Study Protocol

TITLE: The Effect of Voxelotor on Cerebral Hemodynamic Response in Children with Sickle Cell Anemia (VoxSCAN)

NCT NUMBER: NCT05018728

Protocol Date: June 10, 2024

PROTOCOL TITLE:
The Effect of Voxelotor on Cerebral Hemodynamic Response in Children with Sickle Cell Anemia

Protocol Number:
ESR-C007

SHORT TITLE:
Voxelotor SCA NIRS Study (VoxSCAN)

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Agent:
Voxelotor

IND:
155720

VERSION:
2.4 dated 10JUN2024

Drug Supplier:
Global Blood Therapeutics, Inc. (GBT)
181 Oyster Point Boulevard
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REVISION HISTORY

Revision #	Version Date	Summary of Changes	Consent Changes (Y/N)
1.0	18NOV2020	Original	N
1.1	9DEC2020	Original + Template Update	N
1.2	29DEC2020	Original + Updates from Team Review	N
1.3	3MAR2021	Original + Monitoring Plan Update	N
1.4	18MAR2021	Original + Updates from Emory Reviewer	N
1.5	8APR2021	Original + FDA requested update	Y
1.6	14MAY2021	Original + IRB pending approval request	Y
1.7	09MAR2022	Original + Updates from Team Review	Y
1.8	03Aug2022	Original + Changes in PI, enrollment target & study labs	Y
1.9 *draft, not released*	08Sept2023	Original + changes to study labs & subject payment	Y
2.0 *draft, not released*	28Sept2023	Original + Data Safety Monitoring Plan Requirements	N
2.1	25Oct2023	Original + Data Safety Monitoring Plan Monitoring Table edits	N
2.2	12Jan2024	Original + changes to study labs + SAE reporting changes	N
2.3	04MAR2024	Original + changes to assessments + endpoints	N
2.4	06Jun2024	Original + changes to assessments timepoints	

List of Major Changes to Version 1.9: (*draft, not released)*

- Change subject payment to $50/visit
- In Schedule of Assessments, removed CMP & LDH assessments from the 4 and 8w time points, remove Hb electropheresis assessments from the 8 and 12w time points.
- Removing Global Blood Therapeutics Laboratory as the source of analysis for RBC deformability and mitochondrial content assessments.

List of Major Changes to Version 2.0 (*draft, not released)*

- Addition of Data Safety Monitoring Plan Requirements table explaining new process

List of Major Changes to Version 2.1

- Edits to Data Safety Monitoring Plan Requirements monitoring table

List of Major Changes to Version 2.2

- Removing Global Blood Therapeutics Laboratory as the source of analysis for RBC deformability and mitochondrial content assessments. This change was intended in version 1.9, but some references were missed that have now been corrected.
- Changing safety reporting to comply with Pfizer's policies

List of Major Changes to Version 2.3

- Removing Worldwide Therapeutics and Functional Fluidics assessments of some markers of RBC health and hemoglobin modification
- Removing exploratory endpoints associated with RBC oxygen affinity and deformability, as these parameters will no longer be quantified
- Adding 12w electrophoresis assessment

List of Major Changes to Version 2.4

- Removing 4 and 8w timepoints for RBC health and clarified what will be measured to assess RBC health

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STATEMENT OF COMPLIANCE

This pilot study will be conducted in accordance with International Conference on Harmonization Good Clinical Practice (ICH GCP), applicable US Code of Federal Regulations (CFR). As the Sponsor-Investigator, the PI will assure that no deviation from, or changes to the protocol, will take place without prior agreement from the investigational drug supplier Global Blood Therapeutics, a subsidiary of Pfizer as of October 5th 2022, and documented approval from the IRB, except where necessary to eliminate an immediate hazard(s) to the study participants. All personnel involved in the conduct of this study have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the IRB for review and approval. Approval of both the protocol and the consent form will be obtained before any participant is enrolled. Any amendment to the protocol will be reviewed and approved by the IRB before the changes are implemented to the study. All changes to the consent form will be IRB approved.

PROTOCOL SIGNATURE PAGE

Protocol Title:

The Effect of Voxelotor on Cerebral Hemodynamic Response in Children with Sickle Cell Anemia (VoxSCAN)

Version/Date: 2.2 dated December 05, 2023

SPONSOR APPROVAL

I, the Sponsor-Investigator, have read this protocol and agree it contains all necessary information required to conduct the study.

The protocol is being conducted in accordance with International Council on Harmonization (ICH) Good Clinical Practice (GCP) and all applicable federal, state and local regulations governing the conduct of this research, including the Department of Health and Human Services 45 Code of Federal Regulations (CFR) Part 46, FDA 21 CFR Parts 50, 54, 56, 312 and 812.

Sponsor - Investigator (Print):	
Signature:	
Date:	

1. Study Summary

Study Title	ESR-C007: The Effect of Voxelotor on Cerebral Hemodynamic Response in Children with Sickle Cell Anemia
Phase	Single-center, open label pilot study.
Study Design	This study is a pilot, open-label, single-arm study to evaluate the effect of voxelotor on cerebral hemodynamics, as measured by diffuse correlation spectroscopy and frequency domain near-infrared spectroscopy (DCS/FDNIRS) in patients 4 -30 years of age with sickle cell anemia (SCA). We hypothesize that as hemoglobin increases due to voxelotor, cerebral blood flow (CBF) and oxygen extraction fraction (OEF) will decrease. To test this hypothesis, CBF, OEF, and oxygen metabolism (CMRO2) will be assessed by DCS/NIRS in participants prior to, periodically throughout, and after 12 weeks of treatment with voxelotor. The results of this study will be used to inform a larger study.
Primary Objective	The primary objective of this research is to evaluate the effect of voxelotor on CBF, OEF and CMRO2 in children with SCA as assessed by change from baseline in DCS/NIRS at 4 and 12 weeks.
Secondary Objective	The secondary objective of this research is to evaluate the correlation between changes in CBF, OEF and $CMRO_2$ and change in markers of anemia, hemolysis and RBC health at 4 and 12 weeks.
Research Intervention(s)/Interactions	The study intervention is voxelotor, administered orally. It will be used for the approved indication of sickle cell disease. This is a single-arm study. There is no control intervention. <u>Drug Intervention: Voxelotor</u> • Voxelotor tablets: 300 or 500 mg, administered orally • Voxelotor dispersible tablets: 100 and/or 300 mg, administered orally All participants will receive voxelotor. Participants 12 years or older will take 1500 mg/day. Participants younger than 12 years of age will receive a dose based on their body weight to provide exposure corresponding to the adult dose of 1500 mg/day.
Study Population	We plan to recruit male and female children ages 4-30 years with sickle cell anemia (HbSS, HbS/β^0thal) who are followed in the Pediatric Sickle Cell Program at Children's Healthcare of Atlanta

Sample Size	This is a pilot study involving 50 participants to define the magnitude of change. Power cannot be calculated at this time.
Study Duration for Individual Participants	Up to 16 weeks
Total Study Duration	30 months
Funding Source (if any)	Global Blood Therapeutics (GBT)
Study Specific Abbreviations/ Definitions	

ACS	Acute chest syndrome
ALT	Alanine aminotransferase
AE	Adverse Event
ANCOVA	Analysis of Covariance
Bil	Bilirubin
β^0thal	Homozygous sickle cell disease
CBF	Cerebral blood flow
CFR	Code of Federal Regulations
CHOA	Children's Healthcare of Atlanta
CLIA	Clinical Laboratory Improvement Amendments
CMP	Clinical Monitoring Plan
COC	Certificate of Confidentiality
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
DCC	Data Coordinating Center
DCS	Diffuse Correlation Spectroscopy
DHHS	Department of Health and Human Services
DSMB	Data Safety Monitoring Board
DRE	Disease-Related Event
EC	Ethics Committee
eCRF	Electronic Case Report Forms
FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act of 2007
FDNIRS	Frequency domain near-infrared spectroscopy
FFR	Federal Financial Report
GBT	Global Blood Therapeutics
GCP	Good Clinical Practice
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
GWAS	Genome-Wide Association Studies
HbS	Hemoglobin sickle
HbSS	Classic sickle cell disease
HIPAA	Health Insurance Portability and Accountability Act
IB	Investigator's Brochure
ICH	International Conference on Harmonization
ICMJE	International Committee of Medical Journal Editors
IDE	Investigational Device Exemption
IND	Investigational New Drug Application
IRB	Institutional Review Board
ISM	Independent Safety Monitor
ISO	International Organization for Standardization

ITT	Intention-To-Treat
LDH	Lactate Dehydrogenase
LSMEANS	Least-squares Means
MedDRA	Medical Dictionary for Regulatory Activities
MOP	Manual of Procedures
MSDS	Material Safety Data Sheet
NCT	National Clinical Trial
NIH	National Institutes of Health
NIH IC	NIH Institute or Center
NIRS	Near infrared spectroscopy
ODC	Oxygen Dissociation Curve
OEF	Oxygen extraction fraction
OHRP	Office for Human Research Protections
PI	Principal Investigator
QA	Quality Assurance
QC	Quality Control
Retic	Reticulocytes
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SCD	Sickle Cell Disease
SMC	Safety Monitoring Committee
SOA	Schedule of Activities
SOC	System Organ Class
SOP	Standard Operating Procedure
TCD	Transcranial Doppler
ULN	Upper limit of normal
UP	Unanticipated Problem
US	United States
VOC	Vaso-occlusive crisis

2. Objectives and Endpoints

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
Evaluate the cerebral hemodynamic effects of voxelotor	Change in CBF, OEF and CMRO2 from baseline at Week 4 and 12	Voxelotor is a promising new drug that improves hemoglobin levels and reduces the incidence of worsening anemia in SCD. However, it is unclear whether this increase in hemoglobin is associated with reductions in blood flow and oxygen extraction in the brain
Secondary		
Evaluate the relationship between CBF, OEF, CMRO2 and change in total Hb levels	Correlation of CBF, OEF, CMRO2 and change in total Hb levels from baseline	By increasing hemoglobin levels, voxelotor may show improvement in CBF, OEF, CMRO2 akin to the effects of blood transfusion but without the detrimental side effects.
Evaluate the relationship between CBF, OEF, CMRO2 and voxelotor-modified Hb levels	Correlation of CBF, OEF, CMRO2 and level of RBC content of voxelotor-modified Hb	Voxelotor modification of Hb correlate to improvement of anemia. Quantified level of Hb modification (via HPLC or PK) may correlate with improvement in CBF, OEF, CMRO2
Exploratory		
Evaluate the relationship between CBF, OEF, CMRO2 and measures of RBC health	Correlation of CBF, OEF, CMRO2 and change in markers of RBC hemolysis (LDH, indirect Bil) and morphology (blood smear, RBC indices, retic, dense cells, and adhesion) from baseline	Restored RBC health is measured by reduction in hemolysis (measured by blood chemistry labs) and improvement in RBC morphology. Measured improvements in RBC health may correlate with

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
		improvement in CBF, OEF, CMRO2

3. Background

Sickle cell disease (SCD) is a genetic blood disorder that has profound effects on the brain. In the presence of chronic anemia, the brain microvasculature dilates in order to maintain adequate oxygen delivery to the tissue[1–6]. As cerebral blood flow increases and the vasculature becomes maximally dilated, oxygen extraction fraction (OEF, defined as the difference between arterial and venous oxygen content) increases to keep up with metabolic demand [2,7–10]. Unfortunately, this state of maximized response leaves the brain vulnerable and ill-equipped to adapt to increased metabolic demand; consequently, the tissue is susceptible to infarction when blood flow/volume are insufficient to maintain sufficient oxygen metabolism[11] (see **Figure 1**).

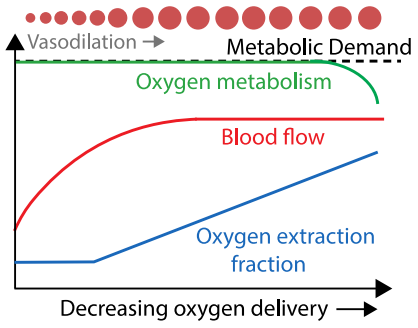


Figure 1, Hypothesized mechanism of ischemic injury in pediatric sickle cell disease (Adapted from Juttukonda et al., JMRI 2019). As anemia worsens, arterial oxygen delivery is reduced. To compensate for this reduction, the cerebrovascular dilates to increase cerebral blood flow (red). As vascular reserve is exhausted and CBF plateaus, oxygen extraction fraction (blue) increases to preserve oxygen metabolism (green). Eventually there is a mismatch between tissue metabolic demand (dotted black) and oxygen metabolism, and infarction ensues. Transfusion acts to increase oxygen delivery and is thought to relieve metabolic stress by reducing oxygen extraction and cerebral blood flow and improving vascular reserve. We hypothesize that the increase in hemoglobin caused by voxelotor will act in a similar fashion to reduce oxygen extraction fraction and blood flow.

Chronic transfusion is used in patients with sickle cell disease to increase arterial hemoglobin concentration, which improves oxygen carrying capacity to the tissue. Recent work with MRI has confirmed that improved oxygen carrying capacity in turn reduces elevated cerebral blood flow (CBF) and oxygen extraction[1,7,9,12]. Although cerebral oxygen metabolism appears to remain unchanged after transfusion, the return of oxygen extraction and blood flow to "normal" levels is thought to alleviate metabolic stress and improve the brain's ability to respond to increased metabolic demand[1]. Indeed, chronic transfusion therapy has been proven to reduce the incidence of both silent and overt infarcts in pediatric SCD patients[13,14]. Despite the beneficial effects of transfusion, the therapy is not without significant risks, i.e., infection, iron overload, alloimmunization, and comes with a substantial burden to patients and their families[15,16]. Moreover, the Silent Cerebral Infarct Multi-Center Clinical Trial (SIT) revealed that new silent infarct is prevented in only 1 out of every 13 children treated with transfusion therapy[13]. Thus, *alternative therapeutic strategies that improve oxygen delivery to the brain without the risks associated with transfusion are needed to alleviate metabolic stress and reduce the risk of stroke*.

Voxelotor (Oxbryta) is a new drug for patients with sickle cell disease that improves hemoglobin levels and reduces the incidence of worsening anemia[17]. However, it is unclear whether this increase in hemoglobin is associated with a reduction in cerebral metabolic stress. ***In this work, we will determine the effects of voxelotor on cerebral hemodynamics.*** We <u>*hypothesize*</u> that as hemoglobin increases due to voxelotor, cerebral blood flow and oxygen extraction fraction will decrease, similar to the effects of blood transfusion. To test this hypothesis, measurements of brain blood flow, oxygen extraction, and oxygen metabolism will be made using the Buckley Lab's customized diffuse correlation spectroscopy and frequency domain near-infrared spectroscopy system (DCS/FDNIRS). These well-validated, non-invasive optical techniques utilize near-infrared

light to penetrate deep into tissue (~1-2 cm) in order to estimate an average oxygen saturation of the microvasculature, i.e., capillaries, arterioles, and venules[18–21]. Our preliminary results with DCS/FDNIRS applied to children with sickle cell disease suggest that the techniques are sensitive to alterations in blood flow and oxygen extraction in these patients associated with changes in hemoglobin.

In a recently ancillary analysis of data from a Phase 2 Open Label Multicenter Trial (HOPE-KIDS 1; NCT02850406) voxelotor therapy in children with SCD was associated with improvements in RBC health as early as 12 weeks of treatment[25]. Compared with pretreatment measures, RBC deformability performed at fixed shear stress and at varying osmolality showed sustained improvement. Upon gradual deoxygenation, RBC deformability under shear stress continued to improve with voxelotor. Furthermore, RBC sickling began at a much lower partial pressure of oxygen (pO2) compared with pretreatment values, which confirmed that voxelotor prevents ex vivo sickling in humans. In addition, the leftward shifts in oxygen dissociation curves (partial pressure p20 and p50) confirmed increased Hb-oxygen affinity with voxelotor treatment[25]. ***In this work, we will explore how measurements of RBC health are associated with cerebral hemodynamics.*** We *hypothesize* that voxelotor modification of hemoglobin and improvements of RBC health will correlate with change in cerebral blood flow and oxygen extraction fraction. To test this hypothesis, measurements of RBC morphology, hemolysis, Hb modification, RBC deformability and adhesion will be made prior and during treatment period.

4. Study Intervention/Investigational Agent

Voxelotor is a promising new drug for patients with sickle cell disease that improves hemoglobin levels and reduces the incidence of worsening anemia. However, it is unclear whether this increase in hemoglobin is associated with a reduction in cerebral metabolic stress. In this work, we will determine the effects of voxelotor on cerebral hemodynamics.

4.1 Dosing and Administration

We plan to treat 50 patients in this study. All participants will receive voxelotor, administered orally once daily as tablets or dispersible tablets at a dose based on their body weight, for the duration of their enrollment in the study. Participants ≥ 12 years will be instructed to take a 1500mg dose of voxelotor consisting of three 500mg tablets once daily. Participants age 4-12 years will be instructed to take dispersible tablets once daily at a dose based on their body weight for the duration of treatment. The participant's weight at enrollment will be used to determine the starting voxelotor dose in this study. Voxelotor, in all formulations, may be taken with or without food. Voxelotor dispersible tablets should be dispersed in clear liquid (e.g., water, clear soda, apple juice, electrolyte drinks, flavored drinks, or sports drinks). Detailed instructions for study drug administration will be provided to participants and their caregivers/legal guardians, as needed.

Voxelotor drug product must be administered orally daily. If a participant misses a dose, the participant should resume normal dosing next day (i.e., the dose on the day after a day of a missed dose should not be increased or decreased).

For AEs that affect safety and tolerability, a trial of reducing or holding (temporarily stopping) the dose may be used. All instances of voxelotor dose modification (dose reduction, interruption, or discontinuation) should be documented in the participant's medical record. If the condition/event leading to the dose modification has resolved, the original dose level should be resumed, unless in the judgement of the Investigator this cannot be done safely.

4.2 Dose Justification

The recommended dosage of Voxelotor is 1500 mg taken orally daily, with or without food. The dose of voxelotor to be used in this study (weight-based 1500 mg equivalent) is based on clinical efficacy and safety results from voxelotor clinical studies in adult and adolescent participants combined with Pop PK modeling, physiologically based PK (PBPK) modeling, and allometric scaling. Dosing for participants <12 years of age will be weight based, as presented in **Table 1**, and is anticipated to provide exposures similar to adolescent participants receiving voxelotor 1500 mg. These doses are based on Pop PK and, PBPK modeling and allometric scaling and are expected to provide exposures of voxelotor equivalent to exposures observed in adults (Study GBT440-031) and adolescents (Studies GBT440-007 and GBT440-031)[26,27]

Table 1: Voxelotor Weight-based Doses for Participants Less than 12 Years of Age

Population	Voxelotor Doses (1500 mg Equivalent)
10 to <20 kg	600 mg
20 to <40 kg	900 mg
≥40 kg	1500 mg

Participants will receive voxelotor administered orally daily as tablets (300 or 500mg) or dispersible tablets (100 mg and 300 mg each). All participants younger than 12 years of age will receive a dose based on their body weight to provide exposure corresponding to the adult dose of 1500 mg/day. Participants 12 years or older will take 1500 mg/day.

4.3 Acquisition and Accountability

All study drug packaging must be returned at each visit, regardless of whether they are empty or contain unused study drug (voxelotor). Unused tablets will be counted and recorded. Dates of receipt, dispense, return, lot numbers, and tablet counts will all be recorded in a log.

In accordance with GCP, the investigational site will account for all supplies of voxelotor drug product. Details of receipt, storage, assembly, and return will be recorded. The Investigator must ensure that all drug product supplies are kept in a secure locked area with access limited to those authorized by the Investigator.

The Sponsor Investigator (or designee) will maintain an accurate record of the receipt of voxelotor drug product as shipped by GBT, including, but not limited to, the date received, storage conditions/location, lot number, amount received, and the disposition of all voxelotor drug product.

Current dispensing records will also be maintained including the date and amount of voxelotor drug product dispensed and the participant receiving the drug product. This drug accountability record will be available for inspection at any time.

All unused supplies of voxelotor drug product will either be destroyed by the site or returned to the shipping depot at the end of the study.

4.4 Formulation, Appearance, Packaging, and Labeling

Voxelotor will be supplied as 300 or 500 mg tablets or as 100 mg and 300 mg dispersible tablets. Voxelotor dispersible tablets will be supplied in HDPE bottles with induction sealed polypropylene child-resistant caps.

Voxelotor tablets will be supplied in high-density polyethylene (HDPE) bottles with induction-sealed polypropylene child-resistant caps. Bottles and/or cartons will be labeled according to applicable regulations.

4.5 Product Storage and Stability

Voxelotor will be stored at controlled room temperature between 15 °C and 25°C (59°F to 77°F) in a secure, temperature-controlled and locked environment with restricted access at the Sponsor-Investigator's site. No special procedures for the safe handling of voxelotor are required.

4.6 Preparation

Voxelotor is a tablet ready to be swallowed. Voxelotor dispersible tablets should be dispersed in clear liquid (e.g., water, clear soda, apple juice, electrolyte drinks, flavored drinks, or sports drinks). Detailed instructions for study drug administration will be provided to participants and their caregivers/legal guardians, as needed.

4.7 Study Intervention Compliance

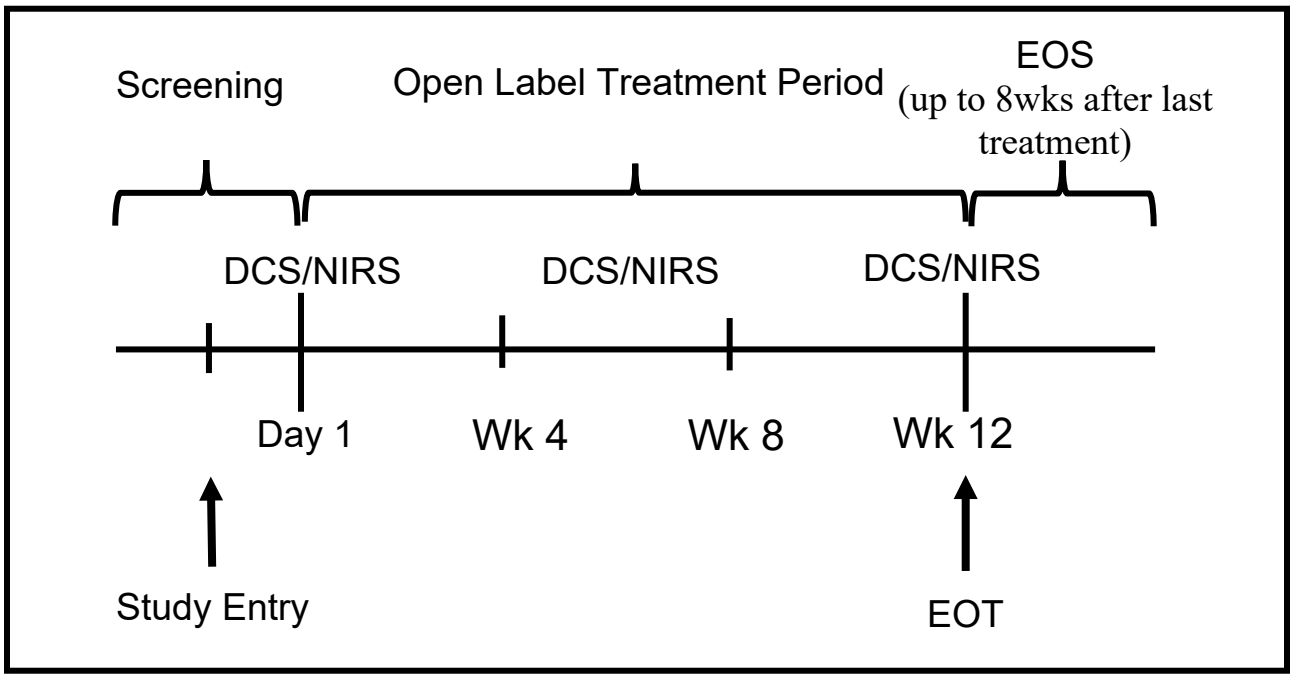
Study drug compliance will be assessed by counting of unused tablets or stick packs. In addition, medication compliance will be reported by the participant and caregiver.

4.8 Concomitant Therapy

A concomitant medication is defined as any prescription or over-the-counter preparation, including vitamins and supplements. All concomitant medications will be recorded in the participants medical record and the study case report form at study visits. Participants will be asked always to check with the PI or clinical study staff before taking a new medication. Prohibited medications include sensitive CYP3A4 substrates with a narrow therapeutic index and strong CYP3A4 inhibitors.

5. Procedures Involved

5.1 Study Schema



Study Population, N=50

- Age 4-30 years
- HbSS, S/β^0 thalassemia
- Stable HU permitted
- No transfusion in prior 8 wks

Study Outcomes

- 1^0: Change in CBF, OEF, $CMRO_2$ from baseline at 12 Weeks
- 2^0: Correlation between CBF, OEF and change in total Hb and Hb modification
- Exploratory: Correlation between CBF, OEF, $CMRO_2$ and change in measures of RBC health

5.2 Schedule of Assessments (SoA)

FDNIRS/DCS and TCD measurements will be made on D1, or within the screening period depending on researcher availability. For the 4w, 8w, and 12w and end of study visits, the time point given spans a window of +/- 7 days. For patients continuing commercial voxelotor after 12w, the end of treatment (12w) visit will also serve as the end of study visit. Patients who will not continue commercial voxelotor after 12w will complete an end of study visit up to 8 weeks after last study dose.

	Screening (up to 28 days)	Treatment				End of Study (up to 8 wks post-treatment)
		D1 (+/- 7d)	4w (+/- 7d)	8w (+/- 7d)	12w (+/- 7d)	
Consent/Assent	X					
Inclusion/Exclusion Criteria	X					
Medical History & PE	X	X			X	
Height, weight	X				X	X
Vital signs, pulse oximetry	X	X	X	X	X	X
CBC diff, retic	X	X	X	X	X	X
CMP & LDH	X				X	X
Hb Electrophoresis	X				X	
Hb morphology	X	X	X	X	X	X
RBC Health		X			X	
FDNIRS/DCS (performed by Buckley Lab staff)		X	X		X	
Transcranial Doppler ultrasound (TCD)	(X)	X			X	
Pregnancy testing (for females of child bearing potential)	X					
Adverse event reporting	X	X	X	X	X	X
Drug dispensing		X	X	X	X	

5.3 Efficacy, Safety, and Other Assessments

Screening will occur up to 28 days prior to the start of the study. The subject's medical chart will be used for screening and for part of collection of trial data, per Health Insurance Portability and Accountability Act (HIPAA) rules, other relevant federal or state laws, and local institutional requirements, as applicable. Information obtained from the medical chart will include:

a) List of current medications and their doses
b) Vital signs

c) Transfusion history
d) Stroke history
e) Previous MRI and/or MRA's
f) Previous sleep studies
g) Previous transcranial Doppler ultrasound results.

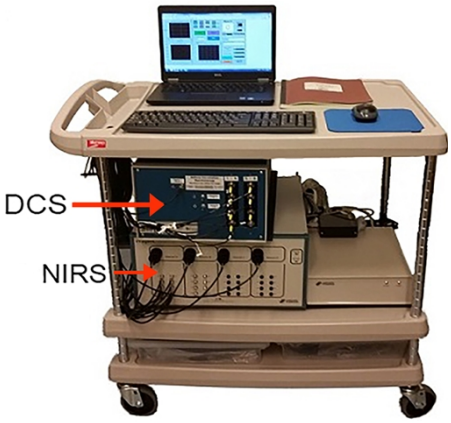


Figure 2, Picture of portable NIRS/DCS instrumentation. The cart takes up a footprint of approximately 1.5ft x 3ft.

The study procedures and evaluations to be done as part of the study to support the determination of efficacy, as per the primary and secondary objectives outlined in this protocol, will include:

- **DCS/FDNIRS assessments**. Measurements of cerebral blood flow, oxygen extraction, and oxygen metabolism will be made by a trained study staff personnel using a customized diffuse correlation spectroscopy combined with frequency domain near infrared spectroscopy oximeter (Figure 2). A DCS/FDNIRS optical sensor will be manually held over the right and left forehead to obtain a brief (<1min) resting state measure of brain hemodynamics.

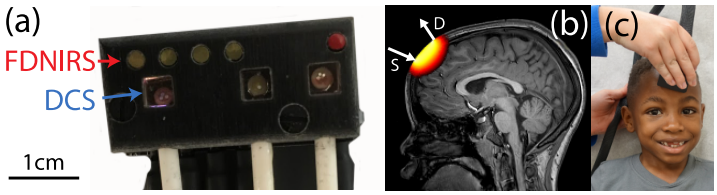


Figure 3, (a) Non-invasive FDNIRS/DCS sensor comprised of fiber optics embedded in a 3D printed holder. The top row of fibers are for FDNIRS and the bottom row are for DCS (b) Depth sensitivity of FDNIRS/DCS measurements in a representative 10 year old child. (c) Patient measurement with a similar hand-held FDNIRS/DCS sensor.

  Measurements will be repeated up to 3 times per hemisphere (Figure 3). Total time of exam is typically 15-30 minutes. Measurements will be made before, and at 4 and 12 weeks after the start of voxelotor. Measurements will be obtained in quiet area of Aflac outpatient clinics using PPE and hand hygiene practices according to hospital policy.
- A research blood specimen will be obtained when a clinical sample is unavailable. Up to sixteen mLs of venous blood will be collected at each visit. Laboratory assessments relevant to measures predictive of voxelotor response and to measures of RBC Health will be performed at local (central CHOA lab) clinical labs. Local laboratory assessments will include complete blood count, reticulocyte count, hemoglobin electrophoresis, RBC dense cells and measures of RBC hemolysis (LDH, indirect bilirubin). RBC health laboratory assessments will include but are not limited to RBC adhesion.
- **Procedures that will be completed during the study as part of regular standard of clinical care**: These include physical examination, vital sign assessment (heart rate, blood pressure, pulse oximetry, etc.), medical history.
- **Transcranial Doppler ultrasound** assessment of blood flow velocity in the main feeding arteries. Measurements will follow standard clinical protocols that are outlined routine screening in children with sickle cell disease. Baseline TCD assessment may occur at screening or at D+1 visit prior to study medication.

5.4 Adverse Events and Serious Adverse Events

Definition of Adverse Events (AE)

Adverse event means any untoward medical occurrence associated with the use of Voxelotor or NIRS/DCS measurements of cerebral hemodynamics, whether or not considered intervention-related (21 CFR 312.32 (a)).

Definition of Serious Adverse Events (SAE)

An adverse event (AE) or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes: death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Classification of an AE (Severity of Event)

For adverse events (AEs), guidelines will be used to describe severity.

- **Mild** – Events require minimal or no treatment and do not interfere with the participant's daily activities.
- **Moderate** – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- **Severe** – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating. Of note, the term "severe" does not necessarily equate to "serious".

Classification of an AE (Relationship to Study Intervention)

All adverse events (AEs) will be assessed for relationship to study intervention as the following:

Definitely Related – There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to taking voxelotor and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the study intervention (dechallenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory rechallenge procedure if necessary.

Probably Related – There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal

laboratory test result, occurs within a reasonable time after taking voxelotor, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfill this definition.

Potentially Related – There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after taking voxelotor). However, other factors may have contributed to the event (e.g., the participant's clinical condition, other concomitant events). Although an AE may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or "definitely related", as appropriate.

Unlikely to be related – A clinical event, including an abnormal laboratory test result, whose temporal relationship to voxelotor makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after taking voxelotor) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the participant's clinical condition, other concomitant treatments).

Not Related – The AE is completely independent of voxelotor, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.

Classification of an AE (Expectedness) *additional information can be found in Attachment A at the end of this document

The PI will be responsible for determining whether an adverse event (AE) is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study intervention.

Expected AEs include the following: headache, diarrhea, abdominal pain, nausea, fatigue, rash, and pyrexia.

5.5 Time Period and Frequency for Event Assessment and Follow-up

The occurrence of an adverse event (AE) or serious adverse event (SAE) may come to the attention of study personnel during study visits and interviews of a study participant presenting for medical care, or upon review by the study PI.

All AEs including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate study database form. Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

The PI or study coordinator will record all reportable events with start dates occurring any time after informed consent is obtained until 7 (for non-serious AEs) or 30 days (for SAEs) after the last day of study participation. At each study visit, the investigator will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization.

5.6 Adverse Event Reporting

AEs will be reported to Pfizer at regular intervals as required by Pfizer. SAEs will be reported to Pfizer within 24 hours after the investigator learns of the event and to the IRB according to IRB Policies & Procedures. The Sponsor-Investigator will be responsible for notifying the FDA of any unexpected fatal or life-threatening suspected SAE as soon as possible, but in no case later than 7 calendar days after Sponsor-Investigator's initial receipt of the information.

Expected AEs will be recorded in CRFs.

5.7 Serious Adverse Event Reporting

The study principal investigator will immediately report to Emory IRB according to their Policies & Procedures as well as to Pfizer any serious adverse event, whether or not considered study intervention related, including those listed in the protocol or investigator brochure and must include an assessment of whether there is a reasonable possibility that the study intervention caused the event within 24 hours of finding out about the event. Study endpoints that are serious adverse events (e.g., all-cause mortality) must be reported in accordance with the protocol unless there is evidence suggesting a causal relationship between the study intervention and the event (e.g., death from anaphylaxis). In that case, the investigator must immediately report the event to the IRB and Pfizer.

All serious adverse events (SAEs) will be followed until satisfactory resolution or until the PI deems the event to be chronic or the participant is stable. Other supporting documentation of the event may be requested by Pfizer and should be provided as soon as possible.

The Sponsor-Investigator will be responsible for notifying the Food and Drug Administration (FDA) of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible, but in no case later than 7 calendar days after the initial receipt of the information from the study principal investigator. The Sponsor-Investigator or Designee must notify the FDA and all participating investigators in an Investigational New Drug (IND) safety report of potential serious

risks, from clinical trials or any other source, as soon as possible, but in no case later than 15 calendar days after the Sponsor-Investigator determines that the information qualifies for reporting. In addition, the Sponsor-Investigator is responsible for notifying Pfizer of the FDA reported safety event and provide all documentation submitted to the FDA, including the safety event narrative.

5.8 Reporting of Pregnancy

In the event of pregnancy that occurs during study, the pregnancy will be reported to GBT Pharmacovigilance or Designee and the decision for the participant to continue on study or not will depend on the specific situation and will be discussed with GBT Pharmacovigilance or Designee. Pregnancies should be monitored for the full duration of pregnancy and/or followed through to a definitive outcome. The outcome of a pregnancy event will be reported to GBT Pharmacovigilance or Designee.

5.9 Unanticipated Problems

Definition of Unanticipated Problems (UP)

The Office for Human Research Protections (OHRP) considers unanticipated problems involving risks to participants or others to include, in general, any incident, experience, or outcome that meets **all** of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the Institutional Review Board (IRB)-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied;
- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

This definition could include an unanticipated adverse device effect, any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects (21 CFR 812.3(s)).

Unanticipated Problems Reporting

The investigator will report unanticipated problems (UPs) to the reviewing Institutional Review Board (IRB) and to the principal investigator (PI). The UP report will include the following information:

- Protocol identifying information: protocol title and number, PI's name, and the IRB project number;
- A detailed description of the event, incident, experience, or outcome;
- An explanation of the basis for determining that the event, incident, experience, or outcome represents an UP;
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UP.

To satisfy the requirement for prompt reporting, UPs will be reported using the following timeline:

- UPs that are serious adverse events (SAEs) will be reported to the IRB and to GBT Pharmacovigilance or Designee within 24 hours of the investigator becoming aware of the event.
- Any other UP will be reported to the IRB and to GBT Pharmacovigilance or Designee within 10 days of the investigator becoming aware of the problem.
- All UPs should be reported to appropriate institutional officials (as required by an institution's written reporting procedures), the supporting agency head (or designee), and the Office for Human Research Protections (OHRP) within 10 days of the IRB's receipt of the report of the problem from the investigator.

5.10 End of Study

A participant is considered to have completed the study if he or she has completed all phases of the study including the last visit or the last scheduled procedure shown in the Schedule of Activities (SoA). For patients continuing commercial Voxelotor after 12w, the end of treatment (12w) visit will also serve as the end of study visit. Patients who will not continue commercial Voxelotor after 12w will complete an end of study visit up to 8 weeks after last study dose.

6. Data and Specimen Banking

Data obtained during this research may be stored for future use. Data will be stored indefinitely on password protected computers on servers managed by Children's Healthcare of Atlanta/Emory University and only accessible be authorized personnel. Paper data is stored in a locked cabinet in a locked office. Only study personnel have access to input data into electronic database. Data collected and stored will include, but not limited to:

- demographic data
- data collected from surveys
- medical treatment data

Data will be maintained for the duration of the study and thereafter as required by funding sources.

Deidentified data from this study may be requested from other researchers after the completion of the primary endpoint by contacting the Principal Investigator and presenting appropriate regulatory approvals.

No biological specimens will be retained, and no genetic material will be collected in this study.

7. Sharing of Results with Participants

In the event that medically significant and clinically actionable results are found, this information will be shared with providers. Results would be provided to the subject's physician who will discuss with the patient or legal guardian and appropriate treatment, repeat testing, genetic counseling or follow-up testing would be discussed and decided locally on a case by-case basis.

8. Study Timelines

The duration of an individual participant's participation in the study is approximately 16 weeks. We anticipated enrolling about 5-8 subjects per month, with 9 month expected duration to enroll all study participants. The estimated date for the investigators to complete this study is 28 months.

9. Inclusion and Exclusion Criteria

9.1 Inclusion Criteria

Subjects will be recruited from the CHOA Aflac Sickle Cell Comprehensive Clinics located at the Scottish Rite campus and the Egleston campus.

In order to be eligible to participate in this study, an individual must meet all of the following criteria:

1. Written informed parental/guardian consent and participant assent for patients < 18y or written informed consent for patients > 18y has been obtained per institutional review board (IRB)/Ethics Committee (EC) policy and requirements, consistent with ICH guidelines.
2. Stated willingness to comply with all study procedures and availability for the duration of the study
3. Male or female participants, ages 4 to 30 years, inclusive
4. Homozygous hemoglobin SS (HbSS) or hemoglobin S/beta^0 thalassemia (HbS/β^0 thal
5. Hemoglobin (Hb): Hb ≤10.5 g/dL at baseline
6. Concomitant hydroxyurea (HU) therapy is allowed if the dose has been stable for at least 3 months with no anticipated need for dose adjustments during the study and no sign of hematological toxicity.
7. Ability to take oral medication and willingness to adhere to daily voxelotor and scheduled DCS/NIRS assessments.

8. If sexually active and female, must agree to abstain from sexual intercourse or to use a highly effective method of contraception throughout the study period and for 30 days after discontinuation of study drug. If sexually active and male, must agree to abstain from sexual intercourse or willing to use barrier methods of contraception throughout the study period and for 30 days after discontinuation of study drug.
9. Females of child-bearing potential, i.e., who have begun menstruation and are sexually active, are required to have a negative pregnancy test at screening before the initial administration of study drug.

9.2 Exclusion Criteria

An individual who meets any of the following criteria will be ineligible for participation in this study:

1. Any one of the following requiring medical attention within 14 days prior to signing the informed consent form (ICF):
    - Vaso-occlusive crisis (VOC)
    - Acute chest syndrome (ACS)
    - Splenic sequestration crisis
    - Dactylitis
2. Requires chronic transfusion therapy
3. RBC transfusion within 60 days of signing the ICF
4. Renal dysfunction requiring chronic dialysis or creatinine ≥1.5 mg/dL.
5. Hepatic dysfunction characterized by alanine aminotransferase (ALT) >4× upper limit of normal (ULN) for age.
6. Clinically relevant cardiac abnormality, in the opinion of the Investigator, such as:
    - Hemodynamically significant heart disease, e.g., congenital heart defect, uncompensated heart failure, or any unstable cardiac condition
    - An arrhythmic heart condition requiring medical therapy
7. QTcF >450 msec, congenital long QT syndrome, second- or third-degree heart block at rest (with the exception of asymptomatic Mobitz type I second degree heart block).
8. Received an investigational drug within 30 days or 5 half-lives, whichever is longer, of signing the ICF.
9. Heavy smoker (defined as smoking more than 10 cigarettes/day or its nicotine equivalent including e-cigarettes).
10. Unlikely to comply with the study procedures.
11. Other medical, psychological, or addictive condition that, in the opinion of the Investigator, would confound or interfere with evaluation of safety and/or PK of the investigational drug, prevent compliance with the study protocol, or preclude informed consent.
12. Any condition affecting drug absorption, such as major surgery involving the stomach or small intestine (prior cholecystectomy is acceptable).

13. History of malignancy within the past 2 years prior to treatment Day 1 requiring chemotherapy and/or radiation (with the exception of local therapy for non-melanoma skin malignancy).
14. Clinically significant bacterial, fungal, parasitic, or viral infection currently receiving or that will require therapy.
    - Participants with acute bacterial infection requiring antibiotic use should delay screening until the course of antibiotic therapy has been completed and the infection has resolved, in the opinion of the investigator.
    - Known active hepatitis A, B, or C infection or human immunodeficiency virus (HIV)-positive; known active
    - Known active malaria.
15. Pregnant patients
16. Evidence of abnormal high blood flow velocities on TCD of 200 cm/sec or more

9.3 Lifestyle Considerations

During this study, participants are asked to:

- Refrain from using herbal medications (e.g., St. John's Wort), or medications that are sensitive cytochrome CYP3A4 substrates or CYP3A4 modulators, such as fluconazole.

10. Vulnerable Populations

There are no restrictions in the inclusion or exclusion criteria in regard to race, ethnicity, or gender. However, SCD is more common in certain ethnic groups including individuals of African descent such as African Americans and Hispanic-Americans from Central and South America and individuals of Middle Eastern, Asian, Indian, and Mediterranean descent. Thus, we anticipate that our cohort will be reflective of this.

This research includes individuals who are not yet adults. Safeguards are in place to protect their rights and welfare. This study research is consistent with the Declaration of Helsinki in that the research is necessary to promote the health of children with sickle cell disease.

Children will not be enrolled without the informed consent of parents and the assent (or consent) of the child per IRB/REB/local ethics committee policies and guidelines.

Declining to participate in the research will not affect their care.

11. Local Number of Participants

Approximately 50 subjects are expected to be accrued in this research. The anticipated accrual rate is 5-8 subjects per month.

12. Recruitment Methods

12.1 Subject Identification

Potential participants will be recruited from outpatient sickle cell clinic at Children's Healthcare of Atlanta. Providers, including physicians, advanced practice providers (APPs-nurse practitioners

or physician assistants), or genetic counselors will identify potential participants based on their diagnoses at the time of their scheduled clinical visits.

Patients under the care of providers at Children's Healthcare of Atlanta (CHOA) sites who have sickle cell disease may be approached for enrollment at any time after being diagnosed.

We plan to enroll children with sickle cell disease with any mix of male or female gender, ranging in age from 4 to 30 years.

IRB approved study team members will identify potential candidates through medical record review. Patients and families of the Children's Healthcare of Atlanta inpatient and outpatient sickle cell disease clinics will be contacted by a study team member prior to their scheduled appointment. Patients and families may be approached in the clinic or via a phone call.

12.2 Screen Failures

Individuals who do not meet the criteria for participation in this trial (screen failure) because recent vaso-occlusive crisis (VOC), acute chest syndrome (ACS), splenic sequestration crisis, or dactylitis may be rescreened. Rescreened participants should be assigned the same participant number as for the initial screening.

Individuals who do not meet the criteria for participation in this study (screen failure) because hydroxyurea dose or hemoglobin had not been stable for 3 months may be rescreened. Rescreened participants should be assigned the same participant number as for the initial screening.

Screen failures are defined as participants who consent to participate in the clinical trial but are not entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

13. Withdrawal of Participants

13.1 Discontinuation of Study Intervention

At any point in the study, the child or parents can withdraw from the study. Discontinuation from voxelotor treatment will end participation in the study.

13.2 Participant Discontinuation/Withdrawal from the Study

Participants are free to withdraw from participation in the study at any time upon request.
An investigator may discontinue or withdraw a participant from the study for the following reasons:

- Pregnancy
- Significant study intervention non-compliance
- If any clinical adverse event (AE), such as hypersensitivity to the study drug, laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant
- If the participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation
- Disease progression which requires discontinuation of the study intervention

The reason for participant discontinuation or withdrawal from the study will be recorded on the Case Report Form (CRF). Subjects who sign the informed consent form and subsequently withdraw, or are withdrawn or discontinued from the study, will not be replaced.

Once dosed, participants who discontinue/withdraw the study will not be replaced. We estimate 40 subjects will be sufficient to achieve the primary, secondary, and exploratory endpoints. Our target sample size of 50 allows for approximately 20% drop out rate. This dropout rate far exceeds the actual dropout rate in the large multicenter Phase 2a study of voxelotor use in pediatrics (GBT440-007).

13.3 Lost to Follow-Up

A participant will be considered lost to follow-up if he or she fails to return for scheduled visits and is unable to be contacted by the study site staff.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant and reschedule the missed visit within 1-2 weeks and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record or study file.
- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

14. Risks to Participants

Treatment with this study drug may also involve risks that we currently don't know about.

As of November 2019, commercial Oxbryta (voxelotor) was approved for treatment of sickle cell disease in adults and pediatrics 12 years and older. In December 2021, the indication was modified to include pediatrics 4 years and older.

The most common adverse reactions (incidence >10%) are headache, diarrhea, abdominal pain, nausea, fatigue, rash, and pyrexia. Other reported side effects include symptoms like a common cold (upper respiratory tract infection), pain (including pain in joints, stomach, chest, and back) and vomiting.

All of these side effects were also reported by people who took placebo (a sugar pill, containing no voxelotor). Adults and children had sickle cell pain crises during previous and current studies with voxelotor, but they occurred as often as in people who received placebo (sugar pill, containing no voxelotor); most of these events were thought to be related to SCD. Acute chest syndrome and priapism also occurred on other studies with voxelotor – these events were less common and are known to be related to SCD. Overall, the sides effects observed in adults and children were similar.

Uncommon side effects (occurring in 0.1 % to 1% of people; between 1 and 10 in a thousand people): Allergic reactions that the study doctors thought were caused by voxelotor were also reported in one person with SCD. Symptoms of this allergic reaction included face swelling and fever. Symptoms went away after the study drug was stopped.

Unknown risks: It is possible that your SCD symptoms will not improve during the study or may even worsen. Treatment with this study drug may also involve risks to your future health that we currently don't know about.

Interaction with other medications: The effects of the study drug may change when taken with other drugs. Among these are carbamazepine, phenytoin, rifampin and St. John's Wort

Blood draw: The risks of drawing blood from a vein include fainting and pain, bruising, swelling, or rarely infection at the needle site. These discomforts are brief and transient.

DCS/NIRS test: The light used in the DCS/NIRS sensor delivers less power than the energy your child would receive from the sun on an outdoor walk on a sunny day. This energy is also below the American National Standards Institute (ANSI) light levels and is in compliance with the United States Food and Drug Administration (FDA). See **Appendix** for more information. Children with sensitive skin might have minor temporary skin irritation from contact with the optical sensor on the forehead.

15. Potential Benefits to Participants

Voxelotor is being offered as a treatment for your SCD for the reasons previously indicated. If it is effective, symptoms of SCD may improve, however participants may or may not receive any benefit from being in this Study. It is possible that your symptoms may get better, stay the same or get worse. This study is designed to learn more about the effects of a novel treatment for sickle cell disease (voxelotor) on the brain. We hope that the study results will be used to help others in the future.

16. Compensation to Participants

Subjects will be compensated for their time. A $50.00 gift card will be given to the subject after completion of each measurement session, for a total up to $250.00 for the completion of all study measures.

17. Statistical Considerations, Data Management and Confidentiality

17.1 Statistical Considerations

Statistical Hypothesis

- Primary Endpoint: CBF and OEF are significantly lower at 4 and 12 weeks of treatment with voxelotor compared to pre-treatment levels. Paired t-test or Wilcoxon signed-rank test will be used to evaluate results, as patients are their own controls.

- Secondary Endpoints: The change in OEF and CBF from pre-treatment levels are significantly correlated with the change in hemoglobin. The change in OEF and CBF from pre-treatment levels are correlated with markers of hemolysis.

Sample Size Determination

This is a pilot study involving 50 participants to define the magnitude of change. Power cannot be calculated at this time.

Populations for Analyses

There will be one dataset for this pilot study of 50 participants.

17.2 Statistical Analyses

General Approach

As the primary objective of this study is to obtain clinical and laboratory information to justify a larger clinical efficacy trial of voxelotor in SCA patients, the results of this study will be descriptive.

Analysis of the Primary Efficacy Endpoint

To test our hypothesis that voxelotor reduces oxygen extraction and blood flow, we will compare changes in the DCS/FDNIRS-measured outcome variables (CBF, OEF, $CMRO_2$) from baseline levels using one-sample paired t-test or Wilcoxon signed-rank test, as appropriate. Measures will be adjusted for the increase in hemoglobin observed (i.e., relative change in OEF per g/dL change in hemoglobin).

Analysis of the Secondary/Exploratory Endpoints

Linear mixed-effects regression models with random-intercept at the patient level will be used to examine the association of each outcome measure (OEF, CBF, and CMRO2) with demographical (sex and age) and laboratory data (Hb, reticulocyte count, bilirubin, LDH, Hb occupancy, oxygen dissociation curves, RBC deformability and

mitochondrial content). These models will be two-level and will account for repeated measures (0, 4, and 12 week measurements) nested within the same patient.

Safety Analysis

AEs will be coded by MedDRA terminology. Each AE will be counted once for a given participant. Severity, relationship to study drug, expectedness, dates, outcome, and duration of AEs will be reported.

Baseline Descriptive Statistics

Due to the anticipated small number of participants descriptive statistics will be used primarily to present the chart-extracted data. Summary of patient characteristics such as age, prior sickle cell complications, disease-modifying therapy, laboratory and radiology findings and outcomes will be presented. Descriptive analyses will involve calculation of proportions (frequencies) for categorical data. For continuous variables, mean and standard deviation (normally distributed data) or median and interquartile ranges (not normally distributed data) will be presented. Chi Square (or Fisher's Exact test as applicable) will be used to compare categorical variables presented as proportions of total. Student's t-test or one-way ANOVA will be used to compare continuous parametric variables and non-parametric variables, respectively. Aid in statistical analysis, if indicated, will be sought via the Emory Biostatistics Core.

Tabulation of Individual Participant Data

Individual participant data will be listed by measure and time point.

17.3 Data Management, Quality Assurance and Quality Control

Data Collection and Management Responsibilities

Data collection is the responsibility of the study team under the supervision of the principal investigator. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

All source documents will be completed in a neat, legible manner to ensure accurate interpretation of data.

Hardcopies of the study visit worksheets will be provided for use as source document worksheets for recording data for each participant enrolled in the study. Data recorded in the electronic case report form (eCRF) derived from source documents should be consistent with the data recorded on the source documents. Data will be recorded in password-protected database accessible only by PI and the study personnel. Any paper source documents will be stored in a locked cabinet in the Investigator's office.

Quality Assurance and Quality Control

Study staff are trained extensively on the operation of the DCS/FDNIRS device prior to performing measurements. Documentation regarding device settings, sensor settings, and sensor positioning is completed at each measurement session. Periodic performance testing of the DCS/FDNIRS device is completed routinely with calibration standards.

The clinical site will perform internal quality management of study conduct, data collection, documentation and completion. The PI will check the quality and accuracy of data entry. The research lab conducts quality control on all its instrumentation and lab protocols.

Summarized data will be provided to GBT in study progress reports.

17.4 Study Record Retention

There is no set time for destroying information collected for this study. As this is an investigational, pilot study, we may use the data for future studies, and we also may need to access additional clinical information of the patient in the future as we delve deeper into the data and discover new aspects of the relationship between cerebral hemodynamics and clinical condition to explore.

17.5 Protocol Deviations

It is the responsibility of the Principal Investigator to use continuous vigilance to identify and report deviations to Emory IRB within 10 working days of identification of the protocol deviation, or within 10 working days of the scheduled protocol-required activity. All deviations must be addressed in study source documents, reported to the Emory University IRB and included in the study progress reports submitted to GBT. Protocol deviations must be sent to the reviewing Institutional Review Board (IRB) per their policies. The site investigator is responsible for knowing and adhering to the reviewing IRB requirements.

18. Provisions to Monitor the Data to Ensure the Safety of Participants

Internal monitoring of the trial for protocol compliance and data accuracy will be the responsibility of the Research Administration QA/QI Analyst. The trial will be monitored for completion of the informed consent forms, clinical data capture forms, IP dispense logs and treatment-related adverse events not related to SCD. In addition, the credentials, delegation of authority and training logs will be monitored for study member. The monitoring will occur according to the following schedule:

- At least every six months while participants are receiving intervention and
- Annually while participants are in follow-up
- Close-Out Study Visit

Additional monitoring may be performed in the event of Serious Adverse Events if deemed necessary by the monitoring committee or if requested by the Principal Investigator.

This study will be conducted in compliance with the Aflac Cancer & Blood Disorders Center Data Safety Monitoring Board (DSMB) for Phase 1 and 2 pediatric studies. In brief, the role of the Data Safety Monitoring Board (DSMB) is to protect the interests of subjects and the scientific integrity for all Phase 1 and 2 studies. The DSMB consists of 7 members including a chair, a statistician, a pharmacist, and at least one external member.

The DSMB meets on a quarterly basis to review current study results, as well as data available to the DSMB from other related studies. The DSMB will provide recommendations for each study reviewed to change the study or to continue the study unchanged and will determine the frequency of future meetings.

Data and Safety Board reports for Institutional Review Boards will be prepared. The Principal Investigator assumes responsibility for assuring that the study is carried out in accordance with the DSMP.

DSMP Requirement	How this Requirement is Met	Frequency	Responsible Party(ies)
Site Monitoring at pre-determined intervals: The Principal Investigator has a responsibility to ensure that the study is following all aspects of the protocol.	*There should be a standard operating procedure to review data (whether a sample or 100%) at pre-determined intervals to ensure that there is adequate documentation of critical elements such as eligibility criteria. Monitoring is required at the following timepoints (but may be done more frequently):* • *study initiation* • *at least every six months while participants are receiving intervention and* • *annually while participants are in follow-up*	*At a minimum, a review is required annually when no one has been enrolled or the study is in long term follow up. Additional interim monitoring at least once every 12-24 weeks based on the site activity, and more as needed, to include the possibility of remote monitoring.*	*Delegate a responsible party for each requirement below*. Self-assessment is NOT acceptable. An experienced, knowledgeable person* ***who is independent of the study team*** *should serve as monitor. A Contract Research Organization (CRO) may be used. Consult the IRB Office regarding acceptable qualifications for the independent monitor, if not using an outside expert such as a CRO.*
Real-time review of participant data during initial data collection.	Inclusion/Exclusion Checklist is completed by the CRC and reviewed by the PI for approval and signoff. The CRC will also signoff	This is completed when study subjects enroll	Study team and PI
100% review of regulatory files	Regulatory binder will be created to house all Regulatory documents for review. Regulatory files will be reviewed by monitor at monitoring visits.	Start of study, close out and as necessary in between	Team coordinators, QA/QI Analyst

100% review of consent forms	Per Aflac policy, all consents are reviewed in real time by 2 separate coordinators. The first review is within 5 days of the consent. The next review is within 5 days of the first review but within 10 days of the consent date. Consents will be reviewed by monitor at monitoring visits.	As consents are signed	Team coordinators QA/QI Analyst
Review of credentials, training records, the delegation of responsibility logs (if applicable)	Study training logs are kept in the Regulatory Binder for review with the upkeep of the coordinators. This information will be reviewed by monitor at monitoring visits.	Reviewed every time there are study team changes as well as study amendments	Team coordinators QA/QI Analyst
Comparison of case report forms (CRF) to source documentation for accuracy and completion	Data will be stored on Erin Buckley's lab's server. The assigned monitor will compare data in the study database to the source data on the CRFs. Discrepant data and queries will be sent to the study team who will be required to respond to each item. CRF documentation will be reviewed by monitor at monitoring visits.	Yearly Review	Buckley Lab, CHOA QA/QI Analyst
Review of documentation of all adverse events	The PI will have oversight on the documentation of AE's which will be logged and tracked by the study Coordinator and kept in the subject's study chart. AE documentation will be reviewed by monitor at monitoring visits.	Every time an AE occurs the Study Coordinator will log it. Monitor will review at specified monitoring intervals.	PI, Team Coordinators, QA/QI Analyst
Monitoring of critical data points (eligibility, study endpoints, etc.)	Data Collection of critical data points is the responsibility of the study coordinators under the supervision of the principal investigator. The coordinators are responsible for ensuring accuracy, completeness, legibility, and timeliness of the data reported. The monitor will	Eligibility will be confirmed at time of patient enrollment. Study endpoints will be reviewed at end of each treatment period.	Principal Investigator, Team Coordinators, and QA/QI Analyst

	review critical data points at monitoring visits. The PI will have oversight over all data collected for the study and will review all monitoring reports upon the completion of a monitoring visit.		
Laboratory review of processing and storage of specimens	Coordinators will process labs. Labs are typically shipped same day of collection but can be stored on site as well.	If samples are stored, they are reviewed at the visits and close-out visit	Buckley Lab, Team Coordinators, QA/QI Analyst
Assessment of laboratory specimens stored locally	The specimens will be stored in Buckley lab (HSRB E169)	Yearly	Buckley Lab
Test article accountability review	Buckley lab is responsible for device maintenance and calibration. Calibration records will be reviewed by monitor at monitoring visits.	Calibration will be done prior to each measurement	Buckley Lab, QA/QI Analyst
Accountability logs, dispensing records, and other participant records	All IP and IP logs are monitored and housed with the Research Pharmacist within NCoup. IP logs will be reviewed by monitor at monitoring visits.	Will be monitored at least once monthly, more frequently if an active patient is on the study	Research Pharmacist, QA/QI Analyst
For FDA regulated studies, the following requirements apply:	**How this Requirement is Met**	**Timing, frequency, and intensity of monitoring**	**Responsible Party(ies)**
Monitoring methods (may include centralized, on-site, and self-monitoring)	Previously, CTAC did a review after the first 10 subjects and the results of that were provided in the comments of the CR already. The QA/QI Analyst will be monitoring this study going forward.	As per protocol	QA/QI Analyst
*For international studies, you are required to engage a CRO that is working in the site country and/or to consult with Emory's legal counsel regarding compliance with the country's clinical research regulations.			

19. Provisions to Protect the Privacy Interests of Participants

Eligible subjects will be identified by treating physicians, APPs, and/or genetic counselors with whom they already have a treating relationship. A description of the study will be provided to potential subjects by the provider or a study staff designee. The provider will be able to answer

any questions about participation and data to be collected and to ensure that participants' privacy interests are protected. Study interactions will be kept strictly confidential.

Data will be stored on a secure server maintained by CHOA/Emory University. All data will be de-identified and aggregated prior to being analyzed and published.

No personnel will be authorized to use study data or specimen for any other purpose other than what falls within the scope of the study. Only authorized personnel will have access to any identifying information. The only other groups that will have access to data and specimens are members of supervisory organizations, such as IRB, and then only as part of routine review or audit.

Any electronic records will be maintained in a de-identified manner in a locked, password-protected data file/database, and access to this will be limited to the research team, requiring both login ID and password. Study data will only be accessed from School of Medicine/ CHOA computers that are compliant with all SOM Data security policies and are both backed up and encrypted. Any email correspondence between study investigators and staff will be completed using emory.edu or choa.org accounts. If study materials need to be transmitted electronically, senders will send the email in an encrypted fashion. All research staff have received extensive training on storing and transmitting secure data.

19.1 Publication and Data Sharing Policy

This study will be conducted in accordance with the following publication and data sharing policies and regulations:

Any final peer-reviewed journal manuscripts that arise from this study will be submitted to the digital archive PubMed Central upon acceptance for publication.

This study will comply with the NIH Data Sharing Policy and Policy on the Dissemination of NIH-Funded Clinical Trial Information and the Clinical Trials Registration and Results Information Submission rule. As such, this trial will be registered at ClinicalTrials.gov, and results information from this trial will be submitted to ClinicalTrials.gov. In addition, every attempt will be made to publish results in peer-reviewed journals.

19.2 Conflict of Interest Policy

All study team members will disclose conflicts of interest, which will be managed in ways that prevents interference with proper conduct of the study.

20. Economic Burden to Participants

Participants and/or their families will be responsible for costs associated with fuel, parking, childcare, or any procedures considered standard of care. Data and samples for this study will be collected at scheduled research visits and/or provided electronically via the internet at their

convenience. While participants are responsible for their regular clinical care, they are not responsible for any costs associated with this study.

21. Consent Process

Informed Consent

1. Informed consent/assent will be obtained prior to the collection of research data. Assent and consent forms will be Institutional Review Board (IRB) approved, and assent and consent will be obtained per IRB policies and guidelines. The participant or parent/legal guardian may withdraw consent at any time throughout the course of the study. A signed copy of the informed consent document will be given to the participants' parent/legal guardian. By signing the informed consent form, the participant's parent/legal guardian agrees that the participant will be enrolled in the study until the participant is withdrawn voluntarily or for any reason.
2. For children under the age of majority or capacity (as locally defined} at the time of parental consent, once the child reaches the age of majority or capacity, informed consent must be obtained from that subject to allow 1) his/her name, other identifying information, data and specimens, to continue to be available to study investigators; 2) ongoing collection of research data; and 3) for future contact.
3. Informed consent will include:
    - o Permission to access and provide clinical data centrally for study-related research
    - o Permission to contact for future research
    - o Permission to contact for return of medically significant and actionable research results (optional)
    - o Permission to store de-identified data in a manner such that it can be efficiently shared with collaborators and other investigators.

Setting of Consent Discussion

Potential subjects identified by providers will be approached about the study by an authorized member of the study staff (e.g. Clinical Research Associate, Research Coordinator) at the time of a regularly scheduled clinic visit. The study will be described in detail to the Potential Subject/family through a conversation expected to take 15-30 minutes. To minimize the possibility of coercion, the voluntary nature of participation will be emphasized and no compensation for participation will be offered. The Potential Subjects/family will be given ample opportunity to ask questions for clarification and will be asked to describe their understanding of the study to ensure their understanding. If desired by the Potential Subject, they will be allowed to consider and discuss participation with other family and sign consent forms to be returned via mail or electronically.

Documentation of Informed Consent

Paper Assent and Consent forms will be IRB approved and serve as the primary documentation of informed consent/assent for the subjects.

Enrollment

The Primary Subject (a child with SCD) will be assigned a Unique Subject Identifier upon entry to study.

Non-English-Speaking Participants

The language(s), other than English, that are understood by prospective participants or representatives are unknown. If the patient and/or family is not able to clearly understand English, they will have access to this program if they meet eligibility criteria. A CHOA certified interpreter will participate in the consent process. CHOA interpreters are available 24 hours/day, 7 days/week. Consent/authorization will be obtained using the IRB-approved English consent, along with a short form in the appropriate language. The interpreter orally translates the English consent. Consent is obtained only if consenting staff and the interpreter are convinced that the patient/family understands the translation, and all questions have been addressed. It will be stressed that treatment is available whether or not the patient/family agrees to participate in the study.

Participants who are not yet adults (children, teenagers)

This study will enroll children and teenagers who have not attained the legal age/capacity for consent for research, as locally defined. Therefore, parents or another Legally Authorized Representative will provide informed consent prior to Subject enrollment.

Assent will be obtained from appropriately aged Subjects who are not yet adults. Local IRB guidelines may vary, but generally, assent is not required from children younger than 6 years old, verbal assent will be obtained from children 6 to 10 years old, and signed assent will be obtained from children aged 10 to <17 years old, subject to Institutional regulations.

Cognitively Impaired Adults

All consenting staff will adhere to consenting guidelines and explain all alternatives to participation. All questions and concerns are addressed during the consenting process. Subjects are encouraged to take home the consent and forms before deciding to participate in this research. The consenting process involves asking questions to ensure the subjects understand the process and the study.

Adults Unable to Consent

Consent will be obtained from a LAR for adults who are unable to consent per institutional policy. We will utilize institutional research SOPs to document consent in writing.

Waiver or Alteration of Consent Process (consent will not be obtained, required information will not be disclosed, or the research involves deception)

We are requesting a waiver of HIPAA authorization to initially identify participants eligible for this research.

22. Setting

Children's Healthcare of Atlanta (CHOA) is the main healthcare entity caring for children across the state of Georgia, and training site for Emory University pediatric residents and fellows. Faculty are employed by Emory University and clinical service occurs at CHOA. Emory University is the academic affiliate of CHOA. Our Sickle Cell Disease Program is the largest pediatric program in the country, caring for more than 2,000 children and young adults. Combining the latest proven technology and research with a caring, child-friendly approach makes the Aflac Cancer and Blood Disorders Center of Children's Healthcare of Atlanta—the county's largest pediatric hematology program—a top choice for the treatment of sickle cell disease. The Aflac Cancer & Blood Disorders Center's Clinical Research Office (CRO) houses clinical research coordinators and data analysts at Egleston and Scottish Rite for faculty within Aflac.

23. Resources Available

The Principal Investigators will have effort designated for the design, oversight, and management of the study. The Principal Investigator and other site personnel will be credentialed and trained to perform research specific activities. Documentation of their training will be maintained in study regulatory files.

All Co-Investigators and Study Staff will be trained in the proper conduct of the protocol, the research procedures and their duties and functions. Training will be conducted by the Principal Investigator or designee via in person or web-based video conferencing.

24. References

1. Liem RI. Balancing exercise risk and benefits: lessons learned from sickle cell trait and sickle cell anemia. *Hematology Am Soc Hematol Educ Program*. 2018 Nov 30;2018(1):418-425. doi: 10.1182/asheducation-2018.1.418. Review. PMID:3050434
2. Omwanghe OA, Muntz DS, Kwon S, Montgomery S, Kemiki O, Hsu LL, Thompson AA, Liem RI. Self-Reported physical activity and exercise patterns in children with sickle cell disease. *Pediatr Exerc Sci*. 2017 Aug;29(3):388-395. doi: 10.1123/pes.2016-0276. Epub 2017 May 22. PMID:28530510
3. Al-Rimawi H, Jallad S. Sport participation in adolescents with sickle cell disease. *Pediatr Endocrinol Rev*. 2008 Oct;6 Suppl 1:214-6. Review. PMID:19337181
4. Connes P, Machado R, Hue O, Reid H. Exercise limitation, exercise testing and exercise recommendations in sickle cell anemia. *Clin Hemorheol Microcirc*. 2011;49(1-4):151-63. doi: 10.3233/CH-2011-1465. Review. PMID:22214686
5. Cooper DM, Liem RI. Translating exercise benefits in sickle cell disease. *Blood*. 2019 Dec 19;134(25):2227-2229. doi: 10.1182/blood.2019003651. No abstract available. PMID:31856272
6. Merlet AN, Chatel B, Hourdé C, Ravelojaona M, Bendahan D, Féasson L, Messonnier LA. How sickle cell disease impairs skeletal muscle function: implications in daily life. *Med Sci Sports Exerc*. 2019 Jan;51(1):4-11. doi: 10.1249/MSS.0000000000001757. Review. PMID:30095751
7. Merlet AN, Messonnier LA, Coudy-Gandilhon C, Béchet D, Gellen B, Rupp T, Galactéros F, Bartolucci P, Féasson L. Beneficial effects of endurance exercise training on skeletal muscle microvasculature in sickle cell disease patients. *Blood*. 2019 Dec 19;134(25):2233-2241. doi: 10.1182/blood.2019001055. PMID:31742587
8. Gellen B, Messonnier LA, Galactéros F, Audureau E, Merlet AN, Rupp T, Peyrot S, Martin C, Féasson L, Bartolucci P; EXDRE collaborative study group. *Moderate-intensity endurance-exercise training in patients with sickle-cell disease without severe chronic complications (EXDRE): an open-label randomised controlled trial.* *Lancet Haematol*. 2018 Nov;5(11):e554-e562. doi: 10.1016/S2352-3026(18)30163-7. PMID:30389037
9. Grau M, Nader E, Jerke M, Schenk A, Renoux C, Dietz T, Collins B, Bizjak DA, Joly P, Bloch W, Prokop A, Connes P. *Impact of a six week training program on ventilatory efficiency, red blood cell rheological parameters and red blood cell nitric oxide signaling in young sickle cell anemia patients: a pilot study.* *J Clin Med*. 2019 Dec 5;8(12). pii: E2155. doi: 10.3390/jcm8122155. PMID: 31817545
10. Mezzani A. Cardiopulmonary exercise testing: Basics of methodology and measurements. *Ann Am Thorac Soc.* 2017 Jul;14(Supplement_1):S3-S11. doi:10.1513/AnnalsATS.201612-997FR. Review.PMID: 28510504
11. Milani RV, Lavie CJ, Mehra MR, Ventura HO. Understanding the basics of cardiopulmonary exercise testing. *Mayo Clin Proc*. 2006 Dec;81(12):1603-11. Review. PMID:17165639
12. Pianosi PT, Liem RI, McMurray RG, Cerny FJ, Falk B, Kemper HC. Pediatric exercise testing: value and implications of peak oxygen uptake. MID:30389037. *Children (Basel).* 2017 Jan 24;4(1). pii: E6. doi: 10.3390/children4010006. Review. PMID:28125022
13. van Beers EJI, van der Plas MN Nur, E, Bogaard HJ, van Steenwijk RP, Biemond BJ, Bresser Pl. Exercise tolerance, lung function abnormalities, anemia, and cardiothoracic ratio in

sickle cell patients. *Am J Hematol.* 2014 Aug;89(8):819-24. doi: 10.1002/ajh.23752. Epub 2014 May 16.

14. Hutchaleelaha A, Patel M, Washington C, Siu V, Allen E, Oksenberg D, Gretler DD, Mant T, Lehrer-Graiwer J. Pharmacokinetics and pharmacodynamics of voxelotor (GBT440) in healthy adults and patients with sickle cell disease. *Br J Clin Pharmacol*. 2019 Jun;85(6):1290-1302. doi: 10.1111/bcp.13896. Epub 2019 Mar 31. PMID:30743314
15. Liem RI, Reddy M, Pelligra SA, Savant AP, Fernhall B, Rodeghier M, Thompson AA. *Reduced fitness and abnormal cardiopulmonary responses to maximal exercise testing in children and young adults with sickle cell anemia.* *Physiol Rep*. 2015 Apr;3(4). pii: e12338. doi: 10.14814/phy2.12338. Erratum in: *Physiol Rep*. 2016 Jan;4(1). pii: e12680. doi: 10.14814/phy2.12680
16. Watson AM, Liem RI, Lu Z, Saville B, *Acra S*, *Shankar S*, *Buchowski M*. Longitudinal differences in aerobic capacity between children with sickle cell anemia and matched controls. *Pediatr Blood Cancer* 2015 Apr;62(4):648-53. doi: 10.1002/pbc.25383. Epub 2014 Dec 31.
17. Powell AW, Alsaied T, Niss O, Fleck RJ, Malik P, Quinn CT, Mays WA, Taylor MD, Chin C. *Abnormal submaximal cardiopulmonary exercise parameters predict impaired peak exercise performance in sickle cell anemia patients.* *Pediatr Blood Cancer*. 2019 Jun;66(6):e27703. doi: 10.1002/pbc.27703. Epub 2019 Mar 7. PMID:30848046
18. Badawy SM, Payne AB, Rodeghier MJ, Liem RI. Exercise Capacity and Clinical Outcomes in Adults Followed in the Cooperative Study for Sickle Cell Disease (CSSCD) *Eur J Haematol*. 2018 October; 101(4): 532–541. doi:10.1111/ejh.13140.
19. *Wright SE*, *Pearce B*, *Snowden CP*, *Anderson H*, *Wallis JP*. Cardiopulmonary exercise testing before and after blood transfusion: a prospective clinical study. Br J Anaesth. 2014 Jul;113(1):91-6. doi: 10.1093/bja/aeu050. Epub 2014 Mar 28
20. Otto JM, O'Doherty AF, Hennis PJ, Cooper JA, Grocott MP, Snowdon C, Carlisle JB, Swart M, Richards T, Montgomery HE. Association between preoperative haemoglobin concentration and cardiopulmonary exercise variables: a multicenter study. *Perioper Med* (Lond). 2013 Sep 13;2(1):18. doi: 10.1186/2047-0525-2-18. PMID:24472426
21. Vichinsky E, Hoppe CC, *Ataga KI*, *Ware RE*, *Nduba V*, *El-Beshlawy A*, *Hassab H*, *Achebe MM*, *Alkindi S*, *Brown RC*, *Diuguid DL*, *Telfer P*, *Tsitsikas DA*, *Elghandour A*, *Gordeuk VR*, *Kanter J*, *Abboud MR*, *Lehrer-Graiwer J*, *Tonda M*, *Intondi A*, *Tong B*, *Howard J*; *HOPE Trial Investigators*. A phase 3 randomized trial of Voxelotor in sickle cell disease. N Engl J Med. 2019 Aug 8;381(6):509-519. doi: 10.1056/NEJMoa1903212. Epub 2019 Jun 14. PMID:31199090
22. Hackney AC, Hezier W, Gulledge TP, Jones S, Strayhorn D, Busby M, Hoffman E, Orringer EP. Effects of hydroxyurea administration on the body weight, body composition and exercise performance of patients with sickle-cell anaemia, *Clin Sci* (Lond) 92 (1997), 481–486. PMID:9176021
23. Dufu K, Lehrer-Graiwer J, Ramos E, Oksenberg D. *GBT440 inhibits sickling of sickle cell trait blood under in vitro conditions mimicking strenuous exercise.* *Hematol Rep*. 2016 Sep 28;8(3):6637. eCollection 2016 Sep 28. PMID:27757216
24. Howard J, Hemmaway CJ, Telfer P, Layton DM, Porter J, Awogbade M, Mant T, Gretler DD, Dufu K, Hutchaleelaha A, Patel M, Siu V, Dixon S, Landsman N, Tonda M, Lehrer-Graiwer

J. A phase 1/2 ascending dose study and open-label extension study of voxelotor in patients with sickle cell disease. *Blood*. 2019 Apr 25;133(17):1865-1875. doi: 10.1182/blood-2018-08-868893. Epub 2019 Jan 17.PMID: 30655275

25. Chonat S, Fields E, Baratz H, Watt A, Patel M, Dixon S, Tonda M, Brown C, Archer D. Improvement in Red Blood Cell Physiology in Children With Sickle Cell Anemia Receiving Voxelotor: An Ancillary Single-Center Pilot Study From the HOPE-KIDS Trial. *Manuscript in preparation.*
26. Green ML, Savic RM, Tonda M, Jorga K, Washington CB. Model-informed drug development of voxelotor in sickle cell disease: Exposure-response analysis to support dosing and confirm mechanism of action. *CPT Pharmacometrics Syst Pharmacol*. 2022 Jun;11(6):698-710. doi: 10.1002/psp4.12780. Epub 2022 Apr 21. PMID: 35447017
27. Estepp JH, Kalpatthi R, Woods G, Trompeter S, Liem RI, Sims K, Inati A, Inusa BPD, Campbell A, Piccone C, Abboud MR, Smith-Whitley K, Dixon S, Tonda M, Washington C, Griffin NM, Brown C. Safety and efficacy of voxelotor in pediatric patients with sickle cell disease aged 4 to 11 years. *Pediatr Blood Cancer*. 2022 Aug;69(8):e29716. doi: 10.1002/pbc.29716. Epub 2022 Apr 21. PMID: 35451176.

25. Appendix

Diffuse Optical Spectroscopy Device and Laser Safety

The Diffuse Optical Spectroscopy device employed herein is a research device constructed in house. It employs two Diffuse Optical Spectroscopy systems, one called Diffuse Correlation Spectroscopy (DCS) and one called frequency domain near-infrared spectroscopy (fdNIRS). The DCS system uses an 852 nm long-coherence length laser, two 4-channel photon-counting avalanche photodiodes, and a hardware or software autocorrelator board. The fdNIRS system is a customized commercial frequency-domain oximeter with 8 near-infrared laser sources ranging from 650 to 830nm and 4 photomultiplier tube (PMT) detectors. Both DCS and fdNIRS components fit on a portable cart that can be wheeled to the subject's bedside. The light from/to these devices is delivered to/from the tissue of the subject through fiber optic cables arranged within a custom-designed optical sensors. The optical fibers are arranged on a rectangular flexible sensor of a size no larger than 3 in^2. The fibers are flexible and are modified to emit and collect light with a 90-degree angle so that the sensor can lay flat against the skin.

The American National Standards Institute provides recommendations for the safe use of lasers and laser systems that operate between at wavelengths of 0.18 μm - 1 mm [103]. According to the ANSI standards, the lasers used in both the FDNIRS and DCS devices fall under Class 3b, meaning that they may be hazardous for direct eye exposure, but there is a low probability of tissue injury for diffuse reflectance. A summary of the ANSI laser classifications can be found here.

The Maximum Permissible Exposure (MPE)

According to ANSI standards, the single most useful number for laser safety calculations is the Maximum Permissible Exposure (MPE, with units of power per area). The MPE is the maximum irradiance or radiant exposure that may be incident upon the eye or the skin without causing biological damage.

- **Skin:**

$$MPE = \begin{cases} 0.2 & ,\lambda < 0.7\mu m \\ 0.2 \cdot C_a & ,\lambda > 0.7\mu m \end{cases}$$

- **Eyes:**

$$MPE = \begin{cases} 0.001 & ,\lambda < 0.7\mu m \\ 0.001 \cdot C_a & ,\lambda > 0.7\mu m \end{cases}$$

where $C_a = 10^{2(\lambda - .7)}$ and λ is the operating wavelength of the laser. Note that in these calculations, λ is measured in μm not nm (i.e. 700 nm = 0.7μm). **Table 1** shows the MPE values for wavelengths in the near-infrared that are routinely used in our FDNIRS/DCS device.

MPE is dependent upon the specifications of the optical system. Here we list laser specifications, along with the specifications for the fiber optic-based sensors that we have custom designed for our FDNIRS and DCS devices.

FDNIRS: up to 16 low power laser diodes. The diodes are multiplexed so that only one diode is "on" at a time. These diodes are commonly used lasers on a wide range of applications and contain the same semiconductor active media as LEDs. They are operated in continuous wave (CW) mode.

Wavelength (nm)	MPE, skin (W/cm2)	MPE, eye (W/cm2)
660	0.20	0.0010
670	0.20	0.0010
680	0.20	0.0010
690	0.20	0.0010
700	0.20	0.0010
710	0.21	0.0010
720	0.22	0.0011
730	0.23	0.0011
740	0.24	0.0012
750	0.25	0.0013
760	0.26	0.0013
770	0.28	0.0014
780	0.29	0.0014
790	0.30	0.0015
800	0.32	0.0016
810	0.33	0.0017
820	0.35	0.0017
830	0.36	0.0018
840	0.38	0.0019
850	0.40	0.0020
860	0.42	0.0021

Table 1

λ = Wavelengths range from 660 to 830 nm (0.66-0.83 um)
Φ_L = Power at the laser <20 mW (0.02 W)
Φ = Power at the probe <10 mW (0.01 W)
a = Beam diameter at the probe ~ 0.30 cm
NA = Fiber's numerical aperture ~ 0.37
ϕ = Beam divergence = $2\arcsin NA$~ 0.76 rad

DCS: A single solid-state, long coherence-length (>5 m) laser, which is essentially a laser diode with a longer cavity. Because the detection fibers required for DCS measurements are single mode fibers, a higher power laser (compared to FDNIRS) is required. The laser is operated in continuous wave mode.

λ = wavelength = 852 nm
Φ_L = Power at the laser = 100 mW (0.1 W)
Φ = Power out of the sensor = 25 mW (0.025 W)
a = Beam diameter at the sensor > 0.30 cm
NA = Fiber's numerical aperture ~ 0.37
ϕ = beam divergence = 1.57 rad (because we add a diffuser to the sensor surface)

Calculation of Irradiance of FDNIRS/DCS Lasers

The following formula is used to calculate what is the irradiance (E) of our lasers:

E=power (W)/spot area (cm^2)

All of our FDNIRS/DCS custom sensors are designed such that the irradiance is less than the stated wavelength-dependent ANSI MPE for skin exposure. For example, for the DCS laser, we use a 0.3cm beam diameter (radius, r = 0.15cm) at the sensor and the laser power (Φ) out of the sensor is 25mW, thus the irradiance to the skin from our laser is:

$$E = {\Phi}/{\pi(r/2)^2} = 0.35 \text{ W/cm}^2$$

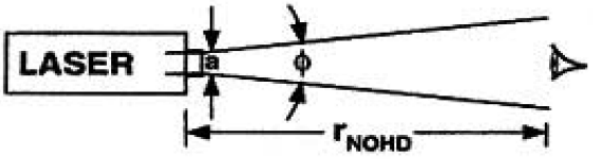
As Table 1 shows, this irradiance of 0.35 W/cm^2 is less than the MPE at 852nm of 0.40 W/cm^2. We have performed similar calculations for all of our FDNIRS lasers to confirm that they also yield irradiance levels that fall well below MPE limits.

Nominal Hazard Zone (NHZ)

In addition to the MPE, the other major specification used in laser safety calculations is the Nominal Hazard Zone (NHZ). The NHZ is the distance within which the irradiance of the laser beam is greater than the MPE. This distance depends not only on the wavelength of light and time of light exposure, but also on the beam's path to your eye, e.g., direct viewing, specular reflectance, or diffuse reflectance. Due to divergence, as the distance from the source or focus of the beam (or its reflection) increases, the irradiance (E) decreases. That boundary defines the NHZ.

The Nominal Ocular Hazard Distance (NOHD) for any particular laser represents the distance (r_{NOHD}) within which it is possible to exceed the MPE:

$$r_{NOHD} = \frac{1}{\varphi}\sqrt{\left(\frac{4\Phi}{\pi MPE}\right) - a^2},$$



where a is the diameter of the beam coming from the light source (cm), and Φ is the laser power (W). φ is the beam divergence (rad) is equal to $2\arcsin NA$, where NA is the numerical aperture of the light source (rad).

Calculation of Nominal Ocular Hazard Distance (NOHD) of Our Lasers

For the FDNIRS lasers, using the following parameters:

Φ = 0.020 W
r = 0.3 cm
ϕ = beam divergence = 0.76 rad = 43°
MPE_e at 785 nm = 0.0014 W/cm^2

We find that the r_{NOHD} = 5.65 cm (6.32 cm for Φ= 0.025 W). For the DCS laser, which has a wider divergence angle (ϕ = 1.57 rad = 90°), r_{NOHD} = 2.73 cm. For comparison, using typical parameters of a laser pointer, we see that these ocular hazard distances are quite small:

Φ = 0.005 W
r = 0.3 cm
ϕ = 1mrad = 0.001 rad
MPE_e=0.001
r_{NOHD}=2,505 cm (25 m)

Calculation of Minimum Optical Density Eyeglasses Needed for the Irradiance of our Lasers:

OD=log(E/MPE_e)=2.3 at 785nm for 20mW power. However, since the NOHD of our lasers is less than 10 cm, our lasers can be considered as class IIIA and therefore safety eyeglasses are not needed [104]. See the excerpts below for more information (page 46):

> Under IEC requirements, the assessment of levels of irradiance or radiant exposure for the purposes of determining the magnitude of exposure in relation to the MPE within the wavelength range from 302.S nm (in the ultraviolet region) to 4000 nm (in the infrared) are made at a distance of 100 mm from the laser source. This is to take account of the likely closest distance of approach and, for the eye, the minimum distance (the near point) at which objects can be clearly focused on the retina. For this reason, when the NOHD for a given laser system has been determined to be less than 100 mm (which can often arise in the case of laser diodes at the lower levels of class 38), protective measures beyond the controlled use of viewing aids may not be necessary, provided that the personnel concerned are fully aware of the nature of the potential hazards and the conditions under which a hazardous exposure could arise.
>
> The NOHD is the distance from the source at which the exposure drops to the level of the MPE. Beyond this distance intra-beam viewing would be safe for the unaided eye but could remain hazardous if magnifying optical instruments were to be used. This is often the case in optical-fiber technology, when microscopes and magnifying lenses may be used for examining the end of optical fibers or for critical work with small components. The extended NOHD is the distance from the source at which the exposure drops to the equivalent of the class I AEL contained within a 50-mm diameter centered on the optical axis. Beyond this distance the exposure can be considered safe for intra-beam viewing even with the use of viewing aids. (Provided, of course, that these do not have a collection aperture greater than 50 mm.) The equations for fiber divergence given earlier can be used to determine these distances using the procedures described in Chapter 6.
>
> We can thus define three zones (illustrated in Figure 1.17). Within the NOHD there is a potential hazard to the eye and eye protection would be needed. Between the NOHD and the extended NOHD unaided viewing is safe but the use of viewing aids needs to be

controlled. Beyond the extended NOHD there is essentially no hazard and no controls are therefore needed.

This concept is particularly useful for dealing with divergent class 3B systems. Class 3B has the *potential* for causing injury to the unaided eye and would thus normally be restricted to operation within enclosed laser areas in a similar manner to class 4. It can be found with divergent emission, however, particularly at the lower levels of class 3B, that the NOHD is considerably less than 100 mm, and that therefore intra-beam viewing would not normally present a hazard to the unaided eye. The wearing of eye protection could therefore be unnecessary. In fact, when work of a similar nature is being regularly undertaken with divergent class 3B systems, it is possible to set up controlled laser working areas based on the degree of hazard rather than simply on the laser class.

Class 3B with NOHD Less than 100 mm
The work could be done alongside class 3A systems and similar conditions would apply. Personal eye protection need not be worn, but the use of any magnifying optics must be restricted and only those viewing aids incorporating suitable eye protection should be permitted.

Class 3B with NOHD Greater than 100 mm But Less than 1m
The 1-m limit here is somewhat arbitrary but is intended to indicate a hazard localized to the area immediately surrounding the laser source. The wearing of personal eye protection is necessary and the area should be enclosed to prevent line-of-sight viewing from outside the area but, provided that appropriate warning signs are clearly displayed that the area is within premises to which access is normally restricted and that equipment is never allowed to be left running while unattended, fully interlocked access should not be necessary.

Attachment A
REPORTING SERIOUS ADVERSE EVENTS

Definitions
"**Adverse Event**" or "**AE**" means an untoward medical occurrence in a Study subject receiving the Pfizer Product, or an individual otherwise exposed to the Pfizer Product, without regard to whether there is a causal relationship between the Pfizer Product and the medical occurrence.

"**Serious Adverse Event**" or "**SAE**" means any Adverse Event, without regard to causality, that is life-threatening (*i.e.*, causes an immediate risk of death) or that results in any of the following outcomes: death; inpatient hospitalization or prolongation of existing hospitalization; persistent or significant disability or incapacity (*i.e.*, substantial disruption of the ability to conduct normal life functions); or a congenital anomaly or birth defect. Any other medical event that, in the medical judgment of Principal Investigator, may jeopardize the health of the subject or may require medical or surgical intervention to prevent one of the outcomes listed above, also is considered an SAE. A planned medical or surgical procedure is not, in itself, an SAE.

"**Device Incident**" is any malfunction or deterioration in the characteristics and/or performance of a medical device made available on the market, including use error due to ergonomic features, as well as any inadequacy in the information supplied by the manufacturer and any undesirable side effect.

"Serious Device Incident" means any incident that directly or indirectly led, might have led or might lead to any of the following: death of a patient, user or other person, temporary or permanent serious deterioration of the patient's, user's or other person's state of health, serious public health threat. A serious deterioration in health would include: a life-threatening illness or injury, a permanent impairment of a body function or permanent damage to a body structure or necessitates medical or surgical intervention to preclude permanent impairment of a body function or permanent damage to a body structure. Permanent means irreversible impairment or damage to a body structure or function, excluding trivial impairment or damage.

"**Reportable Event**" means any SAE, Device Incident or Serious Device Incident, whether individually or collectively.
If the Pfizer Product is, or contains, a medical device, all references to SAEs herein should be read to include the term "Reportable Event."

Reporting Requirements. Additional information about the requirements herein and detailed information about reporting SAEs to Pfizer (including applicable reporting forms and completion guide) has been provided to the Principal Investigator and is available on Pfizer's online grant system. Institution will ensure that this additional, detailed, information is provided to all Study Staff engaged in the reporting of SAEs.

1. Timing and Scope. Within 24 hours of Institution's first awareness of an SAE, or immediately if the SAE is fatal or life-threatening, Institution will notify Pfizer by fax, email or E2B (as concurred with Pfizer) of any SAE for which reporting hereunder is required. SAEs that are subject to these reporting requirements are those that occur in: (i) Study subjects who are assigned or, in the case of a blinded Study, possibly assigned to receive the Pfizer Product; or (ii) individuals otherwise exposed to the Pfizer Product.

Institution will notify Pfizer of any such SAE within the timeframes set forth herein even if complete information is not yet available. Institution will ensure that all Study Staff notify Institution of any SAE immediately upon becoming aware.

2. Reporting Format. Each Study site will report SAEs using one of the following: (i) a reporting form approved by the local regulatory authority, (ii) a CIOMS form, (iii) a Pfizer provided *Investigator Sponsored Research or Clinical Research Collaboration Interventional Serious Adverse Event Report Form* or (iv) any other form prospectively approved by Pfizer. The *Reportable Event Fax Cover Sheet* provided by Pfizer also must be included with each SAE submitted.
3. Specific SAEs. Exposure to the Pfizer Product during pregnancy or lactation, drug overdose, medication error, occupational/environmental exposure to the Pfizer Product and lack of efficacy of the Pfizer Product are reportable to Pfizer.
    3.1. Exposure during pregnancy and lactation are reported independently from any associated SAE. Medication errors and overdoses are reportable only if associated with

an SAE. Occupational/environmental exposure is reported independently from any associated AE/SAE.

3.2. Lack of Efficacy is reportable only if associated with an SAE (except for certain scenarios which are outlined in the Pfizer provided training materials: vaccines, contraceptive and anti-infectives products).

4. Hy's Law: If the Pfizer Product is not approved in the US for the indication being studied, (irrespective of being authorized/approved elsewhere), cases of potential drug-induced liver injury as assessed by laboratory test values ("**Hy's Law Cases**") are SAEs reportable to Pfizer. If a Study subject develops abnormal values in aspartate transaminase ("**AST**") or alanine transaminase ("**ALT**") or both, concurrent with abnormal elevations in total bilirubin and there is no other known cause of liver injury, that event would be classified as a Hy's Law Case.
5. Crizotinib: If Study subjects will receive crizotinib, any AE of potential sight threatening event ("**PSTE**") or severe visual loss ("**SVL**") must be reported as an SAE in subjects treated with crizotinib, even if the study drug is not (or is not otherwise related to) crizotinib.
   5.1. SVL is defined as Grade 3 or Grade 4 eye disorders based on Common Terminology Criteria for Adverse Events ("**CTCAE**") published by the National Cancer Institute. According to CTCAE, Grade 3 eye disorders include symptomatic retinopathy with marked decrease in visual acuity (worse than 20/40) or disabling (limited self-care activities of daily living). Grade 4 eye disorders include blindness (20/200 or worse) in the affected eye.
   5.2. PSTE includes all identified Grade 2 eye disorders listed above (except visual field defect) and the following Grade 2 eye disorders: retinal detachment, retinal edema, maculopathy, iritis, uveitis, and abnormal visual field tests.
6. Exclusions. Specifically excluded from the reporting requirements for SAEs is: (i) any SAE identified in the Protocol as anticipated to occur in the Study population at some frequency independent of drug exposure, unless a Principal Investigator suspects a causal relationship between the SAE and the Pfizer Product; and (ii) if the Pfizer Product is an oncology drug, any SAE judged by a Principal Investigator to represent progression of the malignancy under study, unless it results in death within the SAE reporting period.
7. Reporting Period. SAEs that are subject to these reporting requirements are those that: (i) occur from the first dose of the Pfizer Product or the Study subject's enrollment in the Study (whichever is later) through 28 calendar days after the last administration of the Pfizer Product or the subject's last Study visit (whichever is earlier), or longer if so specified in the Protocol; or (ii) occur any time after the 28-day period (i.e., any time after the completion of the active study collection/reporting period), if Institution or Principal Investigator suspects a causal relationship between the Pfizer Product and the SAE.
8. Follow-Up Information. Institution will assist Pfizer in investigating any SAE and will provide any follow-up information reasonably requested by Pfizer.
9. Regulatory Reporting. Reporting an SAE to Pfizer does not relieve Institution of responsibility for reporting such SAE to appropriate regulatory authorities, if required.
10. IMP and SRSD. Principal Investigator will identify the Pfizer Investigational Medicinal Product(s) and respective Single Reference Safety Documents to be used for expectedness

assessment for the Study and will provide this information via Pfizer's online grants system prior to Study start.

11. Pfizer-Provided Safety Information. Pfizer distributes ISR line listings to Institutions bi-annually, or more frequently based on the risk assessment of the ongoing interventional clinical studies.