NCT05289570

PROTOCOL TITLE

A Phase 2b Open-Label, Single Arm Study to Evaluate the Efficacy of Voxelotor for Improving Oxygen Saturation and Reducing Ventilatory Support Requirements in Adult Patients with New or Increased Oxygen Requirement

Protocol Number
Pro00109353

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Funding Sponsor:	Global Blood Therapeutics 181 Oyster Point Blvd. South San Francisco CA 94080 +1 (650) 864-1754
Study Product:	2-hydroxy-6-((2-(1-isopropyl-1H-pyrazol-5-yl)pyridin-3-yl) methoxy)benzaldehyde [Voxelotor (GBT440)]

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List of Abbreviations

Abbreviation	Definition
AE	Adverse event
ALI	Acute lung injury
ARDS	Acute Respiratory Distress Syndrome
CAS	Chemical abstract services
CR	Child-resistant
CRF	Case Report Form
DPG	Diphosphoglycerate
ECG	Electrocardiogram
ECMO	Extracorporeal membrane oxygenation
eIND	Exempt investigational new drug
EMR	Electronic medical record
ESLD	End -stage lung disease
FDA	Food and drug administration
FEMA	Flavor and Extract Manufacturers Association of the United States
FiO_2	Fraction of inspired oxygen
GBT	Global Blood Therapeutics
GMP	Good manufacturing practice
GRAS	Generally recognized as safe
Hb	Hemoglobin
HbF	Fetal hemoglobin
Hb-O_2	Hemoglobin-oxygen
HbS	Sickle hemoglobin
Hct	Hematocrit
HR	Heart rate
ICU	Intensive care unit
IDS	Investigational drug services
IID	Inactive Ingredients Database
INN	International nonproprietary name
IPF	Idiopathic pulmonary fibrosis
LAR	Legally Authorized Representative
LPM	Liters per minute
NYHA	New York Heart Association Functional Classification
O_2	Oxygen
OEC	Oxygen equilibrium curve(s)
ODC	oxygen dissociation curve
p20	Partial pressure of oxygen resulting in 20% saturation of hemoglobin with oxygen
p50	Partial pressure of oxygen resulting in 50% saturation of hemoglobin with oxygen

$PaCO_2$	Partial pressure of carbon dioxide
PaO_2	Partial pressure of oxygen
PD	Pharmacodynamics
PK	Pharmacokinetics
PO_2	Partial pressure of oxygen
PP	Polypropylene
RBC	Red blood cells
Abbreviation	**Definition**
RN	Registered nurse
SAE	Serious Adverse event
SaO_2	Oxygen saturation
SCD	Sickle cell disease
S/F	SpO_2/FiO_2 ; ratio of inspired oxygen needed to obtain a specific oxygen saturation
SOC	Standard of care
SpO_2	Oxygen saturation
SS	Sickle cell
SSRBC	Sickle cell red blood cell
STRT	Study team respiratory therapist
TEAE	Treatment-emergent adverse event
ULN	Upper limit of normal
VOC	Vaso-occlusive crisis

Study Summary

Title	A Phase 2b Open-Label, Single Arm Study to Evaluate the Efficacy of Voxelotor for Improving Oxygen Saturation and Reducing Ventilatory Support Requirements in Adult Patients with New or Increased Oxygen Requirement
Short Title	GBT Voxelotor
Protocol Number	Pro00109353
Phase	Phase 2b
Methodology	Open label, single arm
Study Duration	24 months
Study Center(s)	Single-center
Objectives	Primary study objective - To evaluate the efficacy of voxelotor for increasing oxygen saturation in patients with hypoxic hypoxemia as a result of end-stage lung disease or acute lung injury. Secondary study objective - To evaluate the efficacy of voxelotor on allowing de-escalation of supplemental oxygen support.
Number of Subjects	20
Diagnosis and Main Inclusion Criteria	Oxygen dependency due to an end-staged lung disease (pre-lung transplant population) or ALI/acute lung injury (e.g. primary allograft dysfunction, infectious pneumonia, aspiration, non-cardiogenic pulmonary edema).
Study Product, Dose, Route, Regimen	2-hydroxy-6-((2-(1-isopropyl-1H-pyrazol-5-yl)pyridin-3-yl) methoxy)benzaldehyde [Voxelotor (GBT440)]
Duration of administration	500 mg of voxelotor two times a day (for a total daily dose of 1000 mg per day) for 5 days. This dose may increase to a total maximum daily dose of 1500 mg (500 mg three times a day), if subject tolerates the initial dose as determined by study doctor.
Reference therapy	Anecdotal evidence from an ECMO dependent Duke intensive care unit (ICU) patient who received Voxelotor on a compassionate use basis, after seeking an eIND from the FDA, who was rapidly weaned from ECMO and ventilatory support.
Statistical Methodology	Descriptive statistics for patient characteristics and baseline factors will be presented as median and interquartile range for continuous variables and number and percentage for categorical variables.

1 Introduction

This document is a protocol for a human research study. This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

Voxelotor (GBT440) is a small molecule inhibitor of sickle hemoglobin (HbS) polymerization which allosterically modifies hemoglobin-oxygen (Hb-O_2) affinity that was developed by Global Blood Therapeutics (GBT) and is FDA-approved for the treatment of sickle cell disease in adults and pediatric patients 12 years of age and older.

1.1 Background

Treatment for decompensation of end-stage lung disease (ESLD) or acute lung injury (ALI) with hypoxemia may include disease modifying drugs (such as anti-fibrotic medications or steroids) and anti-viral/anti-microbial agents. However, escalation of the need for supplemental oxygen, complexity of modality of supplemental oxygen delivery, up to and including invasive mechanical ventilation, may still be required. Transfer to a monitored bed or an intensive care unit is typically triggered by the degree of arterial desaturation in a setting of escalating oxygen requirements, although ICU bed availability may be critically limited. The indication for intubation is typically refractory hypoxemia with falling levels of the saturation of hemoglobin (Hb) with oxygen (SaO_2), yet mortality is high with recovery after intubation being slow and fraught with complications due to the need for heavy sedation and possible ventilator induced lung injury; avoiding intubation would be ideal. This is especially true for the pre-lung transplant population, where “pre-habilitation” is essential for a favorable outcome and loss of ambulatory status (as is often the case with escalating oxygen requirements and intubation) portends an increased mortality after lung transplantation.

As an alternative, additive measure to escalating ventilation modality or the fraction of inspired oxygen (FiO_2), increasing the affinity of hemoglobin for oxygen offers the ability to optimize the SaO_2 for a given partial pressure of oxygen in the arteries (paO_2, i.e. hypoxemia) and, by increasing the SaO_2, postpone or avoid the need for ICU transfer and/or intubation, buying more time for the innate, or treatment induced, resolution of ALI or permitting continued ambulation pre-transplant. This concept is supported by anecdotal evidence from an ECMO dependent ICU patient who received voxelotor on a compassionate use basis, after seeking an eIND from the FDA, who was rapidly weaned from ECMO and ventilatory support.

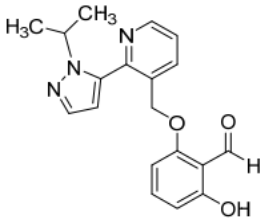
1.2 Investigational Agent

PHYSICAL, CHEMICAL, AND PHARMACEUTICAL PROPERTIES AND FORMULATION

Molecular Structure
Chemical Name: 2-Hydroxy-6-((2-(1-isopropyl-1H-pyrazol-5-yl)pyridin-3-yl) methoxy)benzaldehyde
Company Code: GBT440

Chemical Abstracts Service (CAS) registry number: 1446321-46-5
International Nonproprietary Name (INN): Voxelotor

Structure:



Molecular Formula: C19H19N3O3

Molecular Weight: 337.4

Physical Description Voxelotor is a synthetic small molecule bearing the chemical name 2-hydroxy-6-((2-(1- isopropyl-1H-pyrazol-5-yl)pyridin-3-yl) methoxy)benzaldehyde. The chemical formula is C19H19N3O3 and the molecular weight is 337.4.

Voxelotor is a white-to-yellow-to-beige compound in crystalline Form II of GBT440 free base. It is non-hygroscopic. It is highly soluble in common organic solvents such as acetone and toluene and insoluble in water (approximately 0.03 mg/mL). Voxelotor drug substance is manufactured in accordance with Good Manufacturing Practice (GMP). The chemistry used in the production of voxelotor drug substance is well established. The manufacturing process uses equipment commonly available in the industry.

Nonclinical Pharmacology
The pharmacology program established the mechanism of action by testing effects of voxelotor on HbS and by assessing the benefit on downstream inhibition of HbS polymerization both in vitro and in animal disease models

Primary Pharmacology
The in vitro primary pharmacology studies defined the mechanism of action of voxelotor and the in vivo studies determined the relationship between voxelotor blood concentration and ex vivo effects on Hb-O_2 affinity as well as its physiologic and pathologic effects in a sickle cell mouse model. In addition, using a voxelotor analogue in C57BL/6 and sickle cell disease (SCD) mice, these studies also addressed its ability to preserve oxygen (O_2) extraction in various organs, including the brain, ultimately translating to improved survival. Findings from the primary pharmacological studies demonstrated that voxelotor has the ability to:

- bind specifically to Hb,
- partition to red blood cells (RBCs),
- increase Hb-O_2 affinity and stabilize the oxyHb state,
- inhibit HbS polymerization,
- improve RBC rheology in human sickle cell (SS) blood,
- exhibit ex vivo anti sickling activity in a SCD mouse model,

- increase RBC half-life and reduce reticulocyte counts in a SCD mouse model,
- in addition, a voxelotor analogue preserved O_2 extraction in various organs and improved brain oxygenation and survival in both normal and SCD mice.

Furthermore, these studies showed that voxelotor-modified Hb can release O_2 at lower pH, indicating that voxelotor binding to Hb does not interfere with the ability of Hb to deliver and offload O_2 to metabolically active tissues.

Binding of Voxelotor to Hemoglobin

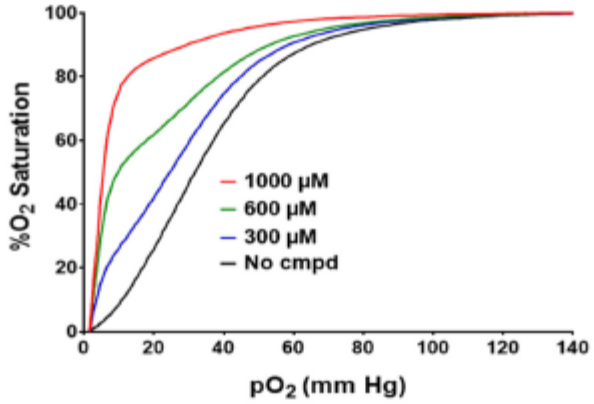
Crystallography studies of cocrystals of carbon monoxide-liganded Hb and voxelotor show that:

- Voxelotor binds to the N-terminal valine residue of an α chain; a single GBT440 molecule binding per Hb tetramer in a 1:1 stoichiometry (Study PRC-18-048; Oksenberg D, 2016;[1] Metcalf B, 2017).[2]
- The voxelotor binding site is distant from heme pockets and therefore does not directly prevent release of O_2 from Hb (Patel M, 2014,[3] data on file).
  Thus, voxelotor increases Hb-O_2 affinity without sterically blocking the release of O_2.

Effect of Voxelotor on Hemoglobin-Oxygen Affinity

The oxygen equilibrium curve (OEC) for whole blood relates the extent of Hb-O_2 saturation to the partial pressure of oxygen (pO_2) and measures the binding affinity of O_2 to Hb. Blood OECs allow for the measurement of the partial pressure of oxygen resulting in 20% saturation of hemoglobin with oxygen (p20) or partial pressure of oxygen resulting in 50% saturation of hemoglobin with oxygen (p50) values, which are the pO_2 at which Hb is 20% or 50% saturated with O_2, respectively. Measurement of OECs using whole blood from patients with SCD (Figure 2) shows a dose-dependent left-shift of the OEC and decrease in p50 and p20 values upon addition of voxelotor (Figure 1).

Figure 1: Effect of Voxelotor on Hemoglobin-Oxygen Affinity in Whole Blood from Patients with SCD



Abbreviations: O_2, oxygen; pO_2, partial pressure of oxygen.
Source: Study PRC-14-027-R

Stabilization of Hemoglobin in a High Oxygen Affinity State by Voxelotor
Hb exists in both a R and a T state with high and low affinity for O_2, respectively. Cooperativity arises from a shift in the population from the low affinity T structure to the high affinity R structure with increasing O_2 affinity (Eaton WA, 1999).[4] Using the oxygen dissociation assay (), voxelotor was shown to effectively maintain a high percent of Hb in the oxyhemoglobin conformation (associated with the R state) under hypoxic conditions (Study PRC-14-033-R; Patel MP, 2018)[5] whilst maintaining the ability of O_2 to offload from Hb.

Voxelotor-modified Hb maintains the physiological mechanisms required for tissue O_2 extraction
The ability of H+ ions and 2,3 diphosphoglycerate (DPG) to reduce Hb-O_2 affinity are 2 of the key physiologic mechanisms that drive O_2 offloading to metabolically active tissues. In vitro studies with voxelotor modified Hb and whole blood in the presence of H+ ions or DPG showed the expected increase in dissociation of O_2. Therefore, physiologic mechanisms of augmenting tissue O_2 extraction, including the Bohr effect and 2,3 DPG binding (Studies PRC-14-029-R and PRC-18-046) are preserved with voxelotor modified Hb.

1.3 Preclinical Data

Prevention of Sickle Hemoglobin Polymerization and Red Blood Cell Sickling In Vitro
The intracellular polymerization of HbS upon deoxygenation is the primary pathogenic event in SCD (Noguchi CT, 1981).[6] Delaying HbS polymerization long enough may prevent the RBCs from damage and sickling while transiting hypoxic tissue capillaries in patients with SCD. Therefore, the ability of voxelotor to delay HbS polymerization in vitro was evaluated (Study PRC-14-040-R). Voxelotor was shown to delay polymerization in a dose-dependent manner and mimicked the profile of polymerization delay afforded by the presence of 20% to 30% fetal hemoglobin (HbF) (Table 1).

Table 1: Delay of Sickle Hemoglobin Polymerization by Voxelotor, and Fetal Hemoglobin

% Inhibitor [a]	HbF ΔDT (min)	GBT440-HbS ΔDT (min)
20	8.8	10
30	13.1	14

Abbreviations: ΔDT, delay time (Delay time of Hb mixture – Delay time of HbS alone); HbF, fetal hemoglobin; min, minutes.
[a] Inhibitor is HbF, or GBT440-HbS added to unmodified HbS. Final concentration of Hb = 50 µM.
Source: Study PRC-14-040-R.

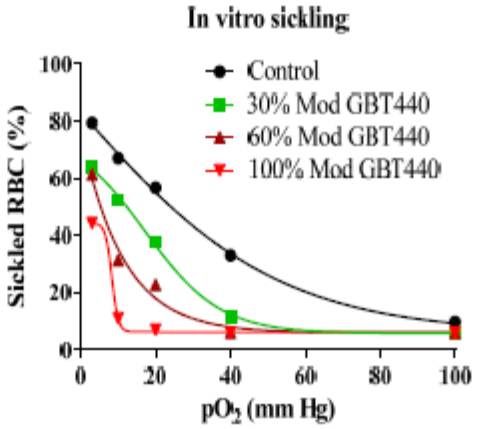
These data indicate that like HbF, voxelotor is a potent inhibitor of in vitro HbS polymerization and suggest that by delaying HbS polymerization, voxelotor may prevent RBC damage and sickling from patients with SCD.

This decrease in polymerization causes a corresponding dose dependent decrease in the number of sickled RBCs (SS RBCs) in blood from patients with sickle cell disease under variable hypoxic conditions. The therapeutic target of 30% modification is sufficient to prevent in vitro

sickling of SS RBCs at an O_2 tension typical of tissue capillaries (40 mmHg) (Study PRC-14-042 R).

Voxelotor dose-dependently reduced the number of sickled RBCs under hypoxic conditions (pO_2 of < 40 mmHg) (Figure 3), mimicking O_2 tensions in tissue capillaries.
Effect on Sickle Blood Viscosity and Deformability In Vitro Furthermore, by inhibiting HbS polymerization, voxelotor improves SS RBC deformability and decreases SS blood hyperviscosity in vitro under hypoxic conditions (Studies PRC-14-044-R and PRC-14-031-R, Dufu K, 2018).[7] The effect of voxelotor on SCD blood viscosity was evaluated under deoxygenated conditions and compared to untreated SCD blood and blood from healthy subjects. Voxelotor reduced the viscosity of the deoxygenated SS blood when compared to untreated deoxygenated SCD blood (Figure 4A) and the viscosity of deoxygenated SCD blood improves with increasing voxelotor concentrations (Figure 2)

Figure 2: Voxelotor Prevents Red Blood Cell Sickling

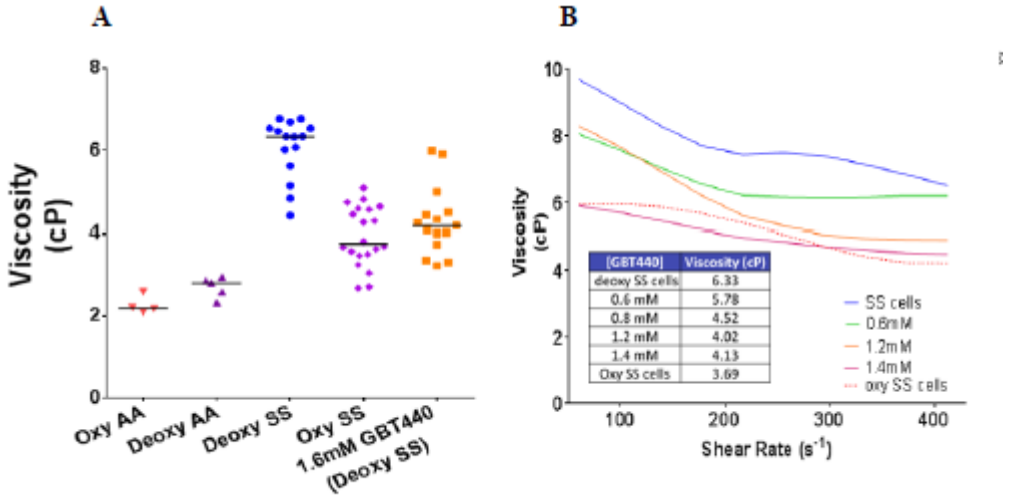


Abbreviations: RBC, red blood cell; pO_2, partial pressure of oxygen.
Source: Study PRC-14-042-R.

Effect on Sickle Blood Viscosity and Deformability In Vitro
Furthermore, by inhibiting HbS polymerization, voxelotor improves SS RBC deformability and decreases SS blood hyperviscosity in vitro under hypoxic conditions (Studies PRC-14-044-R and PRC-14-031-R, Dufu K, 2018).[8] The effect of voxelotor on SCD blood viscosity was evaluated under deoxygenated conditions and compared to untreated SCD blood and blood from healthy subjects.

Voxelotor reduced the viscosity of the deoxygenated SS blood when compared to untreated deoxygenated SCD blood (Figure 3A) and the viscosity of deoxygenated SCD blood improves with increasing voxelotor concentrations (Figure 3B).

Figure 3: Voxelotor Reduces the Hyperviscosity Observed in SCD Blood



AA = Normal blood with HbA; SS = Blood from sickle cell patients.

Source: Study PRC-14-044-R.

Decreased RBC deformability and increased RBC rigidity in SCD have significant positive implications for microcirculatory blood flow and prevention of end-organ damage. Deformability experiments also showed that voxelotor restored deformability of human SS RBC under hypoxic conditions (Study PRC-14-031-R). In vitro results indicate that voxelotor reduces the blood hyperviscosity associated with deoxygenation of HbS. Since the viscosity of SCD blood under hypoxic conditions is primarily determined by polymer load, these data suggest that voxelotor decreased SS blood viscosity by reducing intracellular polymer load resulting from HbS polymerization. Consequently, this suggests a potential to improve the blood flow in the microcirculation, thus improving O_2 delivery to peripheral tissues. As viscosity increases with hematocrit (Hct), and because sickle blood has an increased viscosity compared to normal blood for any given Hct, these results are important in supporting the potential for voxelotor to increase Hct and Hb in SCD patients without causing a clinically adverse worsening in blood viscosity.

1.4 Clinical Data to Date

GBT Sponsored Clinical Studies

As of 05 November 2020, 28 clinical development studies (8 studies in SCD, 2 studies in idiopathic pulmonary fibrosis (IPF), 17 clinical pharmacology studies, and 1 study of healthy participants under hypoxic conditions, Study GBT440-0111) have enrolled an estimated 939 participants who have been exposed to at least 1 dose of voxelotor. An additional 176 clinical study participants received placebo in the voxelotor development program. The 939 participants who received voxelotor consist of:

- 430 participants with SCD: 276 adults and 154 pediatric participants (this includes participants enrolled in the expanded access program)
- 404 healthy participants from the 17 clinical pharmacology studies (including participants with severe renal impairment [not on dialysis] and participants with hepatic impairment)
- 62 healthy participants enrolled in studies other than clinical pharmacology (GBT440-001 and GBT440-0111)
- 43 adult participants with IPF

1.5 Dose Rationale and Risk/Benefits

Based on the clinical studies safety data evaluated to date, voxelotor has an acceptable safety profile and has been well tolerated in adult and pediatric participants (aged 4 to < 18 years) with SCD. The most common adverse reactions in participants with SCD ≥ 12 years old were diarrhea, abdominal pain, nausea, rash, and pyrexia. Other clinically relevant and less frequent adverse reactions included drug hypersensitivity (< 1%). There were no reported events of anaphylaxis or anaphylactoid reactions. The majority of Treatment Emergent Adverse Event (TEAEs) were Grade1 to Grade 2 severity, clinically manageable, reversible or resolved. The TEAE profile of voxelotor for pediatric participants with SCD aged ≥ 4 to 11 years at a dose of 1500 mg or equivalent weight-based dosing is acceptable with no new safety observations and is consistent with the data for adults and adolescent participants.

Overall, the safety profile was similar between adults and pediatric participants aged ≥ 4 years and between participants who were or were not receiving hydroxyurea concomitantly.

In the pivotal Study GBT440-031, over the 72-week treatment period, there was a trend towards a lower overall incidence of vaso-occulsive crisis (VOC) with numerically fewer VOC events in the voxelotor groups compared to the placebo group, although the differences did not reach statistical significance. No increase in the incidence of VOC was observed following discontinuation of voxelotor (up to 28 days after the last dose of study drug).

No clinical safety concerns consistent with inadequate tissue oxygenation were identified in the voxelotor clinical development program, including in SCD study participants.

The most common side effects of voxelotor include:

- Headache
- Diarrhea
- Stomach (abdominal) pain
- Nausea
- Fatigue (Tiredness)
- Rash
- Fever

Less common, but severe, side effects of voxelotor include:

- Pulmonary embolism
- Hypersensitivity reactions (occurring in <1% of patients treated), including the following symptoms:
  - Generalized rash
  - Urticaria (hives)
  - Mild shortness of breath
  - Mild facial swelling
  - Eosinophilia (an increase in one of the white blood cells in your blood called eosinophils)

As voxelotor increases the binding of oxygen to hemoglobin (providing the treatment effect of increased SpO_2 for a given pO_2), we will monitor for the possibility of inadequate oxygen being off-loaded to the tissues by measuring venous blood gases for evidence of metabolic acidosis

from tissue beds and tracking renal function as a reflection of adequacy of tissue oxygen during administration of Voxelotor.

There may also be risks, discomforts, drug interactions, or side effects that are not yet known.

2 Study Objectives

The purpose of the study is to evaluate the efficacy of voxelotor for increasing oxygen saturation in patients with hypoxemia. Specifically, the SpO_2/FiO_2 ratio will be compared before and after voxelotor use at rest and during exercise (ambulatory patients only).

Primary study objective
To evaluate the efficacy of voxelotor for increasing oxygen saturation in patients with hypoxic hypoxemia as a result of end-stage lung disease or acute lung injury.

Secondary study objective
To evaluate the efficacy of voxelotor on allowing de-escalation of supplemental oxygen support.

Exploratory endpoints: Pre and post Voxelotor use compare

- FiO_2 requirements
- Oxygen delivery modality
- Combined end-point of de-escalation of FiO_2 and/or oxygen delivery modality
- Nadir SpO_2
- Oxygen flow
- Healthcare utilization
- Dyspnea scale

3 Study Design

3.1 General Design

Following a screening period and observation that clinical status is stable and requiring substantial oxygen support for 48 hours, subjects with decompensation of ESLD or ALI with hypoxemia, will be approached for informed consent. The nature of the risks and benefits associated with participation in the study will be explained to all potential study subjects or legally authorized representative (LAR). Written informed consent must be obtained before the subject can begin any screening procedures that are not considered standard patient care.

Physiological data: Physiological data at screening, baseline, study day 1 – 5, and up to two days post voxelotor administration will be recorded from standard of care with the only exception being SpO_2/FiO_2 (S/F) ratio measurements, which will be obtained at the same intervals but by the qualified and delegated study staff or medical team through the use of an FiO_2 weaning maneuver.

Physiological measures to be recorded are detailed on the Schedule of Activities table 2.

FiO2 Weaning Maneuver: The process of titrating FiO_2 down slowly until the study patient's SpO_2 measurement is approximately 88%. This will provide the study team with values for an S/F ratio that more closely represents the transition to the steepest part of the oxygen dissociation curve (ODC). Measurements that represent this transition on the ODC will allow for more precise comparison and provide more accurate trending data. Once completed, the oxygen delivery system will be titrated back to achieve SpO_2 goals prescribed by the medical team.

Modified Borg Dyspnea Scale: Study subjects will be asked to rate their dyspnea symptoms daily to record their perceived shortness of breath.

Blood Sampling: Blood samples will be drawn as part of this study. Arterial blood gas samples will only be obtained for patients with an indwelling arterial line. Placement of an Aline is not a requirement for the study, but likely standard of care (SOC) for this patient population. The blood samples will be collected the day prior to voxelotor administration, on study day 1 – 5, and on the 2 wash out days following voxelotor administration. Blood will be collected and sent to a core laboratory for measurement of erythropoetin levels (for evidence of renal medullary hypoxia), drug trough level measurement via pharmacokinetics (PK), and P50. Local lab will be used for Hematology and Chemistry. A total of 109 mls of blood will be collected for this study.

See Schedule of Activities Table 2.

Central lab collection and processing:
PK, P50, and erythropoetin labs will be collected, processed, and stored as described in Study Lab Manual.

Voxelotor administration: The day after obtaining written, informed consent, 500 mg of voxelotor will be administered two times a day (for a total daily dose of 1000 mg per day) for 5 days. This dose may increase to a total maximum daily dose of 1500 mg (500 mg three times a day) if subject tolerates initial dose as determined by Principal Investigator (PI). Subjects will be dosed orally with approved tablets or recently approved dispersible tablet in water and given via feeding tube (when indicated). In the event the voxelotor administration needs to be modified during one of the study administration days for patient safety reasons, the PI will follow the dose modification guidelines for study related adverse events (Table 4). If the PI determines discontinuation of voxeletor is necessary for patient safety, then the protocol will progress to the wash out phase identified in the Schedule of Activities table 2.

If a patient on the lung transplant list receives an "initiation call" from the lung transplant coordinators, study medication will be held until a determination is made to proceed with solid organ transplantation. In the event the study patient does not receive the solid organ transplant, study medication administration will be resumed as previously scheduled and missed doses will be not be considered protocol deviations as the PK and pharmacodynamics (PD) of Voxelotor in the setting of surgery, blood loss, and blood transfusion is unknown. In the event the study patient receives a "go call" for solid organ lung transplant then study medication will be discontinued and the subject will proceed to the study wash out phase.

Voxelotor is oral administration only and the effect of blood transfusion "diluting" the Voxelotor modified RBCs is unknown, so the effect of Voxelotor in this setting will be confounded by the common scenario of blood transfusion and/or hemodilution and they will enter the wash-out period. Also, the indication for Voxelotor, to maintain SaO_2 in the face of a significant Alveolar-arterial pO_2 gradient, will have passed with the implantation of donor lungs.

Study completion is at the end of 2 day wash out period defined by 2 days after the last administration of Voxelotor.

Physiological measures and laboratory data to be recorded from electronic medical record (EMR) are detailed on the Schedule of Activities Table 2.

Table 2: Schedule of Activities and/or other clinical outcomes

	Baseline Measurements		**Dosing Period**					**Wash out Period**	
Study Day	**D-2**	**D-1**	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**
Screening	x								
Consent	x								
Voxelotor Dosing			x	x	x	x	x		
Physiological									
ESLD and ALI cohort (At rest and with exercise*)									
SpO_2	x	x	x	x	x	x	x	x	x
FiO_2	x	x	x	x	x	x	x	x	x
S/F ratio calculation	x	x	x	x	x	x	x	x	x
HR	x	x	x	x	x	x	x	x	x
ALI cohort									
paO_2	x	x	x	x	x	x	x	x	x
SaO_2	x	x	x	x	x	x	x	x	x
P/F ratio calculation	x	x	x	x	x	x	x	x	x
$paCO_2$	x	x	x	x	x	x	x	x	x
A-a gradient calculation	x	x	x	x	x	x	x	x	x
Borg Dyspnea Scale rating	x	x	x	x	x	x	x	x	x
Laboratory									
Erythropoietin (5ml)		x		x				x	
PK (2ml)		x	x	x	x	x	x	x	x
P50 (3ml)		x	x	x	x	x	x	x	x
BMP (4.5ml)			x	x	x	x	x	x	
CMP (4.5ml)		x							x
CBC (3ml)		x	x	x	x	x	x	x	x
Pregnancy test (2 ml)		x							
Con meds	x	x	x	x	x	x	x	x	x
Adverse Events			x	x	x	x	x	x	x

***only if feasible, ambulatory patients only. Exercise protocol is SOC and data collected will be reviewed from medical record.**

**

3.2 Primary Study Endpoints

The primary outcome will be change in S/F ratio from baseline to 2 days after initiation of Voxelotor treatment.

3.3 Secondary Study Endpoints

Secondary study endpoint is change in S/F ratio from baseline to 5 days after initiation of Voxelotor treatment.

***Exploratory endpoints*:** Pre and post Voxelotor use compare

- FiO_2 requirements
- Oxygen delivery modality
- Combined end-point of de-escalation of FiO_2 and/or oxygen delivery modality
- Nadir SpO_2
- Oxygen flow
- healthcare utilization
- Dyspnea scale

3.4 Primary Safety Endpoints

Based on current knowledge of safety profiles, a primary safety end-point will not be specified. Safety will be assessed by tracking SOC clinical laboratory tests (hematology profile, serum chemistry, erythropoietin and urinalysis), physical examinations, vital signs, 12-lead electrocardiogram (ECG) pre-administration and after Day 5. Hematology and chemistry labs will be screened before study drug administration and repeated daily until end of study. Vital signs will be monitored during study drug administration. Adverse events (AEs) and concomitant medications will be monitored and documented throughout the study. Of note, in a recent study in patients with impaired renal function, there was no deterioration of renal function. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7984382/

4 Subject Selection and Withdrawal

4.1 Inclusion Criteria

1. Oxygen dependency due to an end-stage lung disease (pre-lung transplant population) or ALI/acute lung injury (for example but not limited to primary allograft dysfunction, infectious pneumonia, aspiration, non-cardiogenic pulmonary edema). ALI will be defined as per Berlin criteria with a P/F ratio < 100 denoting severe acute respiratory distress syndrome (ARDS), < 200 denoting moderate and < 300 mild ARDS. ALI and mild ARDS are considered synonymous. In the event of inability to obtain arterial blood gas analysis to calculate a P/F ratio, we will consider a range of patients requiring standard nasal cannulae flowing at 6 LPM (liters per minute) to maintain SaO_2 > 90% as ALI, and Salter High -Flow nasal cannulae at 12-15 LPM in order to maintain SaO_2 > 85% as severe ARDS.
2. At least 48 hours of stable, increased oxygen requirement or ventilatory support prior to the start of drug administration if consented.

4.2 Exclusion Criteria

1. Minors (<18 years)
2. Pre-existing congestive cardiac failure (NYHA III or IV)
3. Medically significant, non-revascularized coronary artery disease
4. Inability to obtain informed consent from LAR
5. Pregnancy
6. Incarcerated individual.
7. Failure of another vital organ.
8. Severe hepatic impairment (Childs-Pugh C) or liver enzymes > 4x upper limit of normal (ULN) at screening.
9. Unstable acute kidney injury/rising creatinine.
10. Chronic neuromuscular disease requiring mechanical ventilation
11. Not anticipated to survive >48 hours
12. Limited therapeutic goals (do not resuscitate, etc.)
13. History of Pulmonary Embolism (PE)
14. Requires treatment with Fluconazole or other moderate and strong CYP3A4 inhibitors listed in section 5.6
15. A patient with active bleeding complications requiring more than 1 unit of blood transfusion per day.
16. Participated in another clinical trial of an investigational drug (or medical device) within 30 days or 5-half-lives, whichever is longer, prior to consent, or is currently participating in another trial of an investigational or marketed drug (or medical device)
17. Any condition including the presence of laboratory abnormalities, which places the subject at unacceptable risk if he/she were to participate in the study
18. Any condition or concomitant medication that confounds the ability to interpret data from the study or safely use Voxelotor.

4.3 Subject Recruitment and Screening

A screening period and observation that subject's clinical status is stable and requiring substantial oxygen support will be observed for 48 hours. Research team will be informed of subjects meeting eligibility criteria and plan to approach. A member of the subject's clinical care team will first approach an individual subject or LAR who is a potential candidate for participation. If the subject or LAR is interested in participating in the study, a study team member will explain the purpose, procedures, and intent of the study to each potential study subjects or LAR. Written informed consent must be obtained before the subject can begin any screening procedures that are not considered standard patient care.

In the event a legal representative provides consent at the time of enrollment, the subject's diminished capacity will be assessed throughout study enrollment, and they will be approached for consent if/when they regain the ability to make medical decisions for themselves.

Subjects will not be compensated for participating in the study.

4.4 Early Withdrawal of Subjects

4.4.1 When and How to Withdraw Subjects.

Study completion is at the end of 2 day wash out period defined by 2 days after the last administration of Voxelotor. If a patient withdraws informed consent, the patient will be withdrawn from Voxelotor study treatments and all study related assessments.

A patient will be withdrawn from the study treatment if any of the events listed below occur but should continue to be followed according to the intervention table:

- Decision by the Investigator that it is not in the best interest of a patient to continue treatment.
- Subjects are free to withdraw their consent at any time; the PI will withdraw a subject if any adverse response is thought to be due to Voxelotor.
- If a patient receives a "go call" for solid organ lung transplant then study medication will be discontinued and the subject will proceed to the study wash out phase, as the PK and PD of Voxelotor in the setting of surgery, blood loss, and blood transfusion is unknown. Voxelotor is oral administration only and the effect of blood transfusion "diluting" the Voxelotor modified RBCs is unknown, so the effect of Voxelotor in this setting will be confounded by the common scenario of blood transfusion and/or hemodilution. Also, the indication for Voxelotor, to maintain SaO_2 in the face of a significant Alveolar-arterial pO_2 gradient, will have passed with the implantation of donor lungs.
- If a patient suffers bleeding complications requiring blood transfusion, as the PK and PD of Voxelotor in the setting of blood loss and blood transfusion is unknown. The effect of blood transfusion "diluting" the Voxelotor modified RBCs is unknown, so the effect of Voxelotor in this setting will be confounded and they will enter the wash-out period.
- As Voxelotor increases the binding of oxygen to hemoglobin (providing the treatment effect of increased SpO_2 for a given pO_2), we will monitor for the possibility of inadequate oxygen being off-loaded to the tissues by tracking venous blood gases for evidence of metabolic acidosis from tissue beds and tracking renal function as a reflection of adequacy of tissue oxygen during administration of Voxelotor.

To provide a complete safety review of each patient, participants will be enrolled and end study participation before the next subject is enrolled. This will be done for the first five participants. For the remaining 15 participants, when an SAE occurs with a subject, enrollment of new participants will pause until the SAE is completely reviewed by the PI. After three unexpected, related SAEs are reported in this study, the enrollment of new participants will be halted.

4.4.2 Data Collection and Follow-up for Withdrawn Subjects

Patients enrolled in this study will receive 5 days of Voxelotor treatment, in the perioperative setting of lung transplantation for some subjects. In the event a patient receives a lung transplant during the 5-day treatment period, follow-up of survival status will continue through the 2 day wash out period post-transplant. Many confounding reasons related to the complexity of the recovery and the complication profile of lung transplantation render further follow-up unrealistic beyond the duration that subjects receive Voxelotor, as supported by few safety concerns despite administration to many patients with significant co-morbidities.

5 Study Drug

5.1 Description

Voxelotor (GBT440) is a small molecule inhibitor of HbS polymerization which allosterically modifies Hb-O_2 affinity.

Voxelotor is a white-to-yellow-to-beige compound in crystalline Form II of GBT440 free base. It is non-hygroscopic. It is highly soluble in common organic solvents such as acetone and toluene and insoluble in water (approximately 0.03 mg/mL). Voxelotor drug substance is manufactured in accordance with GMP. The chemistry used in the production of Voxelotor drug substance is well established. The manufacturing process uses equipment commonly available in the industry.

Voxelotor will be supplied as 500 mg tablets, or as 100 mg and 300 mg dispersible tablets for a total daily dose of 1000 mg per day, which may be increased to a total maximum dose of 1500 mg per day if subject tolerates initial dose as determined per PI.

5.2 Treatment Regimen

All participants will receive Voxelotor, administered orally as approved tablets or dispersible tablets for the duration of their enrollment in the study. Subjects will be dosed orally or given via feeding tube (when indicated). Voxelotor in all formulations, may be taken with or without food. Voxelotor dispersible tablets should be dispersed in clear liquid (e.g., water, apple juice).

If the dispersible tablets are given, the instructions for preparation prior to administration are as follows:

Table 3: Volume of Liquid per Dose of Voxelotor Dispersible Tablet

Voxelotor Dispersible Tablet Dose (mg)	**Volume of Water (cc)**	
400 to 600	*No less than*	5
700 to 900	*No less than*	7.5
1000 to 1200	*No less than*	10
1500	*No less than*	12.5

Note: one cubic centimeter (cc) is equivalent to one milliliter (mL)

500 mg of voxelotor will be administered two times daily (for a total daily dose of 1000 mg per day) for 5 days. This dose may increase to a total maximum daily dose of 1500 mg (500 mg three times a day) if subject tolerates initial dose as determined by PI. In the event the voxelotor administration needs to be discontinued for patient safety reasons during one of the study administration days, further doses of voxelotor will be discontinued and the protocol will progress to the wash out phase identified in the schedule of activities (Table 2).

5.3 Method for Assigning Subjects to Treatment Groups

This is an open label single arm study. Subjects will not be randomized.

5.4 Preparation and Administration of Study Drug

Voxelotor will be dispensed from IDS Pharmacy. Drug will be picked up by research team member and delivered to patient's nurse.

5.5 Subject Compliance Monitoring

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).

The subjects will inpatient, and medications will be administered by the RN and noted in the record as given/not given. If study PI plans to dose modify/hold for AEs that affect safety and tolerability, a trial of reducing or holding (temporarily stopping) the dose may be used. In prior sickle cell disease (SCD) studies, modification of dose (or dosage hold) was utilized based upon the exact event and the grade of the event. Dose was reduced for Grade 2 AE and subject reassessed, Dose was held or discontinued (for SAE) for Grade 3, as determined by PI.

Table 4. Dose Modification Guidelines for Study Drug Related Adverse Events

Dose Reduction	
Event	**Recommended Action**
Grade ≥ 2 (NCI grading scale) AE deemed considered related to study drug by the Investigator AND Precludes continued dosing at the current dose level due to safety concern or lack of tolerability (in the Investigator's judgment)	**Study drug:** May be reduced by one (1) tablet. If, in the opinion of the Investigator, a Grade 2 AE has resolved to ≤ Grade 1, participant may resume study drug at the original dose. If, in the opinion of the Investigator, the AE poses a significant safety concern such that a dose hold is considered, the Investigator should contact the Medical Monitor.
Dose Interruption (Hold)	
Event	**Recommended Action**
Grade ≥ 3 (NCI grading scale) AE deemed considered related to study drug by the Investigator AND Precludes continued dosing at the current or at a reduced dose level due to safety concern or lack of tolerability in the Investigator's judgment	**Study drug:** Hold dose until ≤ Grade 2, then resume study drug at original dose. If, in the opinion of the Investigator, dosing should be resumed at a lower dose, contact the Medical Monitor for further discussion. If the AE recurs or worsens, reduce dose by one tablet. Maximum dose hold is 5 continuous days. If, in the opinion of the Investigator, a longer dose hold is clinically needed, the Medical Monitor should be contacted for discussion.
Drug Discontinuation	
Event	**Recommended Action**
Grade ≥ 3 study drug-related AE that, at the discretion of the Investigator, warrants discontinuation of study drug (eg, has not improved or resolved after dose hold).	**Study drug:** Discontinue study drug. If the Investigator considers that the participant would benefit from continuing treatment, the Medical Monitor should be contacted.

Abbreviations: AE, adverse event; SAE, serious adverse event.

All instances of voxelotor dose modification (dose reduction, interruption, or discontinuation) will be documented in the participant's medical record and study case report form (CRF). 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.

Study personnel will review the EMR on a daily basis to ensure compliance or determine reasons for omission.

5.6 Prior and Concomitant Therapy

Prior medications taken within two days before study entry will be collected in CRF. Concomitant medications will be recorded through Study Day 7.

Fluconazole will be prohibited as a concomitant medication and has been listed as an exclusion criterion. The focus on fluconazole is due to its relatively common usage in our patients but we will also censor concomitant use of all moderate and strong CYP3A4 inhibitors in this early stage pilot study. Specifically, we will avoid the following concomitant medications:

- Moderate inhibitors of CYP3A4: amiodarone, erythromycin, fluconazole, miconazole, isavuconazole, diltiazem, verapamil, delavirdine, amprenavir, fosamprenavir, conivaptan.
- Strong inhibitors of CYP3A4: Clarithromycin, telithromycin, nefazodone, itraconazole, ketoconazole, voriconazole/posaconazole, atazanavir, darunavir, indinavir, lopinavir, nelfinavir, ritonavir, saquinavir, tipranavir.

Voxelotor is a weak 3A4 inhibitor so close monitoring of tacrolimus and/or cyclosprorine levels is needed if these drugs are concomitant medications. Daily levels are routine when used for in-patients.

There may also be risks, discomforts, drug interactions, or side effects that are not yet known.

5.7 Packaging

Voxelotor dispersible formulation will be dispensed. Voxelotor 100 mg and 300 mg dispersible tablets contain voxelotor drug substance along with formulation excipients. With the exception of artificial grape flavor, all excipients used in the dispersible tablets are listed in the FDA IID. The artificial grape flavor is listed under the Flavor and Extract Manufacturers Association (FEMA) guidelines and is considered a Generally Recognized as Safe (GRAS) material. All the excipients used in the formulation are either compendial per European Pharmacopoeia (Ph Eur) or are composed of mixtures that are compendial per Ph Eur or accepted by E number or European Commission regulation.

Voxelotor tablets (500 mg) will be packaged in high-density polyethylene (HDPE) bottles with induction sealed polypropylene (PP) child-resistant (CR) caps. As an alternate packaging, tablets may be supplied in multilaminate blisters.

5.8 Blinding of Study Drug

This is an open –label study and subjects will not be randomized. There is no blinding.

5.9 Receiving, Storage, Dispensing and Return

5.9.1 Receipt of Drug Supplies

The initial shipment of voxelotor will be sent to Investigational Drug Services (IDS) Pharmacy once the site has met all requirements for activation by the Sponsor-Investigator and GBT. Initial quantity of study drug will be determined by study-specific forecasting criteria that includes, but not limited to the expected number of subject enrollment, treatment duration and dosing regimen.

Investigational Drug Services (IDS) Pharmacy will receive study drug shipped from sponsor. Upon receipt of the study treatment supplies, an inventory must be performed, and a drug receipt log filled out and signed by the person accepting the shipment. It is important that the designated study staff counts and verifies that the shipment contains all the items noted in the shipment inventory. Any damaged or unusable study drug in a given shipment (active drug or comparator) will be documented in the study files. The investigator must notify study sponsor of any damaged or unusable study treatments that were supplied to the investigator's site.

5.9.2 Storage

Storage and Handling Procedure

All study medications will be stored in IDS pharmacy at controlled room temperature between 15°C to 25°C in the storage area of the investigational site pharmacy, which is a secure, temperature controlled, locked environment with restricted access. No special procedures for the safe handling of voxelotor drug products are required.

5.9.3 Dispensing of Study Drug

IDS pharmacy will maintain a Study Drug Dispensing Log. The following information will be recorded:

- Total amount of study drug dispensed (quantity of tablets dispensed)
- Identification (study number and initials) of the patient to whom the study drug was dispensed;
- Initials of the person who dispensed the study drug;
- Initials of the person who received the study drug for administration to the patient

5.9.4 Return or Destruction of Study Drug

The Investigator is responsible to ensure that all study drug is accurately accounted for. Any discrepancies must be described in writing. At the completion of the study, there will be a final reconciliation of drug shipped, drug consumed, and drug remaining. This reconciliation will be logged on the drug reconciliation form, signed and dated. Any discrepancies noted will be investigated, resolved, and documented prior to return or destruction of unused study drug. Drug destroyed on site will be documented in the study files. Any unused voxelotor will be returned to the Sponsor at the conclusion of the study or destroyed according to clinical site's SOP as mutually agreed upon by the Sponsor and the site.

6 Study Procedures

6.1 Screening (2 days before Voxelotor administration)

The following assessments will be performed during screening:

- Determine eligibility based on inclusion/exclusion criteria
- Obtain voluntarily given, written informed consent from subject or LAR
- Record demographics
- Record medical history
- Collect baseline physiological data for ESLD and ALI at rest and with exercise*
    - SpO_2
    - FiO_2
    - S/F ratio calculation
    - Heart rate
    - Modified Borg Dyspnea scale (MBDs) rating
- Collect additional baseline physiological data with ALI group
    - PaO_2
    - SaO_2
    - P/F ratio calculation
    - $PaCO_2$
    - A-a gradient calculation

6.2 -1 day before Voxelotor administration

The following assessments will be performed during Baseline:

- Collect baseline physiological data for ESLD and ALI at rest and with exercise*
    - SpO_2
    - FiO_2
    - S/F ratio calculation
    - Heart rate
    - MBDs rating
- Collect additional baseline physiological data with ALI group
    - Pa02
    - SaO_2
    - P/F ratio calculation
    - $PaCO_2$
    - A-a gradient calculation
- Blood sampling for central lab
    - Erythropoetin (5ml)
    - P50 (3ml)
    - PK (2ml)
- Blood sampling for local lab
    - Pregnancy test for women of childbearing potential if not done as SOC (2ml)
    - CBC (3ml)
    - CMP (4.5ml)

6.3 Day 1

- Blood sampling for central lab
    - P50 (3ml)
    - PK (2ml)
- Blood sampling for local lab
    - CBC (3ml)
    - BMP (4.5ml)
- Administer 500 mg Volexotor twice daily (or up to three times a day if patient tolerates initial dose per PI)
- Collect daily physiological data for ESLD and ALI at rest and with exercise*
    - SpO_2
    - FiO_2
    - S/F ratio calculation
    - Heart rate
    - MBDs rating
- Collect additional daily physiological data with ALI group
    - PaO_2
    - SaO_2
    - P/F ratio calculation
    - $PaCO_2$
    - A-a gradient calculation
- Record adverse events

6.4 Day 2

- Blood sampling for central lab
    - P50 (3ml)
    - PK (2ml)
    - Erythropoietin (5ml)
- Blood sampling for local lab
    - CBC (3ml)
    - BMP (4.5ml)
- Administer 500 mg Volexotor twice daily (or up to three times a day if patient tolerates initial dose per PI)
- Collect daily physiological data for ESLD and ALI at rest and with exercise*
    - SpO_2
    - FiO_2
    - S/F ratio calculation
    - Heart rate
    - MBDs rating
- Collect additional daily physiological data with ALI group
    - PaO_2
    - SaO_2
    - P/F ratio calculation

    - $PaCO_2$
    - A-a gradient calculation
- Record adverse events

6.5 Day 3-5

- Blood sampling for central lab
    - P50 (3ml)
    - PK (2ml)
- Blood sampling for local lab
    - CBC (3ml)
    - BMP (4.5ml)
- Administer 500 mg Volexotor twice daily (or up to three times a day if patient tolerates initial dose per PI)
- Collect daily physiological data for ESLD and ALI at rest and with exercise*
    - SpO_2
    - FiO_2
    - S/F ratio calculation
    - Heart rate
    - MBDs rating
- Collect additional daily physiological data with ALI group
    - PaO_2
    - SaO_2
    - P/F ratio calculation
    - $PaCO_2$
    - A-a gradient calculation
- Record adverse events

6.6 Day 6 (Wash out period)

- Collect daily physiological data for ESLD and ALI at rest and with exercise*
    - SpO_2
    - FiO_2
    - S/F ratio calculation
    - Heart rate
    - MBDs rating
- Collect additional daily physiological data with ALI group
    - PaO_2
    - SaO_2
    - P/F ratio calculation
    - $PaCO_2$
    - A-a gradient calculation
- Blood sampling for central lab
    - PK (2ml)
    - P50 (3ml)
    - Erythropoetin (5ml)

- Blood sampling for local lab
    - CBC (3ml)
    - BMP (4.5ml)
- Record adverse event

6.7 Day 7 (Wash out period)

- Collect daily physiological data for ESLD and ALI at rest and with exercise*
    - SpO_2
    - FiO_2
    - S/F ratio calculation
    - Heart rate
    - MBDs rating
- Collect additional daily physiological data with ALI group
    - PaO_2
    - SaO_2
    - P/F ratio calculation
    - $PaCO_2$
    - A-a gradient calculation
- Blood sampling for central lab
    - PK (2ml)
    - P50 (3ml)
- Blood sampling for local lab
    - CBC (3ml)
    - CMP (4.5ml)
- Record adverse event

***only if feasible/ ambulatory patients only. Exercise protocol is SOC and data collected will be reviewed from medical record.**

7 ** Statistical Plan

7.1 Sample Size Determination

This is a pilot study and will be used to determine effect size for future prospective study

7.2 Statistical Methods

Descriptive statistics for patient characteristics and baseline factors will be presented as median and interquartile range for continuous variables and number and percentage for categorical variables. The primary outcome will be change in S/F ratio from baseline to 2 days after initiation of voxelotor treatment, and the secondary outcome will be change in S/F ratio from baseline to 5 days after initiation of voxelotor treatment. Each will be compared using the Wilcoxon Signed Rank test. The baseline value will be defined as the median S/F ratio measurement during the 48-hour period before initiation of voxelotor treatment. Similarly, the

day 2 and day 5 S/F ratio values will be defined as the median S/F ratio measurement on the second and fifth days after initiation of treatment.
All S/F ratio values will also be analyzed using a mixed-effects model with a random intercept for each patient and time in hours (relative to initiation of voxelotor treatment) as a fixed effect. Quadratic and cubic terms for time will also be included to allow for flexible modeling of treatment effect over time. We will tabulate and report all observed adverse events occurring during this trial.

For the ALI group with an arterial line in place, the PaO_2/FiO_2 (P/F) ratio will also be compared in a similar manner to identify whether any improvement in S/F ratio is due to clinical improvement of ALI, as reflected in the P/F ratio. The concept of Voxelotor use is an improvement in the S/F ratio (the result of left-shift in p50) for a given P/F ratio (the result of pulmonary function limiting PaO_2). This may be especially important for the ALI sub-study cohort, as ALI could improve during the 5 day dosing period. Realistically, however, such progress is typically slow and, in contrast, the end-stage lung disease cohort would not be expected to improve and S/F ratio alone would reflect voxelotor effect without the need to track P/F ratios.

7.3 Subject Population(s) for Analysis

- Protocol-compliant population: Any subject who was randomized and received the protocol required study drug exposure and required protocol processing will be the primary analysis population.
- All-treated population: Any subject randomized into the study that received at least one dose of study drug will be subject to secondary analyses.

8 Safety and Adverse Events

8.1 Definitions

Adverse Event

An ***adverse event*** (AE) is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries should be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

- results in study withdrawal
- is associated with a serious adverse event
- is associated with clinical signs or symptoms
- leads to additional treatment or to further diagnostic tests
- is considered by the investigator to be of clinical significance

AE Classification. The following definitions were based on guidelines provided by the Office for Human Research Protections (OHRP). The following URL provides useful examples to help clarify classification of adverse events https://www.hhs.gov/ohrp/regulations-and-policy/guidance/reviewing-unanticipated-problems/index.html

An AE is deemed an ***unanticipated problem*** if it is not expected given (a) the known or foreseeable risk of the study procedures that are described in the informed consent document or study protocol, (b) the characteristics of the population being studied, (c) related or possibly related to participation in the study, and (d) suggests the research places subjects are greater risk of harm than previously known.

Serious Adverse Event (SAE)

Adverse events are classified as serious or non-serious. A ***serious adverse event*** is any AE that is:

- fatal
- life-threatening
- requires or prolongs hospital stay
- results in persistent or significant disability or incapacity
- a congenital anomaly or birth defect
- an important medical event

Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the subject, and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in in-patient hospitalization, or intensive treatment of bronchospasm in an emergency department would typically be considered serious.

All adverse events that do not meet any of the criteria for serious should be regarded as ***non-serious adverse events***.

Adverse Event Reporting Period

The study period during which adverse events must be reported is normally defined as the period from the initiation of any study procedures to the end of the study treatment follow-up. For this study, subjects will be followed for AEs from the time of voxelotor administration until 2 days after the last administration of voxelotor as defined by the wash out period.

Preexisting Condition

A preexisting condition is one that is present at the start of the study. A preexisting condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

General Physical Examination Findings

At screening, any clinically significant abnormality should be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event must also be recorded and documented as an adverse event.

Post-study Adverse Event

All unresolved adverse events should be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the investigator should instruct each subject to report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study. The investigator should notify the study sponsor of any death or adverse event occurring at any time after a subject has discontinued or terminated study

participation that may reasonably be related to this study. The sponsor should also be notified if the investigator should become aware of the development of cancer or of a congenital anomaly in a subsequently conceived offspring of a subject that has participated in this study.

Abnormal Laboratory Values

A clinical laboratory abnormality should be documented as an adverse event if any one of the following conditions is met:

- The laboratory abnormality is not otherwise refuted by a repeat test to confirm the abnormality
- The abnormality suggests a disease and/or organ toxicity
- The abnormality is of a degree that requires active management; e.g. change of dose, discontinuation of the drug, more frequent follow-up assessments, further diagnostic investigation, etc.

Hospitalization, Prolonged Hospitalization or Surgery

Any adverse event that results in hospitalization or prolonged hospitalization should be documented and reported as a serious adverse event unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an adverse event if the condition meets the criteria for and adverse event.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an adverse event in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should ***not*** be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study.
- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.

8.2 Recording of Adverse Events

At each contact with the subject, the investigator must seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events should be recorded immediately in the source document of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic procedures results should be recorded in the source document, though should be grouped under one diagnosis.

All adverse events occurring during the study period must be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. Serious adverse events that are still ongoing at the end of the study period must be followed up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study treatment or study participation should be recorded and reported immediately.

Requirements for reporting of an AE to various entities (IRB, FDA, and Sponsor) differ according to whether or not the AE represents an unanticipated problem. This study will follow 21 CFR 312.32 IND Safety Reporting guidelines and DUHS prompt reporting to the IRB policy for events that occur with this study.

Subjects in this study will be followed for AEs from the time of voxelotor administration until 2 days after the administration of voxelotor as defined by study wash out period. The PI will be responsible for ensuring participants' safety.

Dr. Ian Welsby will be the physician responsible for reviewing and evaluating all adverse events. Dr. Welsby will evaluate the safety of voxelotor treatment in all subjects by following adverse events and assigning likelihood of causality. Related AEs will be reported to IRB. Nonrelated AEs will be tracked by the study team.

8.3 Reporting of Serious Adverse Events

8.3.1 Study Sponsor Notification by Investigator

A serious adverse event must be reported to GBT within 24 hours of the event. A Serious Adverse Event (SAE) form must be completed by the investigator and faxed to the study sponsor within 24 hours. The investigator will keep a copy of this SAE form on file at the study site.

Report serious adverse events by email or facsimile to:
globalbloodtx@primevigilance.com or 1-650-243-3433

At the time of the initial report, the following information should be provided:

- Study identifier
- Study Center
- Subject number
- A description of the event
- Date of onset
- Current status
- Whether study treatment was discontinued
- The reason why the event is classified as serious
- Investigator assessment of the association between the event and study treatment

Within the following 48 hours, the investigator must provide further information on the serious adverse event in the form of a written narrative. This should include a copy of the completed Serious Adverse Event form, and any other diagnostic information that will assist the understanding of the event. Significant new information on ongoing serious adverse events should be provided promptly to the study sponsor

8.3.2 EC/IRB Notification by Investigator

Reports of all serious adverse events (including follow-up information) must be submitted to the EC/IRB within 10 working days. Copies of each report and documentation of EC/IRB notification and receipt will be kept in the Clinical Investigator's binder.

8.3.3 FDA Notification by Sponsor

The study sponsor shall notify the FDA by telephone or by facsimile transmission of any unexpected fatal or life-threatening experience associated with the use of the drug as soon as possible but no later than 7 calendar days from the sponsor's original receipt of the information. Other serious, unexpected adverse events associated with the use of study drug shall be reported to the FDA no later than 15 calendar days from the sponsors' original receipt of the information.

If a previous adverse event that was not initially deemed reportable is later found to fit the criteria for reporting, the study sponsor will submit the adverse event in a written report to the FDA as soon as possible, but no later than 15 calendar days from the time the determination is made.

8.4 Stopping Rules

To provide a complete safety review of each patient, participants will be enrolled and end study participation before the next subject is enrolled. This will be done for the first five participants. For the remaining 15 participants, when an SAE occurs with a subject, enrollment of new participants will pause until the SAE is completely review by the PI. After three unexpected, related SAEs are reported in this study, the enrollment of new participants will be halted.

When an unexpected rate of expected SAEs occurs, the enrollment of new participants will be halted.

8.5 Medical Monitoring

It is the responsibility of the Principal Investigator to oversee the safety of the study at his/her site. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above, as well as the construction and implementation of a site data and safety-monitoring plan (see section 9 Auditing, Monitoring and Inspecting). Medical monitoring will include a regular assessment of the number and type of serious adverse events. This study will be subject to Duke clinical quality management plan (CQMP) policy and will have designated review.

9 Data Handling and Record Keeping

9.1 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts

should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

9.2 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

9.3 Case Report Forms

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF must be recorded. All missing data must be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, write "N/D". If the item is not applicable to the individual case, write "N/A". All entries should be printed legibly in black ink. If any entry error has been made, to correct such an error, draw a single straight line through the incorrect entry and enter the correct data above it. All such changes must be initialed and dated. DO NOT ERASE OR WHITE OUT ERRORS. For clarification of illegible or uncertain entries, print the clarification above the item, then initial and date it.

9.4 Records Retention

It is the investigator's responsibility to retain study essential documents for at least 2 years after the last approval of a marketing application in their country and until there are no pending or contemplated marketing applications in their country or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period if required by an agreement with the sponsor. In such an instance, it is the responsibility of the sponsor to inform the investigator/institution as to when these documents no longer need to be retained.

10 Study Monitoring, Auditing, and Inspecting

10.1 Study Monitoring Plan

This study will be monitored according to the monitoring plan in Duke CQMP. The investigator will allocate adequate time for such monitoring activities. The Investigator will also ensure that the monitor or other compliance or quality assurance reviewer is given access to all the above noted study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visit.

10.2 Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the EC/IRB, the sponsor, government regulatory bodies, and University compliance and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable University compliance and quality assurance offices.

11 Ethical Considerations

This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted independent Ethics Committee (EC) or Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the EC/IRB concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be provided to the sponsor before commencement of this study. The investigator should provide a list of EC/IRB members and their affiliate to the sponsor.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. This consent form will be submitted with the protocol for review and approval by the EC/IRB for the study. The formal consent of a subject, using the EC/IRB-approved consent form, must be obtained before that subject is submitted to any study procedure. This consent form must be signed by the subject or legally acceptable surrogate, and the investigator-designated research professional obtaining the consent.

12 Study Finances

12.1 Funding Source

This study is financed through a grant from the Global Blood Therapeutics (GBT). GBT will provide voxelotor to subjects free of charge.

12.2 Conflict of Interest

Any investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed by a properly constituted Conflict of Interest Committee with a Committee-sanctioned conflict management plan that has been reviewed and approved by the study sponsor prior to participation in this study. All University of Pennsylvania investigators will follow the University conflict of interest policy.

13 Publication Plan

The intent is to publish our data in peer-reviewed scientific literature. GBT will have a courtesy review prior to publication. Affiliations and inclusions of study support will be transparent. Neither the complete nor any part of the results of the study carried out under this protocol, nor any of the information provided by the sponsor for the purposes of performing the study, will be published or passed on to any third party without the consent of the study sponsor. Any investigator involved with this study is obligated to provide the sponsor with complete test results and all data derived from the study.

14 References

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