic anemia without increasing vaso-occlusive and viscosity-associated complications. In addition, this study will allow for the identification of predictors of response to EPO and evaluation of potential biomarkers for monitoring the safety and effect of increasing hemoglobin levels on cardiopulmonary status, health-related quality of life (HRQoL), red cell health, and tissue hemodynamics.

If successful, our study will advance SCD care and research in multiple settings:

1) This pilot study will provide prospective evidence of the efficacy and safety of EPO in patients with SCD who remain anemic despite hydroxyurea therapy, potentially broadening the menu of standard therapeutic options for anemia in SCD;

2) Evaluation of baseline biomarkers (e.g., markers of erythropoiesis, hemolysis, renal function, iron homeostasis, and inflammation) in prospectively treated patients will allow for identification of predictors of response to EPO in the SCD population;

3) Evaluation of cardiopulmonary status, neurocognitive function, and HRQoL at baseline and after treatment with EPO will prospectively demonstrate the effect of raising Hb levels on important clinical and HQoL outcomes;

4) Evaluation of *in vivo* biomarkers of tissue hemodynamics using DSM colorimeter, and diffuse optical technologies (i.e., near-infrared spectroscopy (NIRS)), as well as evaluation of neural activity using electroencephalography (EEG), will provide novel insights into tissue-specific physiological effects of raising Hb level in patients with SCD; and finally,

5) Evaluation of biomarkers of hemorheology and cardiovascular and thrombotic risk (e.g., blood viscosity, markers of myocardial strain and hemostatic activation) will help identify potential harmful effects of raising Hb level in patients with SCD and will provide insight into an optimal Hb target range that balances clinical benefit and safety in patients with SCD.

2. Clinical Study Objectives

2.1 Primary objectives

Prospectively evaluate the efficacy and safety of EPO in combination with hydroxyurea in SCD. In SCD patients stabilized on hydroxyurea, we will dose escalate EPO to reach a Hb target of 10-11 g/dL and measure the rates of Hb response during treatment.

2.2 Secondary objectives

Prospectively evaluate the effect of EPO in combination with hydroxyurea on transfusion burden in SCD. We will measure the annualized number of simple red blood cell transfusions received before and during treatment.

2.3 Exploratory Objectives

a) *Evaluate the effect of raising Hb level on clinical outcomes*. We will evaluate changes in cardiopulmonary status, neurocognitive function, and HRQoL after treatment compared to baseline using echocardiography, exercise testing (six-minute walk test), and validated HRQoL questionnaires.

b) *Identify predictors of response to EPO*. We will assess the correlation between Hb response to EPO therapy and a variety of biomarkers at baseline and end of study, including markers of renal function, iron homeostasis, inflammation, erythropoiesis, and hemolysis.

c) Identify biomarkers of the risk and benefit of raising Hb level using EPO therapy. We will evaluate changes in a variety of biomarkers after treatment compared to baseline, including laboratory biomarkers of red cell health, blood viscosity, and cardiovascular or thrombogenic risk, as well as *in vivo* biomarkers of blood flow, tissue oxygen saturation, and vascular tone using near-infrared (NIR) technologies, specifically frequency-domain near-infrared spectroscopy (FD-NIRS) and diffuse correlation spectroscopy (DCS). We will be using DSM III colorimeter, a highly sensitive device for skin color detection to help enhance the accuracy of

data analysis. We will perform these assessments in collaboration with research team members from Carnegie Mellon University. In addition, we will evaluate neural activity after treatment compared to baseline using electroencephalography (EEG).
These measurements will be performed only at study sites where the equipment and expertise are available. For instance, all endpoints will be obtained at Pitt, but only some measurements with NIR technologies will be performed at the Nigeria site due to lack of equipment to perform all measurements (e.g., DCS will not be available in Nigeria). EEG also will not be performed on participants in Nigeria due to lack of the equipment and expertise.

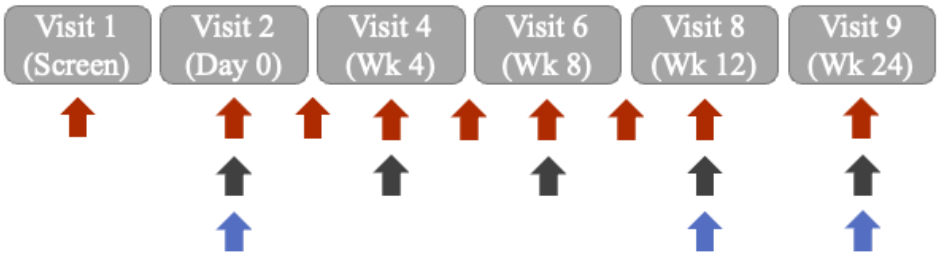
3. Study Design

This is a Phase 1b/2 prospective, single-arm, open-label pilot study of EPO in patients with SCD. Eligible individuals will be consented and undergo baseline testing. EPO dosing will be consistent with the routine clinical practice of the investigators, and as informed by published literature and expert opinion. EPO treatment will be initiated at a standard dose of 10,000 units three times weekly (TIW), with biweekly clinic visits and phlebotomy for clinical monitoring, dose titration, and research laboratory testing (Figure 1 and Table 1). EPO dose will be escalated by 10,000 units per dose every 2 weeks until reaching a maximum dose of 40,000 units TIW or the minimal effective dose for maintaining a Hb level of 10-11 g/dL (Table 2). Dose escalation will not be performed if there are any safety concerns, such as adverse events or clinically significant laboratory abnormalities related to treatment. A dose reduction or temporary holding of therapy may be performed per the PI's discretion and clinical judgment if any safety concerns arise that are assessed by the PI as related to EPO therapy or to increases in hemoglobin level, including the following criteria: Hb increase to > 11.0 g/dL, rapid rise in Hb (defined as a > 2 g/dL increase within 4 weeks), development of increased pain or vaso-occlusive crisis, or any Grade ≥ 3 adverse events assessed as related to treatment (Table 3).

A 12-week treatment period was selected based on guidance from the drug label of EPO stating that a hemoglobin response is expected within 12 weeks. Following this 12-week treatment period, participants who benefit from the therapy may elect to continue EPO treatment clinically in discussion with their primary SCD provider. After the 12-week timepoint (end of treatment (EOT)), all ongoing treatment decisions will be made clinically, and we will collect endpoints at one additional time point at 24 weeks, considered the end of study (EOS) testing. Participants may also elect to stop EPO therapy after completion of the 12-week treatment period. Participants who permanently discontinue EPO therapy prior to the end of the 12-week period will not be considered as completing the study and will complete the EOT testing, after which they will be withdrawn from the study. Participants in whom EPO treatment is temporarily held for any safety reasons will remain on study and be considered completers so long as the drug is not held for more than 4 out of the 12 weeks and treatment is restarted at least 2 weeks prior to the EOT visit and testing. Hydroxyurea and L-glutamine dose titration and holding are not permitted on study, unless otherwise clinically indicated to ensure the participant's safety.

3.1 Study design schematic

Figure 1: Schematic of treatment period and follow-up visits.



= Clinic visit for clinical labs; dose adjustments; Painimation

= NIRS; EEG; research biomarkers

= Cardiopulmonary testing; quality of life questionnaires; neurocognitive testing; research biomarkers

Wk, week; NIRS, near-infrared spectroscopy; EEG, electroencephalography.

Table 1: Schedule of Events

Study Epoch	Screening	Treatment							
Visit Number	1	2	3	4	5	6	7	8	9
Week on Study Intervention	Day -60 to Day 0	Day 0 Baseline and Drug Initiation	Wk 2	Wk 4	Wk 6	Wk 8	Wk 10	Wk 12 EOT	Wk 24 EOS
Assessment Window Study Procedure		-7 d	±3 d	±3 d	±3 d	± 3 d	± 3 d	± 7 d	± 7 d
Informed consent	x								
Demographics and Review of Eligibility	X								
Review of Inclusion & Exclusion criteria	X								
Pregnancy test**	X	X							
Medical History***		X						X	X
Vital signs	X	X	x	x	x	x	x	X	X
Physical assessment (including weight)		X		x		x		X	X
Height		X							
Dosing		X	x	x	x	x	x		
Clinical Labs[#]	x	X	x	x	x	x	x	X	X
Biomarkers[Δ]		X	x	x	x	x	x	X	X
Transthoracic echocardiography (TTE)*		X						X	X
6MWD Test with Borg Dyspnea Scores (performed by research staff)		X						X	X
Quality of Life Questionnaires		X						X	X
Neurocognitive assessment		X						X	X
DSM Colorimeter, NIRS and EEG		X		x		x		X	X
Painimation Assessments		X	x	x	x	x	x	X	X
AE and SAE assessments		X	x	x	x	x	x	X	
Acute care utilization†		X	x	x	x	x	x	X	X

* Clinical TTE obtained within 180 days of the screening date (if obtained at baseline state of health, without transfusion in 60 days prior, and within 1 g/dL of screening hemoglobin level) can be used for the baseline TTE results if all research parameters were captured (i.e., TTE will not be repeated at baseline).

** For women of childbearing potential, a serum pregnancy test will be performed at the Screening Visit, and a urine pregnancy test will be performed at the Baseline visit.

*** Medical history includes the number of acute vaso-occlusive events, thrombotic complications, and transfusions in the 12 months prior to baseline, at 12 week (EOT) and at 24 week (EOS) (Refer to Table 5). General medical history will be captured at baseline and updated as needed.
# Safety labs checked at every visit include CBC/Diff, reticulocyte count, CMP, direct bilirubin, and LDH. Other clinical labs may only be checked at some visits, e.g., at baseline, 12 weeks (EOT), and 24 weeks (EOS) (refer to Table 4).
Δ Biomarkers include the research labs listed in Table 4 (note that some are only drawn at baseline, EOT, and EOS).
† Frequency of and reason for acute care visits, defined as any non-routine visit to an urgent care, infusion center, emergency room, or hospital to seek medical attention. This will be assessed at every study visit, as well as once per month by phone call by a member of the study team in between weeks 12 and 24.

Table 2: Dose Modification Schedule

Dose Modification Schedule			
Dose Level	Dose of EPO	If Hb > 11.0 (confirmed on repeat Hb measurement), reduce to:	If second reduction needed:
Level 3	40,000 units TIW	30,000 units TIW (Level 2)	20,000 units TIW (Level 1)
Level 2	30,000 units TIW	20,000 units TIW (Level 1)	10,000 units TIW (Level 0)
Level 1	20,000 units TIW	10,000 units TIW (Level 0)	10,000 units BIW (Level -1)
Level 0	10,000 units TIW	10,000 units BIW (Level -1)	10,000 units weekly (Level -2)

Table 3: Dose Reduction Criteria

Dose Reduction Criteria
Hb increase to > 11.0 g/dL (confirmed on repeat Hb measurement)
> 2 g/dL Hb increase in 4 weeks
Increased pain/VOC symptoms
Grade 3 hypertension (defined as systolic blood pressure ≥160 mmHg or diastolic blood pressure ≥100 mmHg; medical intervention indicated; more than one drug or more intensive therapy than previously used indicated) on two consecutive measurements
Grade ≥ 3 treatment related adverse events

3.2 Participant Selection:

Participants:

This prospective interventional study proposes to enroll up to 25 participants with SCD, of HbSS and HbS/β^0-thalassemia genotypes, from USA and Nigeria. Participants will be either from the University of Pittsburgh Medical Center (UPMC) Adult Sickle Cell Disease program and UPMC hospitals that serves adult participants with SCD from the Pittsburgh community, or from Lagos University Teaching Hospital and the surrounding community. The goal is to get a combined total of 15 enrolled participants, from both sites, to complete the study. The study will conclude once 15 participants have completed the study. Other genotypes, including HbSC and S/β^+-thalassemia, will not be eligible since these genotypes tend to have milder anemia and potentially different pathophysiology and would be too heterogeneous of a group to include in this pilot study. Male and female adult participants will be recruited in steady state, defined as absence of VOC or acute illness as determined by their treating hematologist, and be on a stable dose of hydroxyurea, defined as no dose adjustments within the 60 days prior to screening and no planned future dose adjustments. The study will conclude once 15 participants have completed the study; a completer is defined as a participant that has completed the 12-week treatment period and testing.

Lagos University Teaching Hospital site: English is the primary language spoken in Nigeria. Lagos is a cosmopolitan metropolis, and most people speak English, therefore, we do not anticipate any language barrier. In an effort to avoid exclusion of disadvantaged populations in Nigeria, we will not exclude individuals who cannot speak, read, or write English. For individuals recruited in Nigeria who do not speak or read English, there will be interpreters available and consent forms will be translated into Yoruba, which is the major local language spoken in the area where the study recruiting will be carried out. In the event that participants cannot read any language, the research nurse will read the ICF out loud to the participant. If the participant cannot write, whenever required, a thumb print will be used in place of a signature.

3.3 Participant inclusion criteria :

SCD Participants :
Inclusion Criteria:

- Aged ≥ 18 years
- Confirmed SCD (HbSS or HbS/β^0-thalassemia) by hemoglobin electrophoresis, high-performance liquid chromatography, DNA genotyping, or other comparable hemoglobin diagnostic testing
- Screening Hb ≤ 9.0 g/dL
- Screening transferrin saturation ≥ 20% and ferritin ≥ 50 ng/mL
- Must be on stable-dose hydroxyurea treatment (i.e., no changes in dose within 60 days prior to screening) and plan to continue taking hydroxyurea at the same dose and schedule during the study
- If receiving L-glutamine or crizanlizumab, must have been receiving the drug at a stable dose for at least 60 days prior to screening and plan to continue taking the drug at the same dose and schedule during the study.

3.4 Participant exclusion criteria :

SCD Participants :
Exclusion Criteria:

- Participating in a chronic transfusion program (pre-planned series of transfusions for prophylactic purposes) and/or planning on undergoing an exchange transfusion during the duration of the study; episodic transfusion in response to worsened anemia or VOC is permitted, but participant should not have received a blood transfusion within 60 days of screening
- Received voxelotor or EPO within 60 days of screening
- Untreated iron deficiency, or had initiation or change in dose of supplemental iron within 30 days of screening
- Ongoing acute illness, infection, or VOC within 2 weeks of screening
- Arterial or venous thrombosis within 180 days of screening
- Grade 3 hypertension (defined as systolic blood pressure ≥160 mmHg or diastolic blood pressure ≥100 mmHg; medical intervention indicated; more than one drug or more intensive therapy than previously used indicated) on two consecutive measurements
- Unstable angina, uncontrolled seizure disorder, or active malignancy
- End-stage renal disease requiring hemodialysis
- Current pregnancy or breast feeding
- Received active treatment on another investigational trial within 30 days (or 5 half-lives of that agent, whichever is greater) prior to screening visit or plans to participate in another investigational drug trial

Participant Recruitment:

Participants in the US will be recruited from the UPMC Adult Sickle Cell Program and other UPMC hospitals while those in Nigeria will be recruited from Lagos University teaching hospital through method(s) approved by the Lagos Teaching University Hospital (LUTH) Health Research Ethics Committee (HREC), an equivalent body to the IRB.

In the US, the physician investigators or a research coordinator will initiate contact with the participants at the time they present for their routine follow up appointments at their respective clinics, by phone, or by flyers distributed in print or electronically. The study team has usual clinical access to the medical records of subjects via Dr. Xu. If the study team identifies an eligible subject who is not known to the study team from prior clinical care, a clinician known to the patient will obtain their permission to approach for research before initiating recruitment discussions. The physician or his/her delegate will then review the participants' records to identify if they meet the inclusion criteria.

In Lagos, participants will be identified and recruited by the study team physicians in the clinics of the Lagos Teaching Hospital study site with direct 1:1 contact with subjects. No phone scripts or flyers will be used for recruitment.

A total of 50 participants will undergo screening across the 2 sites. A total of up to 25 participants will be enrolled, and a combined total of 15 participants, from both sites, will complete study procedures. US Participants will be compensated a total of $ 675 for participation in the study, which translates to $75 for each completed study visit. Participants in Nigeria will be compensated a total of ₦112,500 Naira (an equivalent of about $270) for participation in the study, which is ₦12,500 Naira (about $30) for each visit. This compensation accounts for approximately 3-4 hours' wage, the cost of travel, inflation, and other out-of-pocket expenses incurred by participating in the study.

Consent Process:

The consent process will be conducted by a physician investigator or his/her delegate. Consent agreement will be obtained by the Principal Investigator (PI) or a study co-investigator after a potential participant has had the opportunity to ask the investigator all the questions they have, and have those questions answered to their satisfaction. Prior to performing any of the research study procedures or interventions, participants must provide informed consent. The PI will make certain that an appropriate informed consent process is in place to ensure that potential research participants, or their authorized representatives, are fully informed about the nature and objectives of the clinical study, the potential risks and benefits of study participation, and their rights as research participants. The investigator will obtain the written, signed informed consent of each participant prior to performing any study-specific procedures on the participant. The date and time that the participant signs the informed consent form and a narrative of the issues discussed during the informed consent process will be documented in the participant's case history. The investigator will retain the original copy of the signed informed consent form, and a copy will be provided to the participant.
Participants in Nigeria will be consented through method(s) approved by the LUTH HREC. Consent will be obtained by a physician investigator at the LUTH site.

Screen failures:
After signing the informed consent document, participants will undergo screening tests to ensure they meet all eligibility criteria. Those individuals who are eligible by criteria and have a clinical indication to start treatment with EPO will be provided with a clinical prescription for the drug, which they will need to fill prior to returning for the Day 0/drug initiation study visit. Individuals who do not fill the clinical prescription within the 60-day screening period or whose clinical status changes to meet exclusion criteria during the screening period will be considered screen failures. These screen failures may be consented and rescreened, up to a maximum of 3 total screening attempts. They will be assigned the same study participant number upon rescreening.

Discontinuation of Individual Research Participants:
We will make our best effort to retain participants and to complete their participation in the 12-week treatment period. However, if a participant does not comply with the study procedure/s or treatment with study drug, the study team will communicate with the participant and try to understand the reason. If the participant wishes to discontinue participation or the study team feels that it would be unsafe to continue the participant on the treatment protocol (for instance, for participants who become pregnant while on study and require discontinuation of hydroxyurea), the PI will inform the participant about the decision and will withdraw the participant. All the data and biospecimen collected up to that point will be retained for future analyses.

PI discontinuation of the clinical research study:
The University of Pittsburgh Institutional Review Board (IRB) will be notified promptly of discontinuation of the entire clinical study. All protocol modifications will be submitted prospectively to the University of Pittsburgh IRB for discontinuation of parts of the clinical research study.

Cost to Participant:

It will not cost anything above the standard costs of clinical care for participants to participate in this study. All non-clinical charges for study related activities will be paid for by the study. All

Standard of Care procedure deemed necessary and independent of study activities will be incurred by the participants.

Currently, EPO is used off-label in clinical practice for treatment of SCD patients with persistent anemia despite optimal treatment with hydroxyurea in the US. The cost of the study drug, EPO, will be paid for by the study. EPO will be dispensed to the participants through the UPMC Investigational Drug Services (IDS) pharmacy.

Participants may also qualify for a limited amount of patient assistance while on study, in line with what is offered routinely to patients in the UPMC Adult Sickle Cell Program clinic who express financial need.

EPO is available in Nigeria but is not affordable for the vast majority of the patient population. Therefore, the cost of EPO drug will also be covered by the study for participants at the Nigerian site.

4. Study Interventions

Participants will be treated with escalating doses of EPO, administered subcutaneously three times weekly (TIW) for 12 weeks. EPO dose will be initiated at 10,000 units TIW and escalated as tolerated by 10,000 units per dose every 2 weeks until reaching a Hb target of 10-11 g/dL, up to a maximum dose of 40,000 units TIW (Table 2).

4.1 Drug supplies

4.1.1 Preparing and dispensing

In the US, EPO will be supplied free of cost to the participants by the study team through the UPMC IDS pharmacy. In Nigeria, EPO will be prescribed clinically and dispensed by the study team, because the needed dosages of EPO are not available in the hospital and community pharmacies. It is common practice in Nigeria for patients to source their EPO drug directly from suppliers who are licensed pharmaceutical companies with importation permits rather than a pharmacy. The LUTH study team will therefore regularly source the required doses of EPO directly from a licensed supplier and store the study drug safely in a refrigerator with locks and temperature monitor and alarms. The refrigerator will be located in a limited-access locked research suite accessible to the study team only. The LUTH team will dispense the correct amount and dose of drug to participants at each study visit as indicated by the protocol. This is standard practice in interventional clinical trials conducted in Lagos. The study team's research nurses are trained in study drug storage, dispensing, and record keeping, and only licensed pharmacists are permitted to be drug suppliers/vendors.

4.1.2 Drug administration

After enrollment onto the study and filling of the clinical prescription for EPO, participants will be scheduled for their Day 0/drug initiation study visit. Each study site will use the epoetins available to them at their site and will carry out their visits in designated clinics. For participants in the US, all study activities will be done in the UPMC Adult Sickle Cell Program clinic. At the Day 0 visit, participants will be educated on how to administer subcutaneous injections of EPO by the research or clinical team, as well as provided with a recorded instruction video to review at home. After the participant and the research team are comfortable with the participant's skills for self-administration, they will return home and self-administer doses of EPO at home. If a participant cannot physically or does not feel comfortable self-administering the injections, a

caretaker may be identified and trained to administer the subcutaneous injections at home. The research or clinical team may help the patient with drug administration when the patient is present in the UPMC Adult Sickle Cell Program clinic if needed. The study drug should not be administered via an intravenous catheter.

4.2 Concomitant Medications

A list of concomitant medication will be collected at screening and updated prior to starting study drug for all participants.

5. Study Procedures:

5.1 Assessment of adverse events

Adverse events (AEs) will be graded by severity using CTCAE version 5.0. Assessment of relationship to the study procedure or treatment will be performed by either the PI or a delegate on the clinical research team that is overseen by the PI. All AEs will be collected and compiled in a research database by a member of the research team. Serious AEs (SAEs) will be collected and reported according to reporting protocol in Section 7.

5.2 Blood and Urine Biomarkers

Blood sampling for hematological parameters and circulating biomarkers of hemolysis, renal function, iron status, inflammation, red cell rheology, adhesion, and thrombogenesis will be performed at baseline and throughout the study according to Table 4. Fresh blood will be used for some assays and will be performed depending on availability at each research site. The remainder of the blood will be processed, and frozen plasma or serum will be stored and then batch-tested for the biomarkers outlined in Table 4. Urine will also be collected for baseline and end of study tests including measurements of biomarkers of kidney function as indicated in Table 4.
The study collaborators in Nigeria will perform all common clinical laboratory assays available to them at their site in Nigeria. The remainder of the laboratory assays will be stored and shipped from Nigeria to University of Pittsburgh for research purposes only. An MTA will be signed.

Table 4. Summary of Blood and Urine Biomarkers		**Sample Type**	**Volume of blood (Collection tube)**	**Frequency**
Clinical	Complete blood count, absolute reticulocyte count	Fresh blood	3 mL (EDTA)	Screening, Baseline, Q2 weeks, EOT, EOS
	Lactate dehydrogenase, basic metabolic panel, hepatic function panel	Fresh blood	4.5mL (LtGreen)	Screening, Baseline, Q2 weeks, EOT, EOS
	Iron homeostasis (TIBC, ferritin, haptoglobin)	Fresh blood		Screening, EOT, EOS
	Erythropoietin level	Fresh or frozen serum	5 mL (GoldSST)	Baseline
	Serum and/or Urine Pregnancy Test	Fresh blood or urine	5 mL (GoldSST) or 10 mL (Clean catch up)	Screening (serum), Baseline (urine)

	HPLC	Fresh Blood	3 mL (EDTA)	Baseline, EOT, EOS
	Urine albumin-to-creatinine ratio (random urine creatinine and urine albumin)	Urine	10 mL (Clean catch cup)	Baseline, EOT, EOS
Exploratory/ Research (Optional)	Markers of iron homeostasis (e.g., hepcidin, soluble transferrin receptor, hemopexin, erythroferrone), B-type natriuretic peptide and IL-6	Frozen plasma	6 mL (EDTA)	Baseline, EOT, EOS
	Markers of thrombosis (e.g., D-dimer, prothrombin fragment 1.2, thrombin-antithrombin complexes, soluble P-selectin)	Frozen plasma	3 mL (Blue)	Baseline, EOT, EOS
	Cystatin C, Troponin, C-reactive protein	Frozen Serum	5 mL (GoldSST)	Baseline, EOT, EOS
	Blood samples for future genetic studies	Frozen DNA	Included with other biomarkers (EDTA)	Baseline
	Whole blood viscosity, red cell deformability and point of sickling (Lorrca ektacytometry), F-cell fraction	Fresh blood	6 mL (EDTA)	Baseline, Q4 weeks, EOT, EOS
	Microfluidic flow assays	Fresh blood	6 mL (EDTA)	Baseline, Q4 weeks, EOT, EOS
	Phosphatidylserine exposure, markers of platelet activation (e.g., surface P-selectin/CD62, activated GPIIb/IIIa) using flow cytometry, and platelet bioenergetics (e.g., mitochondrial measures and ROS generation)	Fresh blood	8 mL (ACD)	Baseline, Q4 weeks, EOT, EOS
	Urine Biomarkers (Kidney Injury Molecule-1 (KIM-1), IGFBP7, TIMP-2, Nephrin, Alpha-1-microglobulin (A1M), IL-18, L-Fatty acid binding protein (L-FABP), Heme/Hemoglobin	Urine	Included with the clinical urine sample collected. (Clean catch cup)	Baseline, EOT, EOS

5.3 Transthoracic echocardiography

Evaluation of cardiac anatomical structure and function using standard echocardiographic techniques and equipment will be performed and interpreted by qualified personnel. Specifically, 2D and Doppler echocardiographic assessment of left ventricular systolic/diastolic function, right ventricular systolic function, and right ventricular systolic pressure (tricuspid regurgitant jet velocity): Standard echocardiographic views will be used, with particular attention to specific measurements of interest for this study and depending on the ability to perform specific measurements at each research site: Pulse wave of mitral inflow in 4 chamber views; continuous wave Doppler of TRV in 4 chamber and parasternal views; tissue Doppler of septal and lateral mitral annulus and TV annulus at the RV free wall; left atrial (end-systolic) and left ventricular (end systolic and end diastolic) volume measurements in the apical 2- and 4-chamber views; tricuspid annular plane systolic excursion (TAPSE) by M-mode; and cardiac index estimated by Doppler. Right and left ventricular ejection fraction will be derived as indices

of systolic ventricular function. TAPSE and RV annular velocity will give additional measures of RV systolic function, presence of LV diastolic dysfunction (Grades I-III) and elevated left atrial pressures will be determined according to updated guidelines established by the American Society of Echocardiography. RV systolic pressure will be derived from TRV using the modified Bernoulli equation. A cardiologist will be consulted if necessary to review the transthoracic echocardiograms if there are results of clinical concern.

5.4 Six-minute walk test with Modified Borg Dyspnea Scale

The 6MW 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. The Modified Borg Dyspnea Scale will be measured in conjunction with the 6MW Test to measure the level of severity of breathlessness and fatigue perceived by the patient before and after 6MW test. The severity is measured on a 10-point scale with 0=nothing at all and 10=maximum severity of breathlessness or fatigue. Vital signs will be taken before and after the 6MW test.

5.5 Assessment of health-related quality of life

Participants will be asked to complete a combination of survey items from the Adult Sickle Cell Quality of Life Measurement System (ASCQ-Me), the Patient Reported Outcome Measurement Information System (PROMIS) short form (SF), and the 36-Item Short Form Health Survey (SF-36) questionnaires at baseline (prior to starting study treatment) and at the end of treatment (Figure 1 and Table 1). Survey items will be selected from following questionnaires to cover the primary functional domains affected in adults with SCD, as well as domains hypothesized to be affected by anemia: ASCQ-Me Pain Impact, Stiffness Impact, Pain Episodes, Emotional Impact, and Sleep Impact; PROMIS Pain Interference, Fatigue, Dyspnea, and Cognitive Function. All listed SF questionnaires have been validated in an SCD population, except for the PROMIS Pain Interference and Cognitive Function, which were designed to be applicable across chronic diseases. In addition, the SF-36 questionnaire has been used in and found culturally appropriate for Nigerians with SCD, and this will be administered in its entirety. Questionnaires will be created in an online database (REDCap) built by the study team and administered using a portable electronic device. Questionnaires should be completed by the subject without help from anyone else (with the exception of help with logistical or technological issues that may arise). During the survey, research staff may help to define a term but cannot define a concept where the respondent's subjective interpretation is the goal of the question. Subjects completing the survey in clinic will be provided with sufficient space and time to complete the survey.

5.6 Assessment of Pain using Visual Analog Scale and Painimation

In addition to using the standard Visual Analog Scale for pain, pain will be assessed at baseline and at each clinic visit using a novel pain assessment tool called Painimation. This tool addresses the documented communication challenges associated with SCD pain and allows participants to express their pain quality, intensity, and location using animations and graphical images instead of words and numerical scales. Painimation was validated in 170 participants with a mix of non-SCD pain types and was acceptable, easy to use, and useful for communicating pain among adults with SCD.[38] The assessment will be administered using a portable electronic device. This participant-centered technology-based approach to pain measurement will complement and support data obtained through the questionnaires.

5.7 Neurocognitive Assessment

For a comprehensive assessment of neurocognitive function, subtests of the National Institute of Health (NIH) toolbox, a widely-used and validated computer-based cognitive test will be administered.[39] The NIH toolbox Cognitive Function Battery (CFB) is a brief battery consisting of 7 tests to assess 8 cognitive abilities. These tests include Dimensional Change Card Sort (DCCS, Executive Function-Cognitive Flexibility); Flanker Inhibitory Control and Attention Test (Executive Function-Inhibitory Control and Sustained Attention); Picture Sequence Memory Test (Episodic Memory-Visual); the Picture Vocabulary Test (Language-Vocabulary Comprehension), Oral Reading Recognition Test (Language-Reading Decoding); List Sorting Working Memory Test (Working Memory); and Pattern Comparison Processing Speed Test (Processing speed). These tests will assess domains that have been shown to be affected in SCD. Assessments will be administered using a portable electronic device and will take ~30 minutes. This participant-centered technology-based method of assessing neurocognitive function will complement and support data obtained through the questionnaires and the testing in Section 5.8.

5.8 Near-infrared (NIR) Technologies and electroencephalography (EEG)

Several NIR technologies have been validated in SCD and non-SCD populations.[40-42] In this study, the NIR technologies of diffuse correlation spectroscopy (DCS) and frequency-domain NIRS (FD-NIRS) will be used to assess the impact of raising hemoglobin level on microvascular hemodynamics in SCD. DCS measures temporal fluctuations in light intensity in deep tissues and is sensitive to changes in blood flow.[43] FD-NIRS measures absolute tissue oxy- and deoxyhemoglobin concentrations and tissue oxygen saturation,[41] which allows for both intra-individual and inter-individual comparisons. DCS and FD-NIRS will be performed at baseline and every month thereafter until the end of 12 weeks of treatment. Various non-invasive probes will be used to acquire spatially and temporally co-registered measurements from superficial forearm muscles and the cerebral cortex to obtain steady-state integrated data on tissue hemodynamics. Melanin content in skin has been shown to have an effect on near-infrared spectroscopy (NIRS) recordings. [54,55] For this reason, a DSM III Skin Colorimeter (Cortex Technology) will be used to record the melanin content in participants' skin. This device uses 2 high intensity white LEDs to provide recordings of both erythema and melanin content. The colorimeter is not FDA approved as it is marketed as a cosmetic research device. In addition, electroencephalography (EEG) measurements will be obtained to simultaneously evaluate neuronal activity. These measures will be performed, depending on the availability of these technologies at each research site. In the US site, the FD-NIRS, DCS, and EEG measurements will be performed in collaboration with study team members from the Carnegie Mellon University (CMU). In the Lagos site, some measurements with NIR technologies will be performed as well, but DCS and EEG will not be performed due to lack of the equipment and/or expertise. The CMU or Pitt team will provide the required equipment and material for these non-invasive measurements. For the remainder of the protocol, the terms NIR technologies and NIRS will be used interchangeably and will refer to all non-invasive imaging technologies using NIR.

Prior to performing the NIRS assessment, the DSM Colorimeter device will be used to check the melanin index and erythema value of the participant. It will be placed on the subject's skin for about 5 seconds to record a measurement from each location where NIRS will be recorded (left forehead, right forehead, and left forearm). Once the NIRS and EEG probes are placed and steady-state measurements are obtained, we will have the participant undergo non-invasive experimental tasks that allow for evaluation of the muscle and cerebral vascular response to

perturbations. For instance, participants will undergo a vascular occlusion challenge, in which a standard automated blood pressure cuff will be placed on the same forearm as the NIRS probes and inflated to temporarily occlude arterial blood flow into the extremity, for a maximum of 3-5 minutes per measurement, while participants are seated and at rest. The cuff will then be released, and NIRS will be used to measure the magnitude of hyperemic response and other parameters related to vascular function.

Other tasks include paced breathing and breath holding after exhalation, as well as brief cognitive tests such as the digit symbol substitution test (DSST). Simultaneous measurement of cerebral blood flow, tissue oxygenation, and neuronal activity via EEG will allow for evaluation of cerebral autoregulation, oxygen extraction, and neurovascular coupling. Each task is designed to induce changes in the blood pressure or physiochemical changes in the brain within normal physiologic variation and would therefore be of minimal risk to healthy or at-risk volunteers. Blood pressure oscillations will be induced at a well-defined frequency, according to protocols that have been already proposed, performed, and reported in the literature.[44-47] Paced breathing involves following the cue of a metronome to breath at a well-defined frequency within the range 0.03-0.50Hz. In all cases, the protocol will involve no more than 5 minutes of each breathing task at each frequency considered (within the range 0.03-0.5Hz) or 5 minutes of cognitive tests for a total measurement time not to exceed 60 minutes. Descriptions and procedures of each task will be provided to participants before they are conducted.

DSM colorimeter, EEG and NIRS measurements will be obtained at baseline and repeated at monthly clinic visits during the 12-week treatment period, as well as at EOS (24 weeks). The information obtained through these methodologies will be purely experimental and will not be used to track participant response to drug therapy in real-time. The data collected will be shared between the Pitt and the CMU research teams. The data will be analyzed retrospectively and will not be used in any way to provide feedback to the study team, treating clinician, or participant for the purpose of making clinical or research decisions.

5.9 Assessment of compliance

Participants will self-report doses of EPO taken and any symptoms observed during the course of participation in the study. They will be filling out a daily drug diary for EPO and their compliance will be reviewed at each visit. The level of compliance will be calculated as the percentage of the total number of doses administered out of the total number of doses prescribed during the treatment period. Symptoms reported through the application will be reviewed by the study team and will be used to facilitate AE tracking and reporting.

6. Efficacy and Safety Assessments:

6.1 Safety assessments

The PI will review participant safety data as it is generated and ensure that the IRB is immediately notified of any serious adverse event. The PI will routinely be notified of any medical concerns during the conduct of the study. Expected and unexpected serious (including fatal) adverse reactions and major unresolved disputes between the research investigator(s) and the research participant or between research investigator(s) will be expeditiously reported to the IRB as per Section 3.4 of the University of Pittsburgh IRB reference manual. The PI will promptly report the unanticipated problems that occur during the conduct of the study according to the reporting criteria and timelines under the current IRB policies. At the time of renewal, the

IRB will be provided with a summary of the cumulative adverse event data, information regarding participant safety or ethics changes, confidentiality issues, benefit-to-risk changes and recommendations on continuing, changing or terminating the study.

Risk/Benefit Assessment:

Risks with Study Treatment. Epoetin (EPO) is a 165 amino acid glycoprotein manufactured by recombinant DNA technology with the same biological effect as endogenous erythropoietin. Initially approved in 1989, epoetins have a long safety record and are the standard of care for anemia in the chronic kidney disease (CKD) population. EPO is also indicated for anemia in zidovudine-treated HIV-infected patients, cancer patients on chemotherapy, and surgery patients. In studies of EPO treatment in these patient populations, patients have experienced increases in blood pressure, greater risk of seizures, increased clotting of the vascular access (A-V shunt), and other thrombotic events (e.g., myocardial infarction, cerebrovascular accident, transient ischemic attack), especially when targeting higher Hb levels. In controlled trials, patients with CKD have experienced greater risk of death, serious adverse cardiovascular reactions, and stroke when administering EPO to target a Hb level of > 11 g/dL. Furthermore, increased mortality and/or increased risk of tumor progression or recurrence has been found in patients with cancer. For these reasons, participants with uncontrolled hypertension, uncontrolled seizure disorder, unstable cardiac disorder, active malignancy, or recent thrombosis will be excluded from the study. This study will also avoid targeting a Hb level > 11 g/dL.

In terms of SCD-specific risks, there have been a handful of anecdotal reports of pain and VOCs associated with EPO therapy, but of note, the largest and most recent retrospective cohort studies did not identify any increased risk of VOC or thrombosis,[30,32] suggesting that EPO therapy is safe and efficacious in the SCD population. The typical dose range of EPO used to achieve a Hb response in the SCD population is higher than the range used in the CKD population. Doses of erythropoietin in previous clinical studies have ranged between 100 and 3000 U/kg per dose (i.e., 7000-210000 U per dose for a 70-kg individual), with dosing 1-3 times per week.[26-37] The dose range and rate of dose escalation is largely determined by institutional and provider practices, but the starting treatment dose used in routine clinical practice is typically 10000-20000 U per dose. As higher doses of EPO are associated with increased risk of cardiovascular and thrombotic complications in the CKD population and may also increase risk of VOC, we have chosen to use low- to mid-range dosing of EPO (10000-40000 U per dose, 3 times per week) to further reduce the risk of complications.

Additional rare risks of EPO therapy include pure red cell aplasia, serious allergic reactions, and severe cutaneous reactions. If any of these reactions occur, EPO therapy should be discontinued immediately. Subcutaneous injections of EPO may also be commonly associated with slight pain or bruising at the injection site, and less commonly, a chance of bleeding or hematoma formation. There is a rare chance of infection at the injection site. Common adverse reactions reported in clinical trials for the following non-SCD patient populations are listed in the drug label insert for epoetins (e.g. Epogen):

- Patients with CKD: Adverse reactions in ≥ 5% of Epogen-treated patients in clinical studies were hypertension, arthralgia, muscle spasm, pyrexia, dizziness, medical device malfunction, vascular occlusion, and upper respiratory tract infection.

- Patients on Zidovudine due to HIV-infection: Adverse reactions in ≥ 5% of Epogen-treated patients in clinical studies were pyrexia, cough, rash, and injection site irritation.

- Patients with Cancer on Chemotherapy: Adverse reactions in ≥ 5% of Epogen-treated patients in clinical studies were nausea, vomiting, myalgia, arthralgia, stomatitis, cough, weight decrease, leukopenia, bone pain, rash, hyperglycemia, insomnia, headache, depression, dysphagia, hypokalemia, and thrombosis.

- Surgery Patients: Adverse reactions in ≥ 5% of Epogen-treated patients in clinical studies were nausea, vomiting, pruritus, headache, injection site pain, chills, deep vein thrombosis, cough, and hypertension.

Risks with Increasing Hemoglobin Level. Higher hemoglobin levels have been associated with higher VOC frequency in SCD in a handful of retrospective studies, but no prospective studies have been performed evaluating changes in pain symptoms and VOC frequency with EPO therapy. We anticipate an increase in hemoglobin with study treatment and will monitor the absolute as well as rate of hemoglobin increase very carefully to avoid rapid or excessive increases in hemoglobin that could lead to increased pain or vaso-occlusion in patients with SCD. We will not correct the hemoglobin to normal levels but will instead target a hemoglobin goal of 10-11 g/dL to minimize potential increases in blood viscosity at near-normal hemoglobin levels. As the response to the study drug and tolerance to the level of increased hemoglobin likely varies between individuals, we will monitor patient symptoms and wellness through adverse event (AE) collection, a Visual Analog Scale for pain, the Painimation tool, and quality of life metrics, and will consider dose reduction or discontinuation as indicated for hemoglobin levels above target or rapid increases in hemoglobin (Table 3).

Risks with Blood Sampling. As part of this study, up to 50 mL (or just over 3 tablespoons) of blood will be collected at the time of each research clinic visit, in addition to routine lab work. The risks of collecting blood may include fainting, pain, and/or bruising. Blood will be drawn by trained certified personal. After blood draw participant will be monitored for stability.

Risks with DSM Colorimeter. There are no risks associated with the use of the DSM Colorimeter since it only measures the wavelength absorbance in the visible range of light. No adverse reactions associated with the device have been reported.

Risks with Near-infrared Spectroscopy (NIRS). There are no risks associated with the use of near infrared light as it has been described in this protocol. The amount of optical energy is too low to cause appreciable tissue heating and no damage will occur to the skin. Light sources are used in NIRS technologies and may cause retinal damage upon looking directly into the light source. To prevent such instances from occurring and minimizing this type of risk, the lasers will be “off” until placed on the skin, and participants will be asked to not look into the light. Temporary pain or paresthesias may occur during the approximately 3–5-minute blood pressure cuff inflation. The vascular occlusion challenge may also result in redness or minor bruising of the limb due to cuff inflation. This occlusion is a standard test that is performed in many hemodynamic assessments, such as in the case of peripheral vascular disease. Patients will be monitored closely for any concerning signs and symptoms during the occlusion challenge, and if there are any concerns for safety or if participants experience significant discomfort, the occlusions will be released immediately. Participants may also experience discomforts during the breathing maneuvers, such as lightheadedness. Participants will be monitored closely for symptoms and signs of discomfort during and after measurements, and participants can ask to take breaks between sessions or to stop the maneuver at any time if the discomfort they experience is intolerable.

All non-invasive instruments used in this study are research devices used for experimental studies only; they are not medical devices based on the criteria: (a) these specific devices are

not part of the US Pharmacopeia, (b) these devices are not collecting clinical information for a medical diagnosis – all information is purely for research purposes, and (c) these devices are non-invasive and are not intended to alter the tissue structure or function in any capacity. For these reasons, the devices used in our study are not medical devices and an IDE application is not required.

Risks with EEG: There are no risks of physical injury associated with EEG. Adhesive from the EEG probes might cause skin irritation. Prior to its removal, the conductive gel for the EEG probes may leave residue on the scalp. The conductive gel can easily be removed from the scalp.

Risk of Inconveniences. Participants may not feel comfortable answering some of the questions during questionnaires, pain assessment, or neurocognitive assessment, however this information is important to understand. There are no right or wrong answers to questions, and all answers will remain confidential. There may also be discomfort with checking vital signs and doing physical exams. Although physical tests and checking vital signs do not involve any known risks, taking blood pressure may cause discomfort or bruising to upper arm. To maximize the patients' convenience, the NIRS machine will be brought to a research facility or clinic on the Pitt campus, and all the tests will be performed in a private room by the collaborating CMU research team members.

Confidentiality Risk. All records with identifying information will be stored in a password-protected server. All future research samples (such as blood samples stored for future genetic or biomarker testing) will be deidentified by labeling with a study code and stored in a securely locked laboratory. Although it is highly unlikely, there is still the possibility that personal information could be linked back to research information. For this reason, additional protections will be taken to protect information linking the codes with identity (i.e., name or other information that could be used to link with the information about the participation in this study), and that information will be kept separate from the research records.

Importance of the Knowledge to be Gained:

The PI will be reviewing the data from this research throughout the study. We will promptly tell the participants about new information from this or other studies or changes in the study that may affect their health, welfare, or willingness to continue in the study.

6.1.1 Data Safety Monitoring Plan (DSMP)

Study sites' local data safety and monitoring plan

A local data and safety monitoring plan will be implemented by each site as per their institutional requirements. Oversight of the data and safety monitoring at all sites is the primary responsibility of the study site PI. The PI at each participating site will meet with the participant when they are first enrolled in the study, and then periodically, every four weeks, the PI will look at the individual subject data, as it is generated throughout the study, for any trends in adverse events or other events that may pose a concern. Any adverse events meeting reporting requirements will be reported as they occur per IRB policy guiding each site. Summary information and reportable information required for continuing reviews, will be reported to the IRB annually.

The study team members will monitor participants' recruitment and retention, occurrence of adverse events, and confidentiality of the participants' information at their respective study sites. The AEs will be reported as per CTCAE version 5.0 and will include whether the participant discontinues to take the drug, start and stop dates, any corrective measures that will be taken, and their outcome. All subjects who receive at least one dose of study treatment will be included in the safety analysis. All observations pertinent to the safety of the study treatment will be recorded and included in a final analytical report. All AEs whether considered drug-related or not, will be reported. For all events, the relationship to treatment and the intensity of the event will be determined by the investigator. All subjects who have AEs, whether considered associated with the use of the study product or not, will be monitored during the treatment period of 12 weeks to determine the outcome. They will be referred back to their care provider for continued follow up and management in the event they need more medical attention. Our AE recording and observation period will stop at the end of treatment (at 12 weeks). AEs will not be tracked between end of treatment and end of study, as patient will not be receiving any intervention per protocol during this period.

All local site(s) will participate in monthly meetings with the Coordinating Center, Pitt site, to re-evaluate study goals, subject recruitment, data coding and retention, documentation and identification of adverse events, complaints, and confidentiality of subjects.

For the collaborating study site in Nigeria, the PI for the coordinating center, who is the Chief Investigator, will initially visit the study site for a study initiation visit. That visit may be done remotely if travel restrictions prohibit it. The coordinating center PI will receive updates on the study progress during the monthly PI meetings. In the event the PI needs a detailed look into a local site participants' study record, the study site PI or their delegates will send de-identified copies of participants' information to the coordinating center PI. The local sites will also upload their data into coordinating center database project hosted on Pitt CTSI REDCap which can be accessed.

DATA AND SAFETY MONITORING BOARD

A Data and Safety Monitoring Board (DSMB) will be created to review this study. After initial approval and at periodic intervals (to be determined by the committee) during the course of the study, the DSMB responsibilities are to:

1. Review the research protocol, informed consent documents and plans for data and safety monitoring;
2. Evaluate the progress of the study, including periodic assessments of data quality and timeliness, participant recruitment, accrual and retention, participant risk versus benefit, adverse events, unanticipated problems, performance of the trial sites, and other factors that can affect study outcome;
3. Consider factors external to the study when relevant information becomes available, such as scientific or therapeutic developments that may have an impact on the safety of the participants or the ethics of the study;
4. Review clinical center performance, make recommendations and assist in the resolution of problems reported by the PI;
5. Protect the safety of the study participants;
6. Report on the safety and progress of the study;
7. Make recommendations to the PI, and if required, to the funding agency concerning continuation, termination or other modifications of the study based on the observed beneficial or adverse effects of the treatment under study;
8. Monitor the confidentiality of the study data and the results of monitoring;

9. Assist the PI by commenting on any problems with study conduct, enrollment, sample size and/or data collection.

The DSMB will include experts in *hematology or vascular medicine and clinical trials/biostatistics*. Members will consist of persons independent of the investigators who have no financial, scientific, or other conflict of interest with the study. Written documentation attesting to absence of conflict of interest will be required.

The University of Pittsburgh Office of Clinical Research, Health Sciences / CTSI will provide the logistical management and support of the DSMB. A safety officer (chairperson) will be identified at the first meeting. This person will be the contact person for serious adverse event reporting. Procedures for this will be discussed at the first meeting.

The first meeting will take place before initiation of the study to discuss the protocol, approve the commencement of the study, and to establish guidelines to monitor the study. The follow-up meeting frequency of the DSMB will be determined during the first meeting. An emergency meeting of the DSMB will be called at any time by the Chairperson should questions of patient safety arise.

6.1.2. Data safety monitoring parameters

Adverse events (AE's) will be collected periodically from start of study drug treatment period (baseline visit) until end of treatment (EOT), after which only acute care utilization will be collected until the end of study (EOS). AE reporting procedure is described in Section 8.2.1.

6.1.3 Physical Sign Monitoring

Body temperature (orally), heart rate, and blood pressure will be measured at baseline before initiation of EPO administration as well as at each clinic visit.

6.2 Privacy

Privacy of participants will be maintained through a set of procedures that has worked well at the University of Pittsburgh. All University of Pittsburgh faculty participating in this study have had HIPAA training. Confidentiality will be ensured by assigning each participant a study code and number that will not allow participant name identification; and these will be used to identify the participant's data. Also, all data will be kept in locked file cabinets in a locked and secured area or digitally on a server like REDCap with access by research personnel only.

7. Adverse Event Reporting:

7.1 Adverse event definitions

Adverse event. Any untoward medical occurrence in a clinical study; regardless of the causal relationship of the event with the investigational drug or study treatment(s).

Associated with the use of the investigational drug or study treatment(s). There is a reasonable possibility that the adverse event may have been caused by the investigational drug or study treatment(s).

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

Life-threatening adverse event. Any adverse event that places the participant or participant, in the view of the investigator, at immediate risk of death from the event as it occurred (i.e., does not include an adverse event that, had it actually occurred in a more severe form, might have caused death).

Serious adverse event. Any adverse event occurring at any dose that results in any of the following outcomes: death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity, or a congenital anomaly/birth defect.

Hospitalization. Hospitalization shall include any initial admission (even if less than 24 hours) to a healthcare facility as a result of a precipitating clinical adverse event; to include transfer within the hospital to an intensive care unit. Hospitalization or prolongation of hospitalization in the absence of a precipitating, clinical adverse event (e.g., for a preexisting condition not associated with a new adverse event or with a worsening of the preexisting condition; admission for a protocol-specified procedure) is not, in itself, a serious adverse event.

Unexpected adverse event. Any adverse event, the frequency, specificity or severity of which is not consistent with the risk information described in the clinical protocol(s).

8. Recording/Reporting requirements

8.1 Safety Assessment and Reporting

8.1.1. Eliciting adverse event information

Clinical study participants will be routinely questioned about adverse events at study visits.

8.1.2. Recording requirements

All observed or volunteered adverse drug events (serious or non-serious) and abnormal test findings, regardless of treatment group or suspected causal relationship to the investigational drug or study treatment(s) will be recorded in the participants' case histories. For all adverse events, sufficient information will be pursued and/or obtained so as to permit 1) an adequate determination of the outcome of the event (i.e., whether the event should be classified as a *serious adverse event*); and 2) an assessment of the casual relationship between the adverse event and the study treatment(s).

Adverse events or abnormal test findings felt to be associated with the study drug will be followed until the event (or its sequelae) or the abnormal test finding resolves or stabilizes at a level acceptable to the investigator-sponsor.

8.1.3 Abnormal test findings

An abnormal test finding will be classified as an *adverse event* if one or more of the following criteria are met:

- The test finding is accompanied by clinical symptoms.

- The test finding necessitates additional diagnostic evaluation(s) or medical/surgical intervention, including significant additional concomitant drug treatment or other therapy.
- Note: simply repeating a test finding, in the absence of any of the other listed criteria, does not constitute an adverse event.
- The test finding leads to a change in study dosing or discontinuation of participant participation in the clinical study.
- The test finding is considered an adverse event by the investigator-sponsor.

Serious Adverse Events will be reported to the IRB according to their guidelines.

8.1.4. Causality and severity assessment

The investigator-sponsor will promptly review documented adverse events and abnormal test findings to determine 1) if the abnormal test finding should be classified as an adverse event; 2) if there is a reasonable possibility that the adverse event was caused by the investigational drug or study treatment(s); and 3) if the adverse event meets the criteria for a *serious adverse event*.

If the investigator-sponsor's final determination of causality is "unknown and of questionable relationship to the investigational drug or study treatment(s)", the adverse event will be classified as *associated with the use of the investigational drug or study treatment(s)* for reporting purposes. If the investigator-sponsor's final determination of causality is "unknown but not related to the investigational drug or study treatment(s)", this determination and the rationale for the determination will be documented in the respective participant's case history.

8.2. Reporting of adverse events

8.2.1 Reporting adverse events to the responsible IRB

In accordance with applicable policies of the University of Pittsburgh Institutional Review Board (IRB), the investigator-sponsor will report, to the IRB, any observed or volunteered adverse event that is determined to be 1) *associated with the investigational drug or study treatment(s)*; 2) *serious*; and 3) *unexpected*. Adverse event reports will be submitted to the IRB in accordance with the respective IRB procedures.

Applicable adverse events will be reported to the IRB as soon as possible and, in no event, later than 10 calendar days following the investigator-sponsor's receipt of the respective information. Adverse events which are 1) *associated with the investigational drug or study treatment(s)*; 2) *fatal or life-threatening*; and 3) *unexpected* will be reported to the IRB within 24 hours of the investigator-sponsor's receipt of the respective information.

Follow-up information to reported adverse event will be submitted to the IRB as soon as the relevant information is available. If the results of the sponsor-investigator's follow-up investigation show that an adverse event that was initially determined to not require reporting to the IRB does, in fact, meet the requirements for reporting; the investigator-sponsor will report the adverse event to the IRB as soon as possible, but in no event later than 10 calendar days, after the determination was made.

8.3 Withdrawal of participants due to adverse events

Participants may be withdrawn from participation in the interventional study if they suffer a serious adverse event felt to be related to the study treatment. The decision to withdraw will be made by the PI.

9. Statistical Methods/Data Analysis:

Descriptive statistics will be presented for all baseline and post-treatment characteristics. Graphs showing intra-individual change will be presented for all laboratory parameters. Baseline clinical and laboratory factors, including age, gender and Hb level, will be correlated with Hb response to identify predictors of individual response. Changes in clinical and laboratory factors from baseline to the end of study will be correlated with Hb response as well. Laboratory measures are listed in Table 4.

For quality-of-life measures, individual item raw scores will be calculated for all items, and scores at the end of treatment will be compared to baseline. If an entire instrument is used (e.g., the PROMIS Fatigue short form), then the individual item raw scores will be combined to obtain a total raw score for each subscale, which will then be converted to standardized *t* scores (with 50 as the mean and 10 as standard deviation of the reference population). Descriptive statistics, correlation, and regression models will be used to present changes in clinical measures and their relationship to changes in Hb level.

9.1 Study endpoints

Primary Endpoint: The primary endpoint of Hb response, defined as a Hb increase of ≥ 1.0 g/dL at the end of treatment compared to baseline, was chosen based on the endpoint definition used in the HOPE trial (Phase 3 study of voxelotor for patients with SCD).[17] This endpoint will serve to define responders to EPO, identify laboratory and clinical predictors or modifiers of response, as well as meaningful biological and clinical correlates of the surrogate marker of Hb increase. All enrolled participants who have completed baseline and end of treatment testing, regardless of duration of treatment with EPO, will be included in the analysis. Participants completing the full 12 weeks of EPO treatment per protocol will be evaluated in a planned sub-analysis. Frequency of adverse events (AEs), including treatment-related AEs and SAEs will also be reported. AEs will be tabulated by type, grade, and relatedness to treatment.

Secondary Endpoint: The secondary endpoint will be the annualized number of units of simple red blood cell transfusions received in the 12 months before treatment initiation compared to during active treatment.

Exploratory Endpoints (Optional):

Changes in hematological, hemolytic, and other clinical laboratory parameters will be assessed at EOT and EOS compared to baseline (Table 4). As renal dysfunction can affect endogenous EPO production and may also improve with increased Hb level and tissue perfusion, a pre/post serum creatinine, eGFR (using CKD-EPI and Cystatin C), urine albumin-to-creatinine ratio, and serum EPO level will be assessed at baseline and correlated with Hb response. Iron mobilization and inflammation are other factors that contribute to erythropoietic drive and may predict responsiveness to EPO, and therefore will be assessed at baseline and correlated with Hb response. All laboratory parameters assessed at EOS will be compared to baseline and EOT to evaluate longer-term treatment effects of EPO. Exploratory endpoints of Hb response will include a Hb increase of ≥ 1.0 g/dL above baseline at any time between 4 and 12 weeks, a Hb increase of ≥ 1.0 g/dL at 24 weeks, and sustained Hb response between 4 and 12 weeks (defined as the

percentage of Hb values ≥ 1.0 g/dL above baseline). Modeling of the trajectory and heterogeneity of the Hb response over time will be performed.

Clinical and imaging exploratory endpoints are listed in Table 5. Baseline acute vaso-occlusive, thrombotic events, and transfusions (within past 12 months) will be captured at the baseline visit and compared to the number of events at EOT and EOS. Cardiopulmonary function will be assessed by 6-minute walk test with oximetry and 2-dimensional Doppler echocardiogram. Neurocognitive assessments will also be administered, and a composite global score will be generated from the battery of tests. Quality of life items will be scored as mentioned above. Participants will record Visual Analog Scale and Painimation scores to assess pain level. These clinical endpoints will be assessed at EOT and EOS, compared to baseline and correlated with Hb response, as well as laboratory markers of hemolysis, cardiovascular risk, blood viscosity, and imaging biomarkers (Tables 4 and 5).

Whole blood viscosity (Table 4) will be measured using an automated and/or cone-plate viscometer and used to calculate the hematocrit-to-viscosity ratio (HVR), which has been widely used as a surrogate measure of oxygen transport effectiveness in the microvasculature and appears to correlate with non-invasive measures of tissue oxygen saturation.[48] Therefore, measurements of HVR will be correlated to Hb level and NIRS measures and may help to identify safe target Hb levels at which oxygen delivery and tissue perfusion are maximized in SCD. Whole blood viscosity will also be correlated with measurements obtained using microfluidic flow assays performed by Dr. Umut Gurkan at Case Western Reserve University and/or BioChip Labs, LLC.

A number of other measures obtained from fresh whole blood that potentially influence blood rheology will be correlated with Hb response and NIRS measures, including the following: red cell deformability and point of sickling (PoS) (by Lorrca ektacytometer) measured in the laboratory of the PI (Dr. Julia Xu), red cell phosphatidylserine exposure and platelet activation (by flow cytometry) and/or bioenergetics measured in the laboratory of co-investigator Dr. Sruti Shiva, F-cell fraction measured in the laboratory of co-investigator Dr. Adam Straub. Known markers of myocardial stress, thrombogenesis, and vascular injury[49-51] will be measured using frozen plasma or serum samples collected during the study and batched for analysis to further investigate the safety of EPO therapy and increasing Hb level (Table 4). DNA will also be stored and potentially used to identify genetic polymorphisms that may influence response to EPO. DSM colorimeter and NIRS measurements will be correlated with EEG measurements to evaluate neurovascular coupling.

Table 5. Summary of clinical and imaging endpoints		**Frequency**
Secondary	**Transfusions**: number of units of simple transfusions of packed red blood cells	Baseline, EOT, EOS
Exploratory (Optional)	**Acute vaso-occlusive events**: acute episodes of pain not related to other etiologies and requiring acute pain treatment during a medical encounter (i.e., VOC), acute chest syndrome, hepatic or splenic sequestration, or priapism **Thrombotic events:** Stroke, myocardial infarction, or venous thromboembolism	Baseline, EOT, EOS
	Cardiopulmonary function: 6-minute walk distance and oxygen saturation, TRV, cardiac index, left ventricular (LV) size as measured by LV dimensions and end-diastolic volume	Baseline, EOT, EOS
	Neurocognitive Functioning: composite global score from the NIH Toolbox, a computerized neurocognitive assessment tool	Baseline, EOT, EOS
	Select survey items: ASCQ-Me and PROMIS; SF-36	Baseline, EOT, EOS

	Pain: Scores from Visual analog scale and Painimation	Baseline, Q2 weeks, EOT, EOS
	NIRS and EEG testing: tissue oxygen saturation, blood flow velocity, metabolic rate of oxygen extraction, magnitude of hyperemic response, time to peak hyperemia, EEG frequency band power, evoked potentials	Baseline, Q4 weeks, EOT, EOS

9.2 Sample size determination

This study aims to have 15 participants completing the study (defined as completion of 12 weeks of EPO treatment and end of treatment testing) across both study sites. We will enroll up to 25 participants across both sites (Pitt and LUTH), with the expectation that no more than 10 participants will be lost to follow-up or withdraw from the study prior to completion of the 12 weeks. The goal is to determine an estimate with 95% confidence intervals for the population probability that a randomly chosen individual from the eligible SCD population would show a Hb response (i.e. Hb increase of ≥ 1.0 g/dL from baseline after 12 weeks of EPO therapy). Prior studies of EPO in SCD report a Hb response rate of between 50-60%.[30,32,33] In comparison, a low rate (7%) of Hb response over 6 months in the placebo arm of the voxelotor study was observed.[17] Therefore, rejecting a null hypothesis that the true probability of Hb response is $\leq$ 20% may suggest a relatively robust response. Using these assumptions, if the true probability of a Hb response is 0.5, then for a sample size of 25, there is 94% power to reject the null hypothesis that this probability is$\leq$ 20%, based on an exact one-sided 95% binomial confidence interval.

10. Data Handling and Record-Keeping:

10.1 Data recording/Case Report Forms

Case report forms (CRFs) will be completed for each participant enrolled into the clinical study. The principal investigator of each site will review, approve and sign/date each study visit to certify that all CRFs have been completed accurately; the principal investigator's signature serving as attestation of the principal investigator's responsibility for ensuring that all clinical and laboratory data entered on the CRF are complete, accurate and authentic.

When applicable, information recorded on the CRF shall match the *Source Data* recorded on the *Source Documents*.

10.2 Record maintenance and retention

The investigator-sponsor will maintain records in accordance with Good Clinical Practice guidelines.

At the University of Pittsburgh, the records will be kept in a locked area at all times, to be accessed only by personnel involved in the study. Computer-based files will only be made available to personnel involved in the study through the use of access privileges and passwords. Prior to access to any study-related information, personnel will be required to sign statements agreeing to protect the security and confidentiality of identifiable information, whenever feasible, identifiers will be removed from study-related information, precautions are in place to ensure the data is secure by using passwords and encryption.

At the LUTH site, there is a challenge with the medical record system, which is solely on paper and can be lost with time. Therefore, investigators at LUTH will make a photocopy of all source documentation, and the site Principal Investigator will certify these documents with a wet signature. The LUTH study team will maintain these copies in a separate file in order to make it easier to retrieve source documents, and these records will be kept in a locked area at all times, to be accessed only by personnel involved in the study.

10.3 Future Use of Stored Specimens and Data

Data collected through NIRS, and EEG methodologies will be stored by the CMU team and will be shared with the Pitt team for purposes of comparison and analysis during and at the end of the study. If the participant consents to participate in a separate Pitt IRB-approved study (STUDY22050118), which has overlapping endpoints and testing, data collected through this study may be shared with STUDY22050118 to avoid asking participants to undergo the same testing twice. Coded information from the measurement results will be shared between Pitt, Lagos, and CMU team. Data may be stored without identifiers indefinitely by both the CMU and Pitt teams.

A portion of the blood samples will be stored indefinitely at the University of Pittsburgh's Core Biospecimen Repository for current and future research that might include whole genome sequencing. The individual genetic results or incidental findings will not be shared with subjects as these tests are research-related and these initial finding will need additional research before their clinical significance is understood, and appropriate actions are determined. It is possible that some of the genomics research conducted either by the study investigators or secondary researchers could eventually lead to the development of new diagnostic tests, new drugs or other commercial products. If this should occur, there is no plan for research participants to receive any part of the profits generated from such products.

Samples will be stored without identifiers and may be shared with secondary researchers within or outside the participating centers. We will ship up to 6 mL of whole blood from consented patients without any identifying information to our collaborator Dr. Umut Gurkan at Case Western Reserve University (CWRU) and/or BioChip Labs, LLC which have licensed intellectual property from CWRU and are working with Dr. Gurkan at CWRU to translate these technologies. With these blood samples, the CWRU and/or BioChip Labs team will perform blood cell adhesion, occlusion, and blood rheology experiments, and communicate results of that testing back to the site of sample origination. Any residual blood samples may be used for additional assay development and validation by CWRU and/or BioChip Labs. We may also send a limited data set with the coded blood samples (including clinical, demographic, and experimental data) to CWRU and/or BioChip Labs in order to aid in their analysis of samples and validation of their technology. There will be no other analyses, such as DNA or RNA isolation, performed on the transferred samples.

11. Ethics:

11.1. Institutional Review Board (IRB) approval

The investigator-sponsor will obtain, from the University of Pittsburgh Institutional Review Board (IRB), prospective approval of the clinical protocol and corresponding informed consent form(s); modifications to the clinical protocol and corresponding informed consent forms, and advertisements (i.e., directed at potential research participants) for study recruitment.

The only circumstance in which a deviation from the current IRB-approved clinical protocol/consent form(s) may be initiated in the absence of prospective IRB approval is to eliminate an apparent immediate hazard to the research participant(s). In such circumstances, the investigator-sponsor will promptly notify the University of Pittsburgh IRB of the deviation.

11.2. Ethical and scientific conduct of the clinical study

The clinical study will be conducted in accordance with the current IRB-approved clinical protocol; ICH Guidelines on GCP; and relevant policies, requirements, and regulations of the University of Pittsburgh IRB, University of Pittsburgh and UPMC, Commonwealth of Pennsylvania, and applicable federal agencies.

12. Investigator-Sponsor Discontinuation Criteria:

The University of Pittsburgh Institutional Review Board (IRB) will be notified promptly of discontinuation of the entire clinical study. All protocol modifications will be submitted prospectively to the University of Pittsburgh IRB for discontinuation of parts of the clinical research study.

13. Stopping Rules

13.1. Stopping Rule for Qualifying Adverse Events

The stopping rule is designed to halt enrollment for further evaluation of study data by the safety committee and determination whether enrollment can continue, or the study should be modified or terminated. Qualifying AEs/SAEs include any of the following that are possibly, probably, or definitely related to treatment:

- Arterial or venous thrombosis (including myocardial infarction, cerebrovascular accident, and transient ischemic attack)
- Seizures
- Pure red cell aplasia
- Serious allergic reactions
- Severe cutaneous reactions.

If any of these reactions occur, EPO therapy should be discontinued immediately in the patient.

Based on drug package inserts for epoetin alfa and literature review[52,53], we anticipate that the rate of qualifying adverse events during the study period is no greater than 10%.

- Our stopping rule is determined by a Bayesian approach. The stopping boundary for an experiment is reached if the Bayesian posterior probability that the true probability of developing an AE exceeds this benchmark rate of 20% is at least 70%. We take our prior distribution to be a beta distribution with parameters (α, β) = (1, 4). The parameters are chosen so that the mean α / (α + β) = 0.20 as the maximum acceptable proportion of AE and the sum α + β = 5 as the "worth" we place on our prior clinical opinion. This indicates that the relative weight we place on our prior opinion is 5/25 =20% of the weight we will place on the results if n=25. The following table summarizes the stopping rule:

Number of subjects in the study	Halt if the number of subjects who developed a qualifying adverse event during study
5	2
6-9	3
10-14	4
15-18	5
19-23	6
24-25	7

13.2. Stopping Rule for Mortality

A separate stopping rule is proposed for deaths that are deemed to be possibly, probably, or definitely related to study intervention. Should such an incident occur, both entry of other patients into the study and dose escalation for patients on the study will be suspended, and patients receiving study medication will be closely monitored for occurrences of the same event whilst assessment of the event occurs.

The IRB/DSMB and the investigators will evaluate the full circumstances of the event and determine whether to terminate the study. Should it be determined the study be terminated, enrollment in the study will be halted permanently, and subjects already on study drug will be withdrawn from treatment.

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