PROVENCE TECHNOLOGIES GROUP

NCT#03395223

Provepharm SAS

CLINICAL STUDY PROTOCOL – CONFIDENTIAL

MEBIPAM - ***ME****thylene* ***B****lue* ***I****n* ***P****atients with* ***A****cquired* ***M****ethemoglobinemia*

Protocol Number:	PVP-2016003
Investigational New Drug (IND) Number:	IND: 118,156
Study Medication:	ProvayBlue™ methylene blue
Title:	Open label clinical study to evaluate the safety and efficacy of ProvayBlue™ (methylene blue) for the treatment of acquired methemoglobinemia
Version and Date:	Final Version 3 dated 10 August 2017
Sponsor:	Provepharm SAS 22 Rue Marc Donadille 13013 Marseille, France
Medical Monitor:	[redacted]
Sponsor's Contact:	[redacted]

1. SIGNATURE PAGE

Open label clinical study to evaluate the safety and efficacy of ProvayBlue™ (methylene blue) for the treatment of acquired methemoglobinemia

Sponsor

[redacted]

[redacted]

Signature

[redacted]

Date

Co-ordinating Investigator

[redacted]

[redacted]

Signature

[redacted]

Date

Project Manager

[redacted]

[redacted]

Signature

[redacted]

Date

2. PROTOCOL AGREEMENT PAGE

I confirm that I have read and that I understand this protocol, the Investigator Brochure (IB), and all other product information provided by Provepharm SAS (the Sponsor). I agree to conduct this study in conformity with this protocol and also in accordance with the ethical principles that have their origin in the Declaration of Helsinki, the E6 ICH Harmonized Tripartite Guideline for Good Clinical Practice, all applicable laws and regulations, including, without limitation, data privacy laws and regulations, and the regulatory requirements for reporting serious adverse events defined in Section 15.3 of this protocol.

Principal Investigator

Title and Name: _____

Hospital Name: _____

Signature: _____

Date : _____

3. SYNOPSIS

Study title:	Open label clinical study to evaluate the safety and efficacy of ProvayBlue™ (methylene blue) for the treatment of acquired methemoglobinemia
Protocol number:	PVP-2016003
Type of study:	Phase 4
Sponsor:	Provepharm SAS
Co-ordinating Investigator:	[redacted]
Study medication:	ProvayBlue™ (methylene blue injection USP)
Dose to be studied:	1 mg/kg
Dosage form:	Each ProvayBlue™, 10 mL single-dose ampule contains 50 mg Proveblue® methylene blue and water for injection q.s. Each mL of solution contains 5 mg methylene blue and water for injection q.s.
Route:	ProvayBlue™ is a sterile solution intended for intravenous (IV) administration.
Dosing regimen:	ProvayBlue™ has been approved by the US FDA to be administered intravenously at a dose of 1 mg/kg over 5-30 minutes and if the methemoglobin (metHb) level remains above 30% or if clinical symptoms persist, to give a repeat dose of up to 1 mg/kg 1 hour after the first dose. Depending on the clinical status of the patient, additional doses may be administered at the discretion of the Investigator. The data on these additional doses will be recorded in the study Case Report Form (CRF). If ProvayBlue™ is administered as an infusion, it MUST be diluted in 5% dextrose in water (D5W) only. Dilution in saline will result in precipitation of the product and must not be administered.
Study centers:	The study will include sites which will be based in France, Germany, UK and the USA.
Study objectives:	**Primary Objective** • To confirm that ProvayBlue™ after a single administration is efficacious in patients with acquired methemoglobinemia. **Secondary Objectives** • To evaluate the efficacy of a single dose of 1 mg/kg of ProvayBlue™; • To evaluate the efficacy of a second dose of 1 mg/kg of ProvayBlue™ to further reduce metHb levels when metHb is not fully reduced by a single dose;

	• To evaluate the normalization of the respiratory rate, heart rate and blood pressure of patients who achieve a reduction in metHb level within 2 hours of receiving the first dose of ProvayBlue™; • To confirm the safety and tolerability of ProvayBlue™ injection in patients with acquired methemoglobinemia. **Exploratory Objective** • To collect sparse pharmacokinetic (PK) data in patients.
Study design:	This is an open label, uncontrolled Phase 4 study of 10 patients who present in hospital/urgent care setting with acquired methemoglobinemia. The population may include pediatric and adult patients (males and females of all ages are included). The aim of the study is to confirm safety and efficacy of ProvayBlue™ for the treatment of acquired methemoglobinemia. This study comprises of an analysis of data for patients presenting with acquired methemoglobinemia and requiring ProvayBlue™. The analysis itself will focus on clinical outcome of treatment with ProvayBlue™ alone. If possible, depending on the number of patients, the data will be summarized by age, sex and body weight.
Study population:	Patients who present in hospital/urgent care setting diagnosed with acquired methemoglobinemia. The population may include pediatric and adult patients (males and females of all ages are included).
Diagnosis and main criteria for inclusion:	**Inclusion Criteria** 1. Pediatric or adult patients (males and females of all ages are included) diagnosed with acquired methemoglobinemia and receiving treatment with ProvayBlue™ as per the treating physician's diagnosis and hospital standard of care. Acquired methemoglobinemia is defined as a level of methemoglobinemia >30% or ≤30% in case of clinical symptoms (e.g. sleepiness, cyanosis, dizziness, etc.). 2. Written informed consent obtained prior to any data collection (retrospective and prospective) for this study and study specific assessments. **Exclusion criteria** 1. Known severe hypersensitivity reactions to methylene blue or any other thiazine dye; 2. Known deficiency in glucose-6-phosphate dehydrogenase (G6PD) due to the risk of hemolytic anemia as well as lack of therapeutic effect; 3. Known deficiency in Nicotinamide Adenine Dinucleotide Phosphate Hydrogen (NADPH) reductase.

	4. Known use of selective serotonin reuptake inhibitors (SSRIs), serotonin and norepinephrine reuptake inhibitors (SNRIs), MonoAmine Oxidase (MAO) inhibitors or drugs metabolised via CYP isoenzymes anticipated during the treatment phase of the study. 5. Women who refuse to stop breastfeeding for up to 8 days after receiving the last dose of ProvayBlue™.
Total sample size:	Because this is an ultra-orphan indication, the enrollment will be 10 patients, in adult and pediatric population. Based on evaluation of historical data, the potential enrollment for this study is estimated to be 1/3 patient per site per year (i.e., one patient per site over the study period of approximately 32 months). Thus it is expected that 10 patients may be enrolled during the 32-months-study. A sample size of 10 will provide at least 80% power to detect a 90% response rate and exclude a response rate less than 50%. As this is an open label study with only one treatment there will be no randomization and no blinding of subjects. Study numbers will be allocated to subjects in the order in which they are enrolled into the study and by site.
Number of planned visits:	The trial includes the following visits: • Screening and enrolment • Study Treatment - H0: ProvayBlue™ Dose 1 - H1: 1 hour after the end of ProvayBlue™ infusion. Administration of ProvayBlue™ Dose 2 (if applicable) - H2: 2 hours after the end of ProvayBlue™ Dose 1 infusion - H3: 2 hours after the end of ProvayBlue™ Dose 2 infusion (if applicable) • H24 (24 hours after the end of ProvayBlue™ Dose 1 infusion) • Hospital Discharge (if earlier than H24) • Follow up visit (5 -10 days following the last ProvayBlue™ administration) • Follow up call (10 -15 days following the last ProvayBlue™ administration)
Efficacy parameters:	The following efficacy parameters will be assessed: • A reduction in metHb by 50% within 1 hour of dosing;

	• Concomitant normalization of respiratory rate within 2 hours of the first dose of ProvayBlue™; • Concomitant normalization of heart rate within 2 hours of the first dose of ProvayBlue™; • Concomitant normalization of diastolic blood pressure (DBP) within 2 hours of the first dose of ProvayBlue™; • Concomitant normalization of systolic blood pressure (SBP) within 2 hours of the first dose of ProvayBlue™.
Pharmacokinetic parameters:	• PK samples will be analyzed for methylene blue and azure B. PK samples are to be taken at the discretion of the Investigator based on the condition of the patient. The time blood sample(s) are taken should be recorded, but there is no absolute time window for the PK samples. Suggested PK sampling time points are as follows: • Approximately 1 hour after the end of each ProvayBlue™ infusion (i.e. Dose 1, Dose 2 and any additional dose if applicable); • Prior to Dose 2 ProvayBlue™ infusion (if applicable); • Approximately 24 hours post the end of the last ProvayBlue™ infusion, or just prior to discharge from the clinic if earlier than 24 hours.
Safety parameters:	The following safety parameters will be assessed: • Physical examination; • Vital signs; • Hematology and biochemistry; • Electrocardiogram (ECG); • Adverse events (AEs).
Statistical methods:	Statistical analyses will be performed by Orion under the authority of the Sponsor. Statistical analyses will be carried out using SAS®, Version 9.3 or later, SAS Institute, Cary, North Carolina, USA. The statistical methods for this study will be described in a detailed statistical analysis plan (SAP), which will be finalized prior to database lock. Any deviations from the planned analysis as described in the SAP will be justified and recorded in the final clinical study report.

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5. LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

ADR	Adverse Drug Reaction
ADL	Activities of Daily Living
AE	Adverse Event
BP	Blood Pressure
°C	Degrees Celsius
CI	Confidence Interval
CRA	Clinical Research Associate
CRF	Case Report Form
CTC	Common Toxicity Criteria
CYP	Cytochrome
DBP	Diastolic Blood Pressure
ECG	Electrocardiogram
EMA	European Medicine Agency
FAS	Full Analysis Set
FDA	Food and Drug Administration
FWA	Federal Wide Assurance
g	Gram(s)
G6PD	Glucose-6-phosphate Dehydrogenase
GCP	Good Clinical Practice
IB	Investigator Brochure
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IND	Investigational New Drug
IRB	Institutional Review Board
ITT	Intent-to-Treat
IV	Intravenous
Kg	Kilogram(s)
LMB	Leucomethylene Blue
LPLV	Last Patient Last Visit
MAO	MonoAmine Oxidase
MB	Methylene Blue
MedDRA	Medical Dictionary for Regulatory Activities
metHb	Methemoglobin
mg	Milligram(s)

µl	Microliter(s)
mL	Milliliter(s)
mmHg	Millimeters of Mercury
NADPH	Nicotinamide Adenine Dinucleotide Phosphate Hydrogen
NCI	National Cancer Institute
PI	Principal Investigator
PICD	Patient Informed Consent Document
PK	Pharmacokinetics
PP	Per-Protocol
QA	Quality Assurance
QC	Quality Control
QP	Qualified Person
qs	The Amount which is Enough (*Quantum Satis*)
RA	Regulatory Authority
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SBP	Systolic Blood Pressure
SD	Standard Deviation
SNRIs	Serotonin and Norepinephrine Reuptake Inhibitors
SPC	Summary of Product Characteristics
SSRIs	Serotonin Reuptake Inhibitors
SUSAR	Suspected, Unexpected Serious Adverse Reaction
t½	Elimination half life
TEAE	Treatment-emergent Adverse Event
UK	United Kingdom
US	United States
USP	United States Pharmacopeial Convention
WHO	World Health Organization

6. INTRODUCTION

6.1. RATIONALE FOR THE STUDY

ProvayBlue™[1] (methylene blue injection USP) was approved on 08 April 2016 by the United States (US) Food and Drug Administration (FDA) for the treatment of acquired methemoglobinemia under accelerated approval (see APPENDIX 1: US Package Insert of ProvayBlue™).

As a post-marketing requirement, Provepharm is required to conduct a clinical study to evaluate the safety and efficacy of ProvayBlue™ for the treatment of acquired methemoglobinemia. The minimal efficacy endpoints should include achieving a 50% reduction in methemoglobin (metHb) within 1 hour of the first dose of ProvayBlue™ in addition to normalization of the respiratory rate, heart rate and blood pressure within 2 hours of the first dose of ProvayBlue™.

ProvayBlue™ will be administered as per the APPENDIX 1: US Package Insert of ProvayBlue™.

In this open label, prospectively designed clinical study to evaluate the safety and efficacy of ProvayBlue™ for the treatment of acquired methemoglobinemia, ProvayBlue™ will be administered to patients who present in hospital/urgent care setting with acquired methemoglobinemia. ProvayBlue™ will be administered as per the treating physician's diagnosis and hospital standard of care. ProvayBlue™ has been approved by the US FDA to be administered intravenously at a dose of 1 mg/kg over 5-30 minutes and if the metHb level remains above 30% or if clinical symptoms persist, to give a repeat dose of up to 1 mg/kg 1 hour after the first dose (see APPENDIX 1: US Package Insert of ProvayBlue™ for dosing guidance).

The primary objective in this study is to confirm the efficacy of ProvayBlue™ after a single administration in patients with acquired methemoglobinemia.

6.2. ACQUIRED METHEMOGLOBINEMIA

Methemoglobinemia is generally caused by an abnormal increase in blood concentration of an altered form of hemoglobin, metHb, where one or several iron molecules are found oxidized in the ferric state (Fe3+), instead of the normal ferrous form (Fe2+). This modification makes the hem group unable to bind to a molecule of oxygen. In addition, the change in conformation of the metHb structure reinforces the oxygen affinity with the remaining ferrous hems, increasing the impairment in the oxygen delivery to tissues (Hersh 2004; Lunenfeld and Kane 2004; Beutler 2005; Kane et al. 2007; Umbreit 2007).

Methemoglobinemia is characterized by a reduced ability of the blood to carry oxygen, due to lower than normal levels of hemoglobin (Beutler 2005; Hersh 2004). Methemoglobinemia can be inherited, but is more commonly acquired following exposure to toxic agents (drugs or environmental toxins).

[1] "ProvayBlue™" is the name of the medicinal product registered in the USA. The approved name in European Union for this product is "Methylthioninium chloride Proveblue".

6.3. CLINICAL SYMPTOMS ASSOCIATE WITH METHEMOGLOBINEMIA

Clinical symptoms of methemoglobinemia vary according to the level of metHb in the blood. MetHb is found at a normal range of 1%, as a fraction of the total hemoglobin species (Hersh 2004; Lunenfeld and Kane 2004).

Symptoms typically are proportional to the level of metHb as shown in Table 6-1 below.

Table 6-1: Signs and Symptoms Related to Blood Levels of Methemoglobin (metHb)

Level of Methemoglobin	Symptoms/Outcome
<3%	No symptoms
3-15%	Grayish-blue skin discoloration only (most notably on mucus membranes)
15-30%	Cyanosis, chocolate-colored blood
30-50%	Dyspnea, headache, fatigue, weakness, dizziness, syncope
50-70%	Coma, seizures, depressed central nervous system, arrhythmias, metabolic acidosis
>70%	Death

Source: Do Nascimento 2008

6.4. TREATMENT OF ACQUIRED METHEMOGLOBINEMIA

Currently, a consensus of opinion exists among the medical community regarding use of methylene blue (MB) in cases of methemoglobinemia (Martindale 2007; Therapeutic Drugs 1999). MB allows the rapid conversion of metHb into normal hemoglobin and rapid improvement of methemoglobinemia symptoms. The broad use of MB in this indication is documented by case reports available in the literature and is the current standard of care.

6.5. PROVAYBLUE™

ProvayBlue™ is an oxidation-reduction agent indicated for the treatment of pediatric and adult patients with acquired methemoglobinemia (refer to APPENDIX 1: US Package Insert of ProvayBlue™).

Methylene blue is a water soluble thiazine dye that promotes a non-enzymatic redox conversion of metHb to hemoglobin. *In situ*, methylene blue is first converted to leucomethylene blue (LMB) via nicotinamide adenine dinucleotide phosphate (NADPH) reductase. It is the LMB molecule which then reduces the ferric iron of metHb to the ferrous state of normal hemoglobin.

The efficacy of ProvayBlue™ was assessed on the basis of a metHb decrease of at least 50% within 1 hour after intravenous (IV) administration of 1–2 mg/kg ProvayBlue™ (or a bioequivalent formulation) in 6 patients identified by retrospective chart review or literature search. All 6 patients had a decrease in metHb by at least 50% within 1 hour after treatment.

An additional 41 cases of treatment of methemoglobinemia with a methylene blue class product were identified in the published literature. Of these 41 patients, 37 (90%) had a metHb decrease of at least 50% within 1 hour after intravenous administration of the methylene blue class product.

In a combined analysis of all 47 patients treated intravenously with ProvayBlue™ (or a bioequivalent formulation) or with another methylene blue class product, there was no difference in response rate by dose. The metHb decreased by at least 50% within 1 hour of infusion for 15/17 (88%) of patients treated with 1 mg/kg, 12/13 (92%) treated with 2 mg/kg and 16/17 (94%) treated with a different dose or for those whose dose was not reported.

Refer to APPENDIX 1: US Package Insert of ProvayBlue™ for adverse reactions, clinical pharmacology and non-clinical toxicology information associate with ProvayBlue™.

6.6. RISK-BENEFIT ASSESSMENT

ProvayBlue™ will be administered to the study patients to treat acquired methemoglobinemia resulting in a direct health benefit.

Refer to APPENDIX 1: US Package Insert of ProvayBlue™ for the risks associated with the use of ProvayBlue™.

Entering a patient who requires methylene blue treatment, especially minors, into the protocol is associated with minimal incremental risk to the patient. The treating physician/Investigator and other treating health care professionals should continuously monitor the patient to ensure that the degree of burden and risk to the patient is minimized.

Extreme caution should be exercised when administering to newborns and infants below the age of 3 months due to lower concentrations of NADPH-methemoglobin reductase necessary for reducing methemoglobin to hemoglobin, making these infants more susceptible to methemoglobinemia produced by high doses of methylthioninium chloride.

The approximate volume of blood planned for collection from each patient over the course of the study presents no undue risk to the patients, nor does the collection of additional blood in the event an indwelling cannula is utilized (for wasting to ensure clean sample) or for recheck of safety labs, if deemed necessary by the Investigator.

As per regulations and guidelines, a limited volume of blood will be drawn from each patient during this study. Blood drawn from pediatric patients will not exceed the recommended maximum blood sampling volume for children in clinical trials which should not exceed 1% of total blood volume in one draw and 3% of total blood volume within a 4-week period (European Medicines Agency [EMA] guideline). Refer to APPENDIX 3: estimates of 1% of the patient's blood volume for the maximum volume of blood that can be drawn per patient. The number of attempts of venous punctures in children and adolescents will be restricted to a maximum of 3 attempts.

The safety data collected by this protocol (i.e., physical examination, vital signs, laboratory analysis [including metHb], and adverse event [AE]) are adequate to protect the patient's safety and should detect all expected treatment-emergent AEs (TEAEs).

7. STUDY OBJECTIVES AND ENDPOINTS

7.1. STUDY OBJECTIVES

7.1.1. Primary Objective

- To confirm that ProvayBlue™ after a single administration is efficacious in patients with acquired methemoglobinemia.

7.1.2. Secondary Objectives

- To evaluate the efficacy of a single dose of 1 mg/kg of ProvayBlue™;
- To evaluate the efficacy of a second dose of 1 mg/kg of ProvayBlue™ to further reduce metHb levels when metHb is not fully reduced by a single dose;
- To evaluate the normalization of the respiratory rate, heart rate and blood pressure of patients who achieve a reduction in metHb level within 2 hours of receiving the first dose of ProvayBlue™;
- To confirm the safety and tolerability of ProvayBlue™ injection in patients with acquired methemoglobinemia.

7.1.3. Exploratory Objective

- To collect sparse pharmacokinetic (PK) data in patients.

7.2. STUDY ENDPOINTS

7.2.1. Primary Efficacy Endpoint

- A 50% reduction in metHb level within 1 hour of the first dose of ProvayBlue™ for treatment of acquired methemoglobinemia.

7.2.2. Secondary Efficacy Endpoints

- Concomitant normalization of the respiratory rate, heart rate and blood pressure (see standard values in Section 13.1) within 2 hours of the first dose of ProvayBlue™;
- Evaluation of the patients who achieve a 50% reduction in metHb level after a single dose of ProvayBlue™, in addition to normalization of the respiratory rate, heart rate and blood pressure;
- Evaluation of the number of patients who achieve a 50% reduction in metHb level within one hour after treatment with ProvayBlue™ in addition to normalization of the respiratory rate, heart rate and blood pressure within 2 hours of receiving the first dose of ProvayBlue™;

- Evaluation of the patients who achieve a 50% reduction in metHb level after a second dose of ProvayBlue™, in addition to normalization of the respiratory rate, heart rate and blood pressure, in cases where a single dose did not completely normalize metHb level;

7.2.3. Safety Endpoints

The following safety parameters will be assessed:

- Physical examination;
- Vital signs;
- Hematology and biochemistry (see tests in Section 15.2);
- ECG;
- AEs.

7.2.4. Pharmacokinetic (PK) Endpoints

- PK samples will be analyzed for methylene blue and azure B.

8. INVESTIGATIONAL PLAN

8.1. SUMMARY OF STUDY DESIGN

This is an open label, uncontrolled Phase 4 study of 10 patients who present in hospital/urgent care setting with acquired methemoglobinemia. The population may include pediatric and adult patients (males and females of all ages are included).

The aim of the study is to confirm safety and efficacy of ProvayBlue™ for the treatment of acquired methemoglobinemia.

This study comprises of an analysis of data for patients presenting with acquired methemoglobinemia and requiring ProvayBlue™. The analysis itself will focus on clinical outcome of treatment with ProvayBlue™ alone. If possible, depending on the number of patients, the data will be summarized by age, sex and body weight.

The study will include sites which will be based in France, Germany, UK and the US.

ProvayBlue™ will be administered IV at a dose of 1 mg/kg over 5-30 minutes. If the metHb level remains greater than 30% or if clinical signs and symptoms persist, a repeat dose of 1 mg/kg may be given 1 hour after the first dose.

Depending on the clinical status of the patient, additional doses may be administered at the discretion of the Investigator. The data on these additional doses will be recorded in the study Case Report Form (CRF).

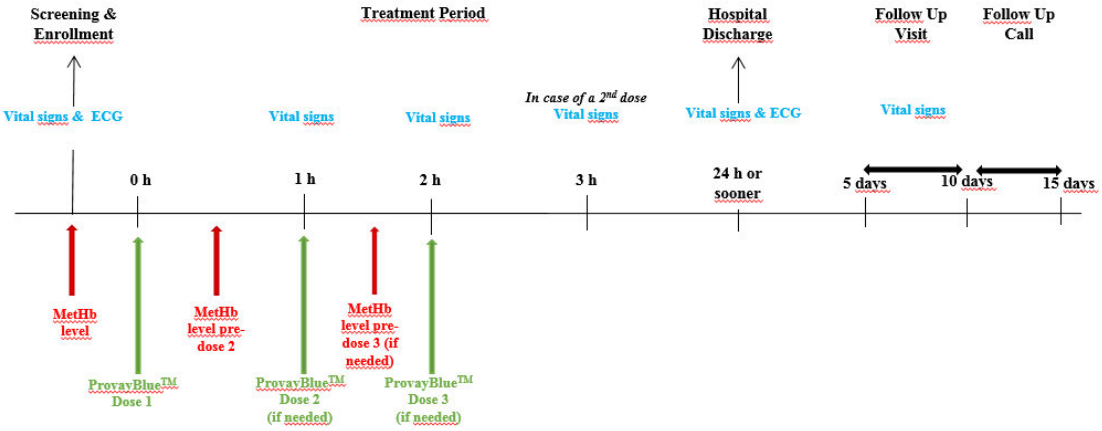
Due to the nature of the disease and indication, the patient's treatment with ProvayBlue™ may take place per the Investigator's standard of care prior to or after Patient Informed Consent Document (PICD) signature. Data collection (retrospective and prospective) for this study and study specific assessments will only take place after PICD signature.

Safety will be monitored throughout the study by repeated clinical and laboratory evaluations according to protocol requirements.

The expected duration of the study will vary for each patient depending on the clinical situation and outcome of treatment. Patients will be followed-up 5 to 10 days (follow-up visit), and 10 to 15 days (follow-up call) after the last administration of ProvayBlue™. End of study is defined as the Last Patient Last Visit (LPLV).

The study design is summarized in Figure 8-1.

Figure 8-1: Study Design



A schedule for the tests and evaluations to be conducted in this study is found in the flow chart in APPENDIX 2:Study Flow Chart.

The study duration of the trial will vary for each patient depending on the clinical situation and outcome of treatment.

8.2. DISCUSSION OF TRIAL DESIGN

As a post-marketing requirement imposed by the US-FDA, Provepharm is required to conduct a clinical study to evaluate the safety and efficacy of ProvayBlue™ for the treatment of acquired methemoglobinemia. The minimal efficacy endpoints should include achieving a 50% reduction in metHb within 1 hour of the first dose of ProvayBlue™ in addition to normalization of the respiratory rate, heart rate and blood pressure within 2 hours of the first dose of ProvayBlue™ (refer to Section 6.1).

This is a prospective, interventional, open label, uncontrolled Phase 4 study to confirm that ProvayBlue™ is efficacious and safe in patients with acquired methemoglobinemia.

The open-label conduct of the trial allows for rapid assessment of the drug-relatedness status of any toxicity.

8.3. TRIAL TIMETABLE (STUDY PERIODS)

The trial includes the following visits:

- Screening and enrolment
- Study Treatment
  - H0: ProvayBlue™ Dose 1
    - H1: 1 hour after the end of ProvayBlue™ infusion. Administration of ProvayBlue™ Dose 2 (if applicable)
  - H2: 2 hours after the end of ProvayBlue™ Dose 1 infusion
  - H3: 2 hours after the end of ProvayBlue™ Dose 2 infusion (if applicable)
- H24 (24 hours after the end of ProvayBlue™ Dose 1 infusion)
- Hospital Discharge (if earlier than H24)
- Follow up visit (5 -10 days following the last ProvayBlue™ administration)
- Follow up call (10 -15 days following the last ProvayBlue™ administration)

9. SELECTION AND WITHDRAWAL OF PATIENTS

10 patients (adults and pediatric population of all ages) diagnosed with acquired methemoglobinemia and treated with ProvayBlue™ will be included in the study.

9.1. INFORMED CONSENT

Each potentially eligible patient (and their parent[s] or legally authorized representative[s] as applicable) will be informed of the study's objectives and overall requirements.

ProvayBlue™ will be administered as per the treating physician's diagnosis and hospital standard of care. It may be possible that the patient will be administered their first dose of ProvayBlue™ as per standard of care prior to obtaining the consent for this study. However, patient consent (and their parent[s] or legally authorized representative[s] as applicable) MUST be obtained prior to any data collection (retrospective and prospective) for this study and study specific assessments.

The Investigator will explain the study fully to the patient (as relevant) and his/her parent(s)/ legally authorized representative(s) (as required) using the PICD approved by the institution's Independent Ethics Committee (IEC)/ Institutional Review Board (IRB). If a parent/legally authorized representative is willing for the patient to participate in the study, (s)he will be requested to give written informed consent. The PICD will be signed and personally dated by **both** a parent/legally authorized representative and the Investigator.

If capable, the patient should provide assent/consent to participate in the study and also sign and personally date the Informed Consent if possible (according to local laws and regulations).

A copy of the signed form will be provided to the parent(s)/ legally authorized representative(s) and the original retained with the source documents.

Although nursing staff may be involved in describing the trial to a patient and his/her parent(s)/legally authorized representative(s), the Investigator must participate in discussions with the patient and his/her parent(s)/legally authorized representative(s) and sign and personally date the PICD.

9.2. INCLUSION CRITERIA

To be eligible for inclusion into this study, each patient must fulfil the following inclusion criteria:

1. Pediatric or adult patients (males and females of all ages are included) diagnosed with acquired methemoglobinemia and receiving treatment with ProvayBlue™ as per the treating physician's diagnosis and hospital standard of care.

   Acquired methemoglobinemia is defined as a level of methemoglobinemia >30% or ≤30% with associated clinical symptoms (e.g. sleepiness, cyanosis, dizziness, etc.).

2. Written informed consent obtained prior to any data collection (retrospective and prospective) for this study and study specific assessments.

9.3. EXCLUSION CRITERIA

To be eligible for inclusion into this study, each patient must violate none of the following exclusion criteria:

1. Known severe hypersensitivity reactions to methylene blue or any other thiazine dye.
2. Known deficiency in glucose-6-phosphate dehydrogenase (G6PD) due to the risk of hemolytic anemia as well as lack of therapeutic effect.
3. Known deficiency in NADPH reductase.
4. Known use of selective serotonin reuptake inhibitors (SSRIs), serotonin and norepinephrine reuptake inhibitors (SNRIs), MonoAmine Oxidase (MAO) inhibitors or drugs metabolised via CYP isoenzymes anticipated during the treatment phase of the study (see Section 11.2).
5. Women who refuse to stop breastfeeding for up to 8 days after receiving the last dose of ProvayBlue™ (see Section 11.2).

9.4. ASSIGNMENT OF PATIENT NUMBER

All patients who sign the PICD will be allocated a patient number sequentially at each center. The number of any patient who is withdrawn from the study will not be reallocated.

9.5. PREMATURE WITHDRAWAL OF PATIENTS FROM STUDY AND REPLACEMENT POLICY

9.5.1. Discontinuation Criteria

Patients (and their parent[s] or legally authorized representative[s] as applicable) will be informed that they have the right to withdraw from the study at any time, without prejudice to their medical care, and are not obliged to state their reasons. Any withdrawals must be fully documented in the CRF and should be followed up by the Investigator.

Additionally, the Investigator may withdraw a patient from the study or from ProvayBlue™ administration at any time if he/she considers this to be in the patient's best interest.

Patients MUST be discontinued from the study for any of the following reasons:

- Severe or life-threatening hypersensitivity/allergic reaction (refer also to Section 11.4.1);
- Hemolytic anemia (refer also to Section 11.4.2);
- Changes in medical status of the patient such that the Investigator believes that patient safety will be compromised;
- Death;
- Withdrawal by patient or at the request of their legally authorized representative;
- Physician decision;
- At the specific request of the Sponsor;
- Study terminated by Sponsor.

Patients MAY be discontinued from the study for any of the following reasons:

- Adverse events including serious intercurrent illness or significant worsening of intercurrent illness;
- Protocol violations, including non-compliance with study assessment and patient refusal;
- Other study-specific reasons;
- If a patient fails to return for a scheduled visit/follow-up, 3 attempts should be made to contact the patient to ensure that the reason for not returning is not an AE. Likewise if a patient declares his/her wish to discontinue from the study, e.g. for personal reasons.

If the ProvayBlue™ therapy is prematurely discontinued, the primary reason for discontinuation must be recorded in the appropriate section of the CRF and all efforts will be made to complete and report the observations as thoroughly as possible. Discontinuing of ProvayBlue™ administration is not a reason to discontinue from the study. Every effort should be made to collect complete information on patients whether ot not they complete the planned treatment.

A complete final evaluation following withdrawal from the study should be made, as described in Section 12.5, and any AEs will be followed 10 to 15 days from the last dose of ProvayBlue™.

The study will be terminated if, in the opinion of the Investigator and the Sponsor, significant safety concerns arise during the conduct of the study.

9.5.2. Replacement Policy

Should a patient drop out or be withdrawn from the study, his/her patient number will not be reallocated. During the study, recruitment may be increased in order to include 10 evaluable patients. A patient will be considered evaluable if he/she is diagnosed with acquired methemoglobinemia (refer to Section 6.3), received a first dose of ProvayBlue™ of 1 mg/kg and had adequate available data to measure the primary efficacy analysis.

10. STUDY MEDICATION

Patients will receive treatment with ProvayBlue™ as per the treating physician's diagnosis and hospital standard of care.

ProvayBlue™ has been approved by the US FDA for patients to be administered IV at a dose of 1 mg/kg over 5-30 minutes and if the metHb level remains above 30% or if clinical symptoms persist, to give a repeat dose of up to 1 mg/kg 1 hour after the first dose.

Depending on the clinical status of the patient, additional doses may be administered at the discretion of the Investigator. The data on these additional doses will be recorded in the study CRF. If ProvayBlue™ is administered as an infusion, it MUST be diluted in 5% dextrose in water (D5W) only. Dilution in saline will result in precipitation of the product and must not be administered.

For further information regarding ProvayBlue™, including packaging, labelling, presentation, storage and preparation prior to patient administration, please refer to the prescribing information for ProvayBlue™ in APPENDIX 1: US Package Insert of ProvayBlue™.

11. CONCOMITANT THERAPY/MEDICAL MANAGEMENT OF ADVERSE EVENTS (AEs)

Medications or clinical interventions, which are considered necessary for the patient welfare or to assist in the management of AEs, may be given at the discretion of the Investigator.

Refer to APPENDIX 1: US Package Insert of ProvayBlue™ for further information on potential drug interactions with ProvayBlue™.

Safety measures are to be considered for certain concomitant medications. These medications are included in Section 11.2. Medications that are prohibited during the course of this study are listed in Section 11.3.

11.1. RECORDING OF PREVIOUS CONCOMITANT THERAPY

All concomitant therapy received during the study, from PICD signature or the start of ProvayBlue™ treatment (whichever occurs sooner) until the follow up visit, will be recorded using the generic terms. The use of herbal/natural products, as well as diagnostic, therapeutic or surgical procedures performed during the study period will be recorded as concomitant therapy.

All prescription, non-prescription, or over-the-counter medications including herbal remedies given to or taken by the patient before inclusion in the study at study entry and within 4 weeks prior to study entry, must be clearly documented on the prior medications CRF.

Investigational ProvayBlue™ should be used for this study/ However, commercial ProvayBlue™ that could be administered for treatment of acquired methemoglobinemia prior to study entry as per hospital standard of care must be retained at the site until the monitoring visit of the Orion Clinical Research Associate (CRA) and all information need to be recorded in the CRF. In particular, batch number, expiration date and reason for not using investigational product should be captured on the drug accountability log.

11.2. PRECAUTIONS FOR USE, INTERACTIONS WITH OTHER MEDICINAL PRODUCTS AND OTHER FORMS OF INTERACTION OF THE IMP

Agents Metabolized by Cytochrome P450 Enzymes

Methylene blue inhibits a range of Cytochrome (CYP) isozymes *in vitro*, including 1A2, 2B6, 2C8, 2C9, 2C19, 2D6 and 3A4/5. This interaction could be more pronounced with narrow therapeutic index drugs that are metabolized by one of these enzymes (e.g., digoxin, warfarin, phenytoin, alfentanil, cyclosporine, dihydroergotamine, ergotamine, fentanyl, pimozide, quinidine, sirolimus and tacrolimus). However, the clinical relevance of these *in vitro* interactions is unknown.

Interaction with other medicinal products and other forms of interaction

Methylthioninium chloride is a potent inhibitor of the transporters OCT2, MATE1 and MATE2-K. The clinical consequences of the inhibition are not known. The administration of methylthioninium chloride Proveblue has the potential to transiently increase the exposure of drugs

primarily cleared by renal transport involving the OCT2/MATE pathway, including cimetidine, metformin and acyclovir.

Methylthioninium chloride is a substrate of P-glycoprotein (P-gp). The clinical consequences are considered likely to be minimal due to the transient and single dose use that normally occurs in the emergency setting.

Contraindications

In Europe patients with chlorate poisoning, nitrite-induced methemoglobinemia during treatment of cyanide poisoning and patients with a deficiency in NADPH (nicotinamide adenine dinucleotide phosphate) reductase are contraindicated for receiving ProvayBlue™ treatment for methemoglobinemia. Although this is not the case in the United States and other countries, special care should be given to ensure the efficacy benefit of administering ProvayBlue™ in patients known to meet these restrictions.

Pregnancy

There are no adequate data from the use of ProvayBlue™ in pregnant women. Studies in animals have shown reproductive toxicity (see section 5.3). The potential risk for humans is unknown. ProvayBlue™ should not be used during pregnancy unless clearly necessary, e.g. in life-threatening methemoglobinemia.

Breastfeeding

It is unknown whether ProvayBlue™ is excreted in human breast milk. The excretion of ProvayBlue™ in milk has not been studied in animals. A risk to the suckling child cannot be excluded. Based on kinetic data, breastfeeding should be discontinued upon receiving ProvayBlue™ and for a period of 8 days after the administration of the last dose of ProvayBlue™. Otherwise, breastfeeding patients must be excluded for safety reasons (see also Section 9.3). In case a breastfeeding woman needs specific therapy that is urgently indicated, drugs shall be administered as current standard therapy.

Fertility

In vitro, ProvayBlue™ has been shown to reduce motility of human sperm in a dose dependent manner.

Contraception

Both patients and their partners with child-bearing potential must employ a reliable method of contraception, for example,

- Oral Contraceptive + Condom with Spermicide;
- Inter Uterine Device (IUD) + Condom with Spermicide;
- Diaphragm with Spermicide + Condom with Spermicide;
- Injectable progesterone + Condom with Spermicide;
- Subdermal Implant + Condom with Spermicide;
- Sexual abstinence.

11.3. MEDICATIONS THAT MUST NOT BE ADMINISTERED

Methemoglobinemia may not resolve or may rebound after response to treatment with ProvayBlue™ in patients with methemoglobinemia due to aryl amines such as aniline or sulfa drugs such as dapsone.

Serotonergic Drugs

Avoid concomitant use of ProvayBlue™ with medicinal products that enhance serotonergic transmission including SSRIs, SNRIs, MAO inhibitors, bupropion, buspirone, clomipramine, mirtazapine and venlafaxine; because of the potential for serious CNS reactions, including potentially fatal serotonin syndrome. Although the mechanism is not clearly, understood, literature reports suggest inhibition of MAO by methylene blue may be involved. In addition, in vitro studies cannot rule out the potential involvement of CYP 2D6 inhibition by methylene blue. If the intravenous use of ProvayBlue™ cannot be avoided in patients treated with serotonergic medicinal products, choose the lowest possible dose and observe closely the patient for CNS effects for up to 4 hours after administration [see Warning and Precautions (5.1), Clinical Pharmacology (12.3) in APPENDIX 1: US Package Insert of ProvayBlue™].

11.4. GUIDANCE ON MEDICATIONS TO BE ADMINISTERED/PATIENT MANAGEMENT FOR EXPECTED ADVERSE EVENTS (AEs)

11.4.1. Hypersensitivity

Anaphylactic reactions to methylene blue class products have been reported. Patients treated with ProvayBlue™ should be monitored for anaphylaxis. If severe or life threatening allergic reaction (e.g., angioedema, urticaria, bronchospasm) should occur, discontinue ProvayBlue™ and initiate supportive treatment. Refer also to APPENDIX 1: US Package Insert of ProvayBlue™ for further information.

11.4.2. Hemolytic Anemia

Hemolysis can occur during treatment of methemoglobinemia with ProvayBlue™. Laboratory testing may show Heinz bodies, elevated indirect bilirubin and low haptoglobin, but the Coombs test is negative. The onset of anemia may be delayed 1 or more days after treatment with ProvayBlue™. In the event that a patient has hemolytic anemia, discontinue ProvayBlue™ and initiate appropriate treatment (e.g. red blood cell transfusion). Refer also to APPENDIX 1: US Package Insert of ProvayBlue™ for further information.

12. SCHEDULE OF ASSESSMENTS

12.1. SCREENING AND ENROLMENT

Refer to APPENDIX 2: Study Flow Chart for data collection time points and schedule of assessments.

Once the patient (and/or the patient's parent[s]/legal guardian[s]) has signed the PICD, the formal screening assessments to determine the patient's study eligibility must be completed. These assessments and relevant collection of data (as per standard of care) include the following:

- Date of signed informed consent(s);
- Demographics – date of birth, sex and race (as allowed by local regulations);
- Medical history (including all concomitant disease[s] and previous medications);
- Date, time and initial reason for hospitalization;
- Methemoglobinemia diagnosis (including name of toxic agents responsible for methemoglobinemia if available, clinical symptoms related to methemoglobinemia [e.g. sleepiness, cyanosis, dizziness, etc.], methemoglobinemia level at presentation in hospital);
- Confirmation of study eligibility (inclusion and exclusion criteria);
- Physical examination including weight (refer to Section 15.2.1);
- Vital signs (refer to Section 15.2.2 for the list of parameters) If the patient is treated prior to consent, all available pre-treatment vital sign information should be retrieved where possible;
- Pregnancy test: All females of childbearing potential will be submitted to a pregnancy test (<u>both</u> urine and serum unless one is not possible);
- Blood sample for:
    - Biochemistry:
        - Sodium
        - Potassium
        - Chloride
        - Urea
        - Creatinine
        - Calcium
        - Inorganic phosphorous
        - Total Protein
        - Albumin
        - Gamma glutamyl transferase
        - Bilirubin (Total)
        - Alkaline phosphatase
        - Aspartate transaminase

- Alanine aminotransferase
- Glucose

Additional tests (for example blood gas) to be done at admission according to hospital standard of care (Refer to Section 15.2.3).

- Hematology:
  - Haemoglobin
  - Red blood cell count
  - White blood cells
  - Platelets
  - Differential white cell count, neutrophils, lymphocytes, monocytes, basophils and eosinophils

  Additional tests to be done at admission according to hospital standard of care (Refer to Section 15.2.4);
  - metHb (Refer to Section 15.2.5);

- 12-lead ECG (refer to Section 15.2.6);
- Review of concomitant therapy since PICD signature or the start of ProvayBlue™ treatment (whichever occurs sooner) (refer to Section 11);
- Collect of AEs that may have occurred since the start of ProvayBlue™ treatment (refer to Section 15.3).

Results of all the screening evaluations and procedures must be reviewed by the Investigator to ensure that all eligibility criteria have been satisfied prior to patient enrolment.

12.2. TREATMENT

Due to the nature of the disease indication, the patient's treatment with ProvayBlue™ as per standard of care can take place prior to or after PICD signature. Data collection (retrospective and prospective) for this study and study specific assessments will only take place after PICD signature.

Refer to APPENDIX 2: Study Flow Chart for data collection time points and schedule of assessments.

Assessments during treatment and the relevant collection of data (as per standard of care) include the following:

- ProvayBlue™ administration (including dose, lot number, date, time and route of administration, and if any dilution took place);
- Clinical symptoms related to methemoglobinemia and severity:
  - Within 1 hour after the end of each ProvayBlue™ infusion (i.e. both Dose 1, Dose 2 and any additional dose if applicable);

  - Within 2 hours after the end of each ProvayBlue™ infusion (i.e. both Dose 1, Dose 2 and any additional dose if applicable);
  - Approximately 24 hours post the end of the last ProvayBlue™ infusion, or just prior to discharge from the clinic if earlier than 24 hours.
- Vital signs (refer to Section 15.2.2 for the list of parameters):
  - Within 1 hour after the end of each ProvayBlue™ infusion (i.e. both Dose 1, Dose 2 and any additional dose if applicable);
  - Within 2 hours after the end of each ProvayBlue™ infusion (i.e. both Dose 1, Dose 2 and any additional dose if applicable);
  - Approximately 24 hours post the end of the last ProvayBlue™ infusion, or just prior to discharge from the clinic if earlier than 24 hours.
- Blood sample for:
  - Biochemistry, hematology and metHb (refer to Section 15.2):
    - Within 1 hour after the end of each ProvayBlue™ Dose 1 infusion (i.e. Dose 1, Dose 2 and any additional dose if applicable);
    - Prior to ProvayBlue™ Dose 2 if applicable (i.e., if the metHb level is acceptable within 1 hour after the end of Dose 1 ProvayBlue™ infusion, the repeat dose of ProvayBlue™ will not be needed);
    - Approximately 24 hours post the end of the last ProvayBlue™ infusion, or just prior to discharge from the clinic if earlier than 24 hours.
  - PK analysis (refer to Section 14):
    - Approximately 1 hour after the end of each ProvayBlue™ infusion (i.e. Dose 1, Dose 2 and any additional dose if applicable);
    - Prior to Dose 2 (or any additional dose) ProvayBlue™ infusion (if applicable);
    - Approximately 24 hours post the end of the last ProvayBlue™ infusion, or just prior to discharge from the clinic if earlier than 24 hours.

  Additional tests to be done according to hospital standard of care.
- 12-lead ECG (refer to Section 15.2.6):
  - Approximately 24 hours post the end of the last ProvayBlue™ infusion, or just prior to discharge from the clinic if earlier than 24 hours.
- Review of concomitant therapy until follow-up (refer to Section 11);
- Review of AEs (refer to Section 15.3).

Should any of the blood samples collected for the purposes of the study be compromised or destroyed in error, a repeat sample should be taken during the visit. Care should be taken to ensure that the maximum amount of blood drawn does not exceed what is stated in the PICD.

12.3. DISCHARGE

Assessments prior to discharge from the clinic and the relevant collection of data (as per standard of care) include the following:

- Clinical symptoms related to methemoglobinemia;
- Physical examination;
- Vital signs (refer to Section 15.2.2 for the list of parameters);
- Blood sample for:
    - Biochemistry
    - Hematology
    - metHb
    - PK analysis (refer to Section 14)

  Additional tests to be done according to hospital standard of care
- 12-lead ECG (refer to Section 15.2.6);
- Review of concomitant therapy (refer to Section 11);
- Review of AEs (refer to Section 15.3).

12.4. FOLLOW-UP

Patients will be followed up with:

- A visit with blood sample for hematology and biochemistry, and assessment of clinical symptoms, physical examination and vital signs 5 to 10 days following the last ProvayBlue™ administration.
- A telephone call to take place 10 to 15 days following the last ProvayBlue™ administration.

Refer to APPENDIX 2: Study Flow Chart for data collection time points and schedule of assessments.

Additional follow-up assessments include the following:

- Review of concomitant therapy (refer to Section 11);
- Review of AEs (refer to Section 15.3).

12.5. WITHDRAWAL/PREMATURE STUDY DISCONTINUATION

Should the patient discontinue prematurely and choose to withdraw from the study, the following procedures should be performed:

- Clinical symptoms related to methemoglobinemia;

- Vital signs (refer to Section 15.2.2 for the list of parameters);
- Blood sample for:
    - Biochemistry
    - Hematology
    - MetHb

  Additional tests to be done according to hospital standard of care.
- Review of concomitant therapy (refer to Section 11);
- Review of AEs (refer to Section 15.3).

The Investigator should document the reason for study discontinuation in the CRF.

In the case of an ongoing AE, appropriate safety evaluations should be repeated more frequently and/or additional tests performed at any time when clinically indicated or at the discretion of the Investigator. All ongoing AEs and SAEs will be followed up until resolution, until the condition stabilizes, until the event is otherwise explained or until the patient is lost to follow up. If the patient is lost to follow up, then this should be noted in the CRF.

13. ASSESSMENTS OF EFFICACY

13.1. SPECIFICATION OF EFFICACY PARAMETERS

The following efficacy parameters will be assessed:

- A reduction in metHb by 50% within 1 hour of dosing;
- Concomitant normalization of respiratory rate within 2 hours of the first dose of ProvayBlue™;
- Concomitant normalization of heart rate within 2 hours of the first dose of ProvayBlue™;
- Concomitant normalization of diastolic blood pressure (DBP) within 2 hours of the first dose of ProvayBlue™;
- Concomitant normalization of systolic blood pressure (SBP) within 2 hours of the first dose of ProvayBlue™.

Examples of standard values for vital signs are provided in Table 13-1 below (Chameides 2011; Kliegman 2015).

Table 13-1: Examples of standard ranges for vital signs assessment

Population	Systolic Blood Pressure (SBP) mmHg	Diastolic Blood Pressure (DBP) mmHg	Respiratory rate (RC) Breaths/min	Heart rate (HR) Beats/min
Birth (12 hours)	60-76	31-45	30-60	120-140
Neonate (96 hours)	67-84	35-53		
Infant (1 to 12 months)	72-104	37-56		110-130
Children (1 to 2 years)	86-106	42-63	22-40	80-110
Children (3 to 5 years)	89-112	46-72	20-28	60-70
Children (6 to 9 years)	97-115	57-76	18-25	70-80
Children (10 to 11 years)	102-120	61-80		
Adolescent (12-15 years)	110-131	64-83	12-20	60-70
Adults (≥ 16 years)	90-140	60-90	12-20	50-100

13.2. METHODS AND TIMING FOR ASSESSING, RECORDING AND ANALYSING EFFICACY PARAMETERS

The primary endpoint is assessed over a period of 1 hour after administration of the first dose of ProvayBlue™. The secondary endpoints are assessed over a period of 2 hours after administration of each dose of ProvayBlue™.

14. ASSESSMENTS OF PHARMACOKINETIC (PK)

Whenever feasible and ONLY if the total blood drawn for any patient does not exceed the recommended maximum blood sampling volume (refer also to Section 6.6 for regulation and guidance), PK samples are to be taken at the discretion of the Investigator based on the condition of the patient. The time blood sample(s) are taken should be recorded, but there is no absolute time window for the PK samples. Suggested PK sampling time points are as follows:

- Approximately 1 hour after the end of each ProvayBlue™ infusion (i.e. Dose 1, Dose 2 and any additional dose if applicable);
- Prior to Dose 2 (or any additional dose) ProvayBlue™ infusion (if applicable);
- Approximately 24 hours post the end of the last ProvayBlue™ infusion, or just prior to discharge from the clinic if earlier than 24 hours.

PK blood samples (venous) must be drawn from the arm opposite to the ProvayBlue™ dosing arm. Whole blood samples will be analyzed for methylene blue and azure B using a validated bioanalytical method. Arterial blood taken should be submitted for analysis with a note that it is an arterial sample.

Further instruction on PK sampling will be provided in a separate laboratory manual.

The volume of whole blood taken per venipuncture sample is no more than 2 ml. The number of samples drawn from any patient up to 2 years of age should be limited (refer also to Section 6.6 for regulation and guidance). Care should be taken to ensure that the maximum amount of blood drawn does not exceed what is stated in Section 6.6 (refer also to APPENDIX 3: estimates of 1% of the patient's blood volume) and in the PICD.

15. ASSESSMENTS OF SAFETY

15.1. SPECIFICATION OF SAFETY PARAMETERS

The following safety parameters will be assessed:

- Physical examination;
- Vital signs;
- Hematology and biochemistry;
- ECG;
- AEs.

15.2. METHODS AND TIMING FOR ASSESSING, RECORDING AND ANALYSING SAFETY PARAMETERS

Refer to the Study Flow Chart (APPENDIX 2: Study Flow Chart) for data collection time points for the safety parameters.

15.2.1. Physical Examination

Physical examinations during the study will be based on patient standard of care as appropriate to determine general condition.

Weight will be recorded at screening.

New or worsening clinically significant abnormalities following PICD signature will be reported as an AE.

15.2.2. Vital Signs

Vital signs will include:

- SBP and DBP (mmHg) (controlled posture if possible);
- Heart rate (beats per minute [bmp]);
- Body temperature (°C or °F);
- Respiration rate (breaths per minute);
- Oxygen saturation by co-oximetry.

New or worsening clinically significant abnormalities following PICD signature will be reported as an AE.

15.2.3. Biochemistry

Biochemistry samples will be analyzed by the hospital laboratory.

The following biochemistry parameters will be collected:

- Sodium
- Potassium
- Chloride
- Urea
- Creatinine
- Calcium
- Inorganic phosphorous
- Total Protein
- Albumin
- Gamma glutamyl transferase
- Bilirubin (Total)
- Alkaline phosphatase
- Aspartate transaminase
- Alanine aminotransferase
- Glucose

Additional (optional) biochemistry parameters (for example blood gas) will be collected based on available data from patient standard of care.

15.2.4. Hematology

Hematology samples will be analyzed by the hospital laboratory.

The following hematology parameters will be collected:

- Haemoglobin
- Red blood cell count
- White blood cells
- Platelets
- Differential white cell count, neutrophils, lymphocytes, monocytes, basophils and eosinophils

Additional (optional) hematology parameters will be collected based on available data from patient standard of care.

15.2.5. Methemoglobin

Methemoglobin samples will be analyzed by the hospital laboratory.

15.2.6. 12-lead Electrocardiogram (ECG)

Collection of ECG data is mandatory at admission and at the time points defined in Section 8.3. The collection of additional data will be based on available 12-lead ECG recordings performed as per patient standard of care as appropriate to determine general condition.

Any new or worsening clinically significant abnormalities following PICD signature will be reported as an AE.

15.2.7. Adverse Events (AEs)

AEs (disease related and/or treatment related) reported by the patients (and/or their parent[s]/legal guardian[s]) will be recorded in the CRF (refer to Section 15.3 for more information).

15.3. ADVERSE EVENT (AE) REPORTING

It is of the utmost importance that all staff involved in the study are familiar with the content of this section. It is the Investigator's responsibility to ensure compliance with reporting of AEs from his/her site.

Adverse events will be collected from the start of ProvayBlue™ treatment up to follow up 10 to 15 days after the last administration of ProvayBlue™.

Any AE that occurs after the 10 to 15 days following the last administration of ProvayBlue™ which the Investigator assesses as related to a study procedure and/or ProvayBlue™, should also be reported as an AE or a SAE.

All AEs should be followed until they are resolved or a clinically-stable endpoint is reached if they are considered chronic or until the patient is lost to follow-up.

15.3.1. Definition of an Adverse Event (AE)

An AE is defined as: Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment.

An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

An AE can also refer to an untoward response to the administration of ProvayBlue™, but can also occur as a result of the protocol-required procedures or be unrelated to both and include worsening of pre-existing conditions, for example.

AEs include the following:

- Suspected adverse medication reactions;
- Reactions from medication overdose, abuse, sensitivity, or toxicity;
- Apparently unrelated illnesses, including the worsening of a pre-existing illness;
- Injury or accidents;

  Note: If a documented medical condition is known to have caused the injury or accident, only the accident should be reported as an AE;
- New or aggravated clinically relevant abnormal medical finding at a physical examination as compared with previous assessments;
- Laboratory abnormalities or other abnormal assessments (e.g. physical examination, vital signs, ECG) that require clinical intervention or further investigation (beyond ordering a repeat [confirmatory] test) unless they are associated with an already reported clinical event.

A suspected adverse drug reaction (ADR) means any AE for which there is a reasonable possibility that the medicinal product caused the AE. Reasonable possibility means there is evidence to suggest a causal relationship between the medicinal product and the AE.

15.3.2. Definition of a Serious Adverse Events (SAE)

An SAE is defined as any untoward medical occurrence that:

- Results in death;

  Note: Death is an outcome of an AE, and not an AE in itself. Event which led to death should be recorded with fatal outcome.

  All deaths occurring on the study or up to 10 to 15 days following the last administration of ProvayBlue™ or patient withdrawal must be reported.
- Is life-threatening;

  A life-threatening event places the patient at risk of death at the time of the event. It does not refer to an event which hypothetically might have caused death if it were more severe).
- Requires inpatient hospitalization or prolongation of existing hospitalization;

  Note: In general, hospitalization signifies that the patient has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfils any other serious criteria, the event is serious. When in doubt as to whether other events meet the serious criteria, the event is to be considered serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious.

  Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE.

- Results in persistent or significant disability/incapacity;

  Note: The term "significant disability" means a substantial disruption of a person's ability to conduct normal life functions. This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, hospital, influenza, and accidental trauma (e.g., sprained ankle) which may interfere or prevent everyday life functions, but do not constitute a substantial disruption.
- Is a congenital anomaly/birth defect;
- Is an important medical event(s) that may not be immediately life threatening or result in death or hospitalization but that may jeopardize the patient or require intervention to prevent one of the above outcomes. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse. Medical and scientific judgement should be exercised in deciding whether expedited reporting is appropriate in such instances.

Refer to Section 15.3.3.9 for reporting SAEs to IEC(s), IRB(s) and regulatory authorities (RAs).

15.3.3. Recording and Reporting of Adverse Events (AEs)

All AEs, as defined above, encountered during the clinical study as well as any **SAEs** (see Section 15.3.2) will be reported in the appropriate section of the CRF. Information will include the following:

- Duration of the AE (onset date and time and resolution date);
- Relationship to ProvayBlue™ (refer to Section 15.3.3.5);
- Time relationship to ProvayBlue™ dosing time;
- Assessment of whether AE is an infusion site reaction;
- Severity (refer to Section 15.3.3.6);
- Outcome of the AE;
- Concomitant treatment dispensed (or other action taken);
- Action taken with respect to the ProvayBlue™.

If an AE increases in severity it will be recorded as a new record with the same AE identifier.

AE data should be obtained through observation of the patient, from any information volunteered by the patient (and the patient's parent[s]/legal guardian[s]) and through patient (and the patient's parent[s]/legal guardian[s]) questioning. The patient may be asked "Do you have any health problems?" or "Have you had any health problems since your last site visit?"

Refer to Section 15.3.3.9 for guidance relating to reporting of suspected, unexpected serious adverse reactions (SUSARs) to regulatory agencies.

15.3.3.1. Adverse Event (AE) Reporting Period

ALL AEs or the worsening of any ongoing events which occur from PICD signature or the start of ProvayBlue™ treatment (whichever occurs sooner) up to 10 to 15 days after the last administration of ProvayBlue™ will be recorded in the CRF. All ongoing AEs and SAEs will be followed up until resolution, until the condition stabilizes, until the event is otherwise explained or until the patient is lost to follow up.

All AEs experienced by a patient, irrespective of the suspected causality, will be monitored until the event has resolved, any abnormal laboratory values have returned to baseline or stabilized at a level acceptable to the Investigator and Medical Monitor, until there is a satisfactory explanation for the changes observed, or until the patient is lost to follow-up.

Any untoward event that occurs after the AE reporting period but which the Investigator assesses as possibly related to ProvayBlue™ should also be reported as an AE or SAE.

15.3.3.2. Reporting of Signs and Symptoms versus a Diagnosis

Recording a diagnosis (when possible) is preferred to recording a list of associated signs and symptoms. However, if a diagnosis is known but there are associated signs or symptoms not generally attributed to the diagnosis, the diagnosis and each sign or symptom must be recorded separately.

15.3.3.3. Death

All deaths that occur during the AE Reporting period (refer to Section 15.3.3.1) must be reported as follows:

- Will be reported as a SAE within 24 hours. The report should detail the main and contributory causes of death. This information should also be accompanied by a death certificate or autopsy report (if available).

15.3.3.4. Pregnancy

Should a pregnancy occur in a female patient or the partner of a male patient, it must be reported in accordance with the procedures described in Section 15.3.3.7. Pregnancy in itself is not regarded as an AE unless there is a suspicion that ProvayBlue™ may have interfered with the effectiveness of a contraceptive medication.

15.3.3.5. Definition of Relationship of Adverse Events (AEs) to Study Medication

The Investigator must assess the possible relationship between the AE and ProvayBlue™ and record that assessment in the CRF. The Investigator is to make his/her own assessment of each SAE to be recorded on the CRF and on the SAE form.

The Investigator should provide a Yes or No assessment as to whether there is a reasonable possibility that the event may have been caused by ProvayBlue™.

The relationship should be assessed according to the criteria in Table 15-1 below:

Table 15-1: Relationship of the Adverse Event (AE) to Study Medication

Unrelated	• The AE must clearly be caused by the participants clinical state, or the study procedure/conditions; • Definitely not related to ProvayBlue™; • Temporal sequence of an AE onset relative to administration of ProvayBlue™ not reasonable; • Another obvious cause of an AE.
Unlikely	• Time sequence is unreasonable; • There is another more likely cause for an AE.
Possibly	• Corresponds to what is known about ProvayBlue™; • Time sequence is reasonable; • Could have been due to another equally, likely cause.
Probably	• Is a known effect of ProvayBlue™; • Time sequence from taking ProvayBlue™ is reasonable; • Ceases on stopping ProvayBlue™; • Cannot be reasonably explained by the known characteristics of the participant's clinical state.
Likely	• Is a known effect of ProvayBlue™ (e.g. listed in APPENDIX 1: US Package Insert of ProvayBlue™); • Time sequence from taking ProvayBlue™ is reasonable; • Event stops upon stopping ProvayBlue™, event returns upon restarting ProvayBlue™;

15.3.3.6. Definition of Severity of Adverse Events (AEs)

Severity of any AE will be recorded in the CRF.

Severity of any AE will be graded according to the National Cancer Institute - Common Terminology Criteria for Adverse Event (NCI-CTCAE) (Version 4.03) as reported in Table 15-2.

Table 15-2: Definition of Severity of Adverse Events (AEs)

Grade 1	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
Grade 2	Moderate; minimal, local or non-invasive intervention indicated; limiting age-appropriate instrumental ADL*.
Grade 3	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL**.
Grade 4	Life-threatening consequences; urgent intervention indicated.
Grade 5	Death related to AE.

National Cancer Institute - Common Terminology Criteria for Adverse Event (NCI-CTCAE) Version 4.03

A Semi-colon indicates 'or' within the description of the grade.

ADL: Activities of daily living

*Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.

**Self-care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

Note the distinction between serious and severe AEs: **Severe** is a measure of intensity whereas an event must meet one of the criteria for serious events listed in Section 15.3.3.6 to be considered **serious**; thus, a **severe** reaction is not necessarily a **serious** reaction. For example, a headache may be severe in intensity, but would not be classified as serious unless it met one of the criteria for serious events listed in Section 15.3.3.6. An AE that is assessed as Grade 3 (severe) or Grade 4 (potentially life-threatening) should not be confused with a SAE.

15.3.3.7. Serious Adverse Event (SAE) Reporting Procedure for Investigators to Regulis

The Investigator must report (by fax, telephone or email) all initial and follow-up SAE and pregnancy reports to Regulis' Pharmacovigilance Department within 24 hours of awareness of an SAE.

Study Contact for Reporting Serious Adverse Events

[redacted]

If, for any reason, it is not possible to complete all sections of the SAE form within 24 hours, transmission of the form must not be delayed and the outstanding information should be sent on a follow-up SAE form.

If the SAE is reported by telephone, all points on the SAE form should be covered in the initial telephone report and followed by a completed and signed SAE form to verify the verbal information given previously. In addition, the event must be documented in the CRF.

Blank copies of the SAE forms are included in the Investigator Site File.

The SAE form must be completed as fully as possible with information relevant to the SAE(s) being reported. All fields should be populated or marked accordingly if no information is available.

Copies of relevant Case Report Form (CRF) pages, such as concomitant medications and medical history may be sent as attachments to the SAE forms.

Death and life threatening SAEs must be reported by the Investigator to the Medical Monitor immediately by phone on +44 7590893277 or +44 1753 578080 (out of hours the main reception will contact 'first call' who will take a message and contact the medical monitor if they are unavailable by mobile). The SAE Form must then be e-mailed or faxed to Regulis Safety Department as above.

All incidents of pregnancy (of the patient or patient's partner) during the study must be reported and follow-up of the pregnancy outcome must be made. Specific pregnancy forms should be completed.

15.3.3.8. Follow-up SAE Reports

For all SAEs where important or relevant information is missing, active follow-up should be undertaken. Investigators or other site personnel should inform Regulis of any follow-up information on a previously reported SAE immediately but no later than 24 hours after they become aware of the SAE. The follow-up information must be presented on an SAE form marked as follow-up. It is necessary only to provide the new information, with the SAE form signed by an Investigator.

Investigators or other site personnel should send relevant or requested anonymized supporting documentation (e.g. ECG, laboratory results, autopsy report) to Regulis.

The Investigator will ensure that all the necessary information is provided within the timelines stipulated by Regulis when the request for information is made.

Follow-up reports (as many as required) should be completed and faxed following the same procedure above.

15.3.3.9. Reporting Serious Adverse Events (SAEs) to Independent Ethics Committee(s) (IEC[s])/ Institutional Review Board(s) (IRB[s])/ Regulatory Authorities (RA[s])

The Sponsor is responsible for informing local IEC(s)/IRB(s)/RA(s) of the applicable safety reports in compliance with local regulations. Copies of all correspondence relating to reporting of any safety reports to the IEC/IRB/RA[s] should be maintained in the Investigator Site File and provided to Orion.

With respect to SUSARs, the reference document for definition of expectedness is the Package Insert of ProvayBlue™ (refer to APPENDIX 1: US Package Insert of ProvayBlue™).

The Sponsor, or its designee, Orion, will inform Investigators, central IEC(s)/IRB(s) and RAs of applicable safety reports, as required.

16. EVALUATION OF RESULTS

16.1. SAMPLE SIZE AND STUDY POWER

Because this is an ultra-orphan indication, the enrollment will be 10 patients, in adult and pediatric population.

Based on evaluation of historical data, the potential enrollment for this study is estimated to be 1/3 patient per site per year (i.e., one patient per site over the study period of approximately 32 months). Thus it is expected that 10 patients may be enrolled during the 3-year study.

A sample size of 10 will provide at least 80% power to detect a 90% response rate and exclude a response rate less than 50%.

As this is an open label study with only one treatment there will be no randomization and no blinding of subjects. Study numbers will be allocated to subjects in the order in which they are enrolled into the study.

16.2. STATISTICAL METHODS

Statistical analyses will be performed by Orion under the authority of the Sponsor. Statistical analyses will be carried out using SAS®, Version 9.3 or later, SAS Institute, Cary, North Carolina, USA.

The statistical methods for this study, summarized below, will be described in a detailed statistical analysis plan (SAP), which will be finalized prior to database lock. Any deviations from the planned analysis as described in the SAP will be justified and recorded in the final clinical study report.

16.2.1. General Considerations

Continuous variables will be summarized by descriptive statistics (number of patients, mean, standard deviation [SD], minimum, median and maximum). Geometric mean and coefficient of variation (CV) will be presented for PK parameters when appropriate. Categorical data will be summarized by absolute and relative frequencies.

A 95% confidence interval (CI) for the responder rate will be presented for each of the primary and secondary endpoints.

All variables will be listed on a patient level.

Unless indicated otherwise, summary statistics will be reported for observed data only. Missing data will not be imputed. If a baseline value is missing, no change from baseline will be calculated.

Baseline for all assessments will be defined as the last measurement taken prior to dosing with study drug.

16.2.2. Analysis Sets

The following analysis sets will be analyzed:

Full Analysis Set (FAS)

The Full Analysis Set (FAS) is defined as all patients who are enrolled. The FAS will be used for all efficacy analyses.

Safety Set

The Safety Set is defined as all patients who receive at least one dose of ProvayBlue™. The Safety Set will be used for all safety analyses.

PK Analysis Set

The PK Analysis Set is defined as those patients in the FAS who have any PK results.

16.3. EVALUATION OF BASELINE DATA

All data will be listed and summary tables will be provided by means of summary statistics and frequency tables as appropriate.

16.4. EVALUATION OF EFFICACY

Refer to Sections 7.2.1 and 7.2.2 for the primary and secondary efficacy endpoints, respectively.

Efficacy evaluations will be performed using the FAS.

Efficacy will be assessed based on the metHb level and on the normalization of respiratory rate, heart rate and blood pressure after administration of ProvayBlue™ (single- or repeat dose).

The results will be descriptive only. A 95% CI for the responder rate will be presented for each of the primary and secondary endpoints. The results will be compared with historical methylene blue results to confirm that ProvayBlue™'s results are not unexpected.

16.5. EVALUATION OF SAFETY

Refer to Section 7.2.3 for the safety endpoints.

All AEs will be displayed in summary tables, by Medical Dictionary for Regulatory Activities (MedDRA) system organ class and preferred term. Other safety data (including laboratory data) data will be summarized using appropriate descriptive statistics.

16.6. EVALUATION OF PHARMACOKINETICS (PK)

Refer to Section 7.2.4 for further details.

Results from PK analyses will be summarized and presented in the clinical study report.

16.7. INTERIM ANALYSIS

No interim analysis is planned.

17. OBLIGATIONS OF THE PRINCIPAL INVESTIGATOR (PI)

The study will be performed in accordance with

- The protocol;
- The Declaration of Helsinki (Version 1996);
- The International Council on Harmonization (ICH) Harmonized Tripartite Guideline for Good Clinical Practice (GCP)[1];
- All local regulations and IEC/IRB guidance.

17.1. INDEPENDENT ETHICS COMMITTEE/ INSTITUTIONAL REVIEW BOARD (IEC/IRB)

It is the responsibility of the Investigator to obtain approval of the trial protocol/amendments from the IEC/IRB of each participating Institution. Prior to the initiation of the study, the Investigator/Orion will submit the following documents to the appropriate IECs/IRBs for approval:

- Study protocol and any amendments;
- PICD and any other written documents to be provided to the patient;
- Package Insert of ProvayBlue™ (refer to APPENDIX 1: US Package Insert of ProvayBlue™);
- Details of any compensation to patients;
- Current *Curriculum Vitae* of the PI;
- Any other requested document(s).

A copy of the approval will be sent to Orion along with **all** other correspondence with the IEC/IRB, including the submission documents. The Investigator should file all IEC/IRB correspondence in the Investigator Site File.

The study will not start until approval of the protocol and the PICD has been obtained from the appropriate IECs/IRBs. The letter of approval should be dated, and should specify the protocol number and date of the protocol or amendment that was reviewed and approved. It should also specify the date of the PICD that was reviewed and approved.

For sites in the United States, the Federal Wide Assurance (FWA) number and appropriate documentation that study staff was not involved in the voting should be provided by the Investigator to Orion prior to study initiation.

A dated list of the voting members of the IEC/IRB who were present when the protocol was reviewed and approved, including their titles/occupations and institutional affiliations should be provided by the Investigator to Orion prior to study initiation. The Investigator will make all

[1] ICH Harmonized Tripartite Guideline for Good Clinical Practice (June 1996), as recommended for adoption to the three regulatory parties to ICH. Transmitted to the CPMP in July 1996 and approved on 17th July 1996 (CPMP/ICH/135/95). The proposed date for coming into operation is (for studies commencing after) 17th January 1997. Approved by FDA on 9th May 1997 and effective 9th May 1997 (62 FR 25692).

attempts to ensure that the IEC/IRB is constituted and operates in accordance with the ICH GCP and any local regulations.

The Investigator will submit any protocol amendments to the IEC/IRB (and other local authorities, according to local regulations) prior to implementation.

The Investigator will submit required progress reports to the IEC/IRB that approved the protocol at least annually, as well as report any SAEs, life-threatening problems or deaths, to comply with ICH GCP. The Investigator will also inform the IEC/IRB of reports of SAEs (provided to him/her by the Sponsor/Orion) that occurred in other clinical studies conducted with ProvayBlue™.

The Investigator must inform the IEC/IRB of the termination of the study.

17.2. REGULATORY BODY APPROVAL

The study will not start until receipt by the Sponsor/Orion of approval from the relevant regulatory authorities.

17.3. INFORMED CONSENT

PICDs will be based on a master document provided by Orion who must approve it prior to submission to the IEC/IRB. The content of the PICD should reflect the information described in Section 4.8.10 of the ICH Guidelines and any local requirements. Any change requested by the IEC/IRB must be approved by the Sponsor/Orion prior to the documents being used. A copy of the final, IEC/IRB-approved PICD must be submitted to the Sponsor/Orion prior to initiation and activation (green light approval) of this study.

Written informed consent will be obtained from each patient prior to inclusion in the trial, and prior to any study-related assessments are performed, as described in Section 12.

17.4. CASE REPORT FORMS AND SOURCE DOCUMENT VERIFICATION

CRFs of a design mutually agreed upon by the Sponsor and Orion will be supplied by Orion. CRFs are the sole property of the Sponsor and should not be made available in any form to third parties, except for authorized representatives of appropriate Health/Regulatory Authorities, without written permission from the Sponsor.

A CRF is required and should be completed for each included (consented) patient. The Investigator will be responsible for the accuracy of the data entered into the CRFs. All data must be entered in English in black ballpoint pen. Corrections of data should be made using one single line, leaving the corrected data clearly visible. The accurate data should be entered next to the inaccurate data. All changes must be initialled and dated. Correction fluids are not allowed. The Investigator will sign the relevant CRF pages as clearly indicated in the CRF. If a clinically significant change is made on any of the CRF pages after the Investigator has signed it, the Investigator must re-sign the relevant page to document that he agrees with the change.

The relevant completed CRF pages must be available for review/collection to designated Orion representatives at each scheduled monitoring visit.

The Investigator will allow designated Orion representatives and regulatory bodies to have direct access to the source documents to verify the data reported in the CRFs.

Source documents (e.g., medical records, raw data collection forms, pharmacy dispensing records, recorded data from automated instruments, laboratory data) are the originals of any documents used by the Investigator or hospital/institution that allow verification of the existence of the patient and substantiate the integrity of the data collected during the trial. Source documents should be available to support all the data recorded in the CRF. The Investigator will prepare and maintain adequate and accurate source documents. These documents are designed to record all observations and other pertinent data for each patient included in this clinical trial.

17.5. CONFIDENTIALITY

The Investigator must ensure that the patients' anonymity will be maintained. On the CRFs or other documents submitted to the Sponsor/Orion, patients should NOT be identified by their names, but by the assigned patient number.

If patient names are included on copies of documents submitted to Orion, the names will be obliterated and the assigned patient number added to the document.

The Investigator should keep a separate log (Patient Master List) of patient's codes (assigned patient number), names, addresses, telephone numbers and hospital numbers (if applicable). Documents not for submission to Orion (e.g. signed PICDs) should be maintained by the Investigator in strict confidence.

17.6. STAFF INFORMATION AND RESPONSIBILITIES

It is the responsibility of the Investigator to ensure that all personnel involved in the study are fully informed of all relevant aspects of the study, including detailed knowledge of and training in all procedures to be followed to allow collection of accurate, consistent, complete and reliable data.

The Investigator will provide a list of delegated responsibility to Orion, detailing the various study tasks to be performed by each member of his/her study staff. Each staff member should sign in agreement to their performing each of the tasks delegated to them on the list. Orion should ensure that all staff members have the required knowledge and training for the tasks delegated to them.

17.7. ESSENTIAL DOCUMENT RETENTION

All clinical information shall be recorded, handled and stored in such a way that it can be accurately reported, interpreted and verified, while ensuring confidentiality of the trial patients' personal data. Documents that enable both the conduct of the clinical trial and the quality of the data produced to be evaluated; and show whether the trial is, or has been, conducted in accordance with ICH-GCP and applicable regulatory requirements are considered essential documents.

The Investigator will retain copies of all the essential documents (as defined by ICH-GCP) until at least 2 years after the last approval of a marketing application in an ICH region, and until there are no pending or contemplated marketing applications in an ICH region, or at least 2 years have elapsed since the formal discontinuation of clinical development of ProvayBlue™. These

documents should be retained for a longer period, however, if required by the applicable regulatory requirements (e.g. EEC Directive 91/507 requires retention of patient codes for at least 15 years after the completion or discontinuation of a trial and retention of hospital records and other source data for the maximum time permitted by the institution where the study takes place). The Investigator should take measures to prevent accidental or premature destruction of these documents.

The essential documents include, but are not limited to: the signed protocol, copies of the completed CRFs, signed PICDs from all patients (and/or the patient's parent[s]/legal guardian[s]) who consented, hospital records, and other source documents, IRB/IEC approval and all related correspondence, including approved documents, and all other documentation included in the Investigator Site File.

The Investigator will inform the Sponsor of the storage location of these essential documents and must contact the Sponsor before disposing of any. If the Investigator wishes to assign the files to someone else (e.g. if he/she retires) or to remove them to another location, the Sponsor should be consulted about this change.

The Sponsor will inform the Investigator in writing when these documents no longer need to be retained.

18. STUDY MANAGEMENT

18.1. MONITORING

Prior to study commencement, the Investigator will be informed of the anticipated frequency of the monitoring visits. He/she will also receive a notification prior to each monitoring visit during the course of the study. It is expected that the Investigator and/or his/her Sub-investigator(s) and other appropriate staff will be available on the day of the visit to discuss study conduct. Orion is responsible for ensuring the proper conduct of the clinical trial with regards protocol adherence and validity of the data recorded in the CRFs.

A site initiation visit must be conducted by Orion and the site must be activated prior to the commencement of any study activities requiring informed consent.

18.2. QUALITY ASSURANCE (QA) AND QUALITY CONTROL (QC)

An independent audit of the study may be conducted during the study or after completion. The audit may be conducted by either Orion or the Sponsor's Quality Assurance (QA) department or an independent auditor or a regulatory authority.

18.2.1. Quality Control (QC)

Quality control (QC) is defined as the operational techniques and activities undertaken within the QA system to verify that the requirements for quality of the trial-related activities have been fulfilled.

QC should be applied to each stage of data handling to ensure that all data are reliable and have been processed correctly.

18.2.2. Quality Assurance (QA)

Quality assurance (QA) is defined as the planned and systematic actions that are established to ensure that the trial is performed and the data are generated, documented (recorded) and reported in compliance with GCP and the applicable regulatory requirements.

18.2.3. Audit

The Investigator will permit an audit mandated by the Sponsor after reasonable notice. The purpose of an audit is to confirm that the study is conducted as per protocol, GCP and applicable regulatory requirements, that the rights and well-being of patients enrolled have been protected and that all data relevant for the evaluation of ProvayBlue™ have been captured, processed and reported in compliance with the planned arrangements. The Investigator will permit direct access to all study documents, ProvayBlue™ accountability records, medical records and source data. The Investigator and his/her study team will also be available for discussion regarding study progress and procedures during the audit (both during the audit and at the end of the audit for an "exit" discussion).

18.3. DATA QUERY PROCESS

Data management of the CRF will be performed by Orion on behalf of the Sponsor.

Sites will enter the data from source documents into the CRF and the data will be verified for missing data, inconsistencies, and for any necessary medical clarifications. Queries arising from these checks will be flagged within the CRF. The site staff will correct, confirm or clarify the data as appropriate. All possible attempts should be made by the site staff to resolve the queries within the requested timeframes. If the site staff are unsure about the meaning of a query, or what data is required, then they should seek clarification from the Orion CRA assigned to their site.

Once all data queries have been resolved, the study will be declared to be "clean", and the CRF will be locked ready for statistical analysis. After clean-file status has been achieved, Orion will provide copies of each patient's CRF to the Investigator for archiving. Copies of each patient's CRF will also be archived by the Sponsor/Orion.

The data management, data handling, and analysis will be conducted in accordance with good clinical, scientific and data management principles and in compliance with Orion's Standard Operating Procedures.

18.4. PROTOCOL DEVIATIONS/AMENDMENTS

The trial must be conducted in accordance with:

- The protocol;
- Applicable regulatory requirement(s) or conditions linked to the approval(s) of the study;
- Applicable IEC/IRB requirement or conditions linked to the approval(s) of the study;
- Any particulars or documents, other than the protocol, accompanying the regulatory or IEC/IRB request or that application.

Any deviation from the protocol that has not been approved by the Sponsor and the relevant regulatory authority/IRB/IEC could result in a discontinuation from the study at the site involved. Any amendment(s) to the protocol must be approved by both the Sponsor and the relevant regulatory authority/IRB/IEC which granted the original approval of the study prior to their implementation (unless only logistical or administrative aspects of the trial are involved). All substantial amendments to the protocol must be approved by the applicable regulatory bodies prior to their implementation.

However, in the event of any medical emergency, the Investigator is free to institute any medical procedure he/she deems appropriate. Such events and procedures must be promptly reported to representatives of the Sponsor and Orion.

18.5. DISCONTINUATION OF THE STUDY

The Sponsor reserves the right to stop the study at any time on the basis of new information regarding safety or efficacy, or if study progress is unsatisfactory, or for other valid administrative reasons. After such a decision is made, the Investigator must inform all patients being screened or in the study as soon as possible. All delivered study materials must be collected and all CRFs completed to the extent possible.

18.6. PUBLICATIONS

Any manuscript, abstract or other publication or presentation of results or information arising in connection with the study (including any ancillary studies involving trial patients) must be prepared in conjunction with the Sponsor and must be submitted to the Sponsor for review and comment at least 8 weeks prior to submission for publication or presentation.

19. REFERENCES

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20. APPENDICES

APPENDIX 1: US PACKAGE INSERT OF PROVAYBLUE™

HIGHLIGHTS OF PRESCRIBING INFORMATION

These highlights do not include all the information needed to use (PROVAYBLUE®) safely and effectively. See full prescribing information for (PROVAYBLUE®).

PROVAYBLUE® (methylene blue) injection USP, for intravenous use

Initial U.S. Approval: 2016

.....INDICATIONS AND USAGE.....

PROVAYBLUE® (methylene blue) is an oxidation-reduction agent indicated for the treatment of pediatric and adult patients with acquired methemoglobinemia. This indication is approved under accelerated approval. Continued approval for this indication may be contingent upon verification of clinical benefit in subsequent trials. (1, 14)

..... DOSAGE AND ADMINISTRATION.....

- Administer 1 mg/kg intravenously over 5-30 minutes. (2.1)
- If methemoglobin level remains above 30% or if clinical symptoms persist, give a repeat dose of up to 1 mg/kg one hour after the first dose. (2.1)

.....DOSAGE FORMS AND STRENGTHS.....

50 mg/10 mL (5 mg/mL) single-dose ampule. (3)

.....CONTRAINDICATIONS.....

PROVAYBLUE® is contraindicated in the following conditions (4):

- Severe hypersensitivity to methylene blue
- Patients with glucose-6-phosphate dehydrogenase deficiency (G6PD) due to the risk of hemolytic anemia

.....WARNINGS AND PRECAUTIONS.....

- Hypersensitivity: If severe or life threatening allergic reaction occurs, discontinue PROVAYBLUE®, treat the allergic reaction, and monitor until signs and symptoms resolve (5.2)
- Lack of Effectiveness: Consider alternative treatments if there is no resolution of methemoglobinemia after 2 doses (2.1, 5.3)
- Hemolytic Anemia: Discontinue PROVAYBLUE® and transfuse (5.4)
- Interference with In-Vivo Monitoring Devices: Use methods other than pulse oximetry to assess oxygen saturation (5.5)
- Effects on Ability to Drive and Operate Machinery: Advise patients to refrain from these activities until neurologic and visual symptoms have resolved (5.6)

..... ADVERSE REACTIONS.....

The most commonly reported adverse reactions (>10%) are pain in extremity, chromaturia, dysgeusia, feeling hot, dizziness, hyperhidrosis, nausea, skin, discoloration and headache. (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact American Regent at 1-800-734-9236 or FDA at 1-800-FDA-1088 or www.fda.gov/medwatch.

.....USE IN SPECIFIC POPULATIONS.....

- Pregnancy: Only use during pregnancy if the potential benefit justifies the potential risk to the fetus. (8.1)
- Lactation: Discontinue breast-feeding for up to 8 days after treatment. (8.2).
- Renal Insufficiency: Monitor patients longer for toxicity and drug interactions due to delayed clearance. (8.6)
- Hepatic Impairment: Monitor patients longer for toxicity and drug interactions due to delayed clearance. (8.7)

See 17 for PATIENT COUNSELING INFORMATION

Revised: 7/2017

FULL PRESCRIBING INFORMATION: CONTENTS*

***Sections or subsections omitted from the full prescribing information are not listed**

FULL PRESCRIBING INFORMATION

WARNING: SEROTONIN SYNDROME WITH CONCOMITANT USE OF SEROTONERGIC DRUGS

PROVAYBLUE® may cause serious or fatal serotonergic syndrome when used in combination with serotonergic drugs. Avoid concomitant use of PROVAYBLUE® with selective serotonin reuptake inhibitors (SSRIs), serotonin and norepinephrine reuptake inhibitors (SNRIs), and monoamine oxidase inhibitors (5.1, 7.1). *[see Warnings and Precautions (5.1) and Drug Interactions (7.1)]*

1 INDICATIONS AND USAGE

PROVAYBLUE® USP is indicated for the treatment of pediatric and adult patients with acquired methemoglobinemia.

This indication is approved under accelerated approval. Continued approval for this indication may be contingent upon verification of clinical benefit in subsequent trials *[see Clinical Studies (14.1)]*.

2 DOSAGE AND ADMINISTRATION

2.1 Dosage and Administration

- Ensure patent venous access prior to administration of PROVAYBLUE®. Do not administer PROVAYBLUE® subcutaneously.
- Monitor vital signs, electrocardiogram and methemoglobin levels during treatment with PROVAYBLUE® and through resolution of methemoglobinemia.
- Administer PROVAYBLUE® 1 mg/kg intravenously over 5-30 minutes.
- If the methemoglobin level remains greater than 30% or if clinical signs and symptoms persist, a repeat dose of PROVAYBLUE® 1 mg/kg may be given one hour after the first dose.
- If methemoglobinemia does not resolve after 2 doses of PROVAYBLUE®, consider initiating alternative interventions for treatment of methemoglobinemia.

2.2 Preparation and Storage

Each mL of PROVAYBLUE® contains 5 mg methylene blue

Each 10 mL ampule of PROVAYBLUE® contains 50 mg methylene blue.

PROVAYBLUE® is hypotonic and may be diluted before use in a solution of 50 mL 5% Dextrose in Water (D5W) in order to avoid local pain, particularly in the pediatric population. Use the diluted solution immediately after preparation.

Do not mix with sodium chloride 9 mg/mL (0.9%) solution for injection, because it has been demonstrated that chloride reduces the solubility of methylene blue.

Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit.

Keep the ampule in the original package to protect from light.

3 DOSAGE FORMS AND STRENGTHS

Injection: 50 mg/10 mL (5 mg/mL) clear dark blue solution in single-dose ampules

4 CONTRAINDICATIONS

PROVAYBLUE® is contraindicated in the following conditions:

- Severe hypersensitivity reactions to methylene blue or any other thiazine dye *[see Warnings and Precautions (5.2)]*.
- Patients with glucose-6-phosphate dehydrogenase deficiency (G6PD) due to the risk of hemolytic anemia *[see Warnings and Precautions (5.3, 5.4)]*

5 WARNINGS AND PRECAUTIONS

5.1 Serotonin Syndrome with Concomitant Use of Serotonergic Drugs

The development of serotonin syndrome has been reported with use of methylene blue class products. Most reports have been associated with concomitant use of serotonergic drugs (e.g., selective serotonin reuptake inhibitors (SSRIs), serotonin and norepinephrine reuptake inhibitors (SNRIs), monoamine oxidase inhibitors). Some of the reported cases were fatal. Symptoms associated with serotonin syndrome may include the following combination of signs and symptoms: mental status changes (e.g., agitation, hallucinations, delirium, and coma), autonomic instability (e.g., tachycardia, labile blood pressure, dizziness, diaphoresis, flushing, and hyperthermia), neuromuscular symptoms (e.g., tremor, rigidity, myoclonus, hyperreflexia, and incoordination), seizures, and/or gastrointestinal symptoms (e.g., nausea, vomiting, diarrhea). Avoid concomitant use of PROVAYBLUE® with serotonergic drugs.

Patients treated with PROVAYBLUE® should be monitored for the emergence of serotonin syndrome. If symptoms of serotonin syndrome occur, discontinue use of PROVAYBLUE®, and initiate supportive treatment. Inform patients of the increased risk of serotonin syndrome and advise them to not to take serotonergic drugs within 72 hours after the last dose of PROVAYBLUE® *[see Drug Interactions (7), Patient Counseling Information (17)]*.

5.2 Hypersensitivity

Anaphylactic reactions to methylene blue class products have been reported. Patients treated with PROVAYBLUE® should be monitored for anaphylaxis. If anaphylaxis or other severe hypersensitivity reactions (e.g., angioedema, urticaria, bronchospasm) should occur, discontinue use of PROVAYBLUE® and initiate supportive treatment. PROVAYBLUE® is contraindicated in patients who have experienced anaphylaxis or other severe hypersensitivity reactions to a methylene blue class product in the past.

5.3 Lack of Effectiveness

Methemoglobinemia may not resolve or may rebound after response to treatment with PROVAYBLUE® in patients with methemoglobinemia due to aryl amines such as aniline or sulfa drugs such as dapsone. Monitor response to therapy with PROVAYBLUE® through resolution of methemoglobinemia. If methemoglobinemia does not respond to 2 doses of PROVAYBLUE® or if methemoglobinemia rebounds after a response, consider additional treatment options *[see Dosage and Administration (2.2)]*.

Patients with glucose-6-phosphate dehydrogenase deficiency may not reduce PROVAYBLUE® to its active form in vivo. PROVAYBLUE® may not be effective in patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency.

5.4 Hemolytic Anemia

Hemolysis can occur during treatment of methemoglobinemia with PROVAYBLUE®. Laboratory testing may show Heinz bodies, elevated indirect bilirubin and low haptoglobin, but the Coombs test is negative. The onset of anemia may be delayed 1 or more days after treatment with PROVAYBLUE®. The anemia may require red blood cell transfusions. *[see Adverse Reactions (6.1)]*. Use the lowest effective number of doses of PROVAYBLUE® to treat methemoglobinemia. Discontinue PROVAYBLUE® and consider alternative treatments of methemoglobinemia if severe hemolysis occurs.

Treatment of patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency with PROVAYBLUE® may result in severe hemolysis and severe anemia. PROVAYBLUE® is contraindicated for use in patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency *[see Contraindications (4)]*.

5.5 Interference with In Vivo Monitoring Devices

- Inaccurate Pulse Oximeter Readings

The presence of methylene blue in the blood may result in an underestimation of the oxygen saturation reading by pulse oximetry. If a measure of oxygen saturation is required during or shortly after infusion of PROVAYBLUE®, it is advisable to obtain an arterial blood sample for testing by an alternative method.

- Bispectral index monitor

A fall in the Bispectral Index (BIS) has been reported following administration of methylene blue class products. If PROVAYBLUE® is administered during surgery, alternative methods for assessing the depth of anesthesia should be employed.

5.6 Effects on Ability to Drive and Operate Machinery

Treatment with PROVAYBLUE® may cause confusion, dizziness and disturbances in vision *[see Adverse Reactions (6)]*. Advise patients to refrain from driving or engaging in hazardous occupations or activities such as operating heavy or potentially dangerous machinery until such adverse reactions to PROVAYBLUE® have resolved.

5.7 Interference with Laboratory Tests

PROVAYBLUE® is a blue dye which passes freely into the urine and may interfere with the interpretation of any urine test which relies on a blue indicator, such as the dipstick test for leucocyte esterase.

6 ADVERSE REACTIONS

The following adverse reactions are discussed in greater detail in other sections of the labeling:

- Serotonin Syndrome with Concomitant Use of Serotonergic Drugs *[see Warnings and Precautions (5.1)]*
- Anaphylaxis *[see Warnings and Precautions (5.2)]*
- Lack of Effectiveness *[see Warnings and Precautions (5.3)]*
- Hemolytic Anemia *[see Warnings and Precautions (5.4)]*
- Interference with In-Vivo Monitoring Devices *[see Warnings and Precautions (5.5)]*
- Effects on Ability to Drive and Operate Machinery *[see Warnings and Precautions (5.6)]*
- Interference with Laboratory Tests *[see Warnings and Precautions (5.7)]*

6.1 Clinical Trials Experience

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a drug cannot be directly compared to rates in the clinical trials of another drug and may not reflect the rates observed in practice.

The safety of PROVAYBLUE® was determined in 82 healthy adults of median age of 36 years (range, 19-55 years); 54% were male, and 68% were white. Each individual in the safety population received a single dose of PROVAYBLUE® 2 mg/kg intravenously. There was one serious adverse reaction reported (syncope due to sinus pauses of 3-14 seconds). The most common (>2%) moderate or severe adverse reactions were pain in the extremity (56%), headache (7%), feeling hot (6%), syncope (4%), back pain (2%), hyperhidrosis (2%) and nausea (2%). Table 1 lists the adverse reactions of any severity that occurred in at least 2% of individuals who received PROVAYBLUE®.

Table 1. Adverse Reactions Following Infusion of PROVAYBLUE® 2 mg/kg

Adverse Reaction	Any Grade TEAE (n=82)		Moderate-Severe TEAE (n=82)	
Pain in extremity	69	84%	46	56%
Chromaturia	61	74%	0	
Dysgeusia	16	20%	1	1%
Feeling hot	14	17%	5	6%
Dizziness	13	16%	4	5%
Hyperhidrosis	11	13%	2	2%
Nausea	11	13%	2	2%
Skin discoloration	11	13%	0	
Headache	8	10%	6	7%
Musculoskeletal pain	7	9%	0	
Paresthesia oral	7	9%	0	
Paresthesia	7	9%	0	
Infusion site pain	5	6%	1	1%
Feeling cold	5	6%	0	
Pallor	4	5%	0	
Dermatitis contact	4	5%	0	
Syncope	3	4%	3	4%
Influenza like illness	3	4%	1	1%
Pruritus	3	4%	1	1%
Anxiety	3	4%	0	
Decreased appetite	3	4%	0	
Chest discomfort	3	4%	0	
Back pain	2	2%	2	2%
Cold sweat	2	2%	1	1%
Dizziness postural	2	2%	1	1%
Muscle spasms	2	2%	1	1%
Presyncope	2	2%	1	1%
Vomiting	2	2%	1	1%
Arthralgia	2	2%	1	1%
Chills	2	2%	0	
Diarrhea	2	2%	0	
Discomfort	2	2%	0	
Dyspnea	2	2%	0	
Erythema	2	2%	0	
Hypoesthesia oral	2	2%	0	
Infusion site discomfort	2	2%	0	
Limb discomfort	2	2%	0	
Oral discomfort	2	2%	0	
Catheter site pain	2	2%	0	
Ecchymosis	2	2%	0	

Other adverse reactions reported to occur following administration of methylene blue class products include the following:
Blood and lymphatic system disorders: hemolytic anemia, hemolysis, hyperbilirubinemia, methemoglobinemia
Cardiac disorders: palpitations, tachycardia
Eye disorders: eye pruritus, ocular hyperemia, vision blurred
Gastrointestinal disorders: abdominal pain lower, dry mouth, flatulence, glossodynia, tongue eruption
General disorders and administration site conditions: death, infusion site extravasation, infusion site induration, infusion site pruritus, infusion site swelling, infusion site urticaria, peripheral swelling, thirst
Investigations: elevated liver enzymes
Musculoskeletal and connective tissue disorders: myalgia
Renal and urinary disorders: dysuria
Respiratory, thoracic and mediastinal disorders: nasal congestion, oropharyngeal pain, rhinorrhea, sneezing
Skin and subcutaneous tissue disorders: necrotic ulcer, papule, phototoxicity
Vascular disorders: hypertension

7 DRUG INTERACTIONS

7.1 Serotonergic Drugs

Avoid concomitant use of PROVAYBLUE® with medicinal products that enhance serotonergic transmission including SSRIs (selective serotonin reuptake inhibitors), MAO inhibitors, bupropion, buspirone, clomipramine, mirtazapine and venlafaxine; because of the potential for serious CNS reactions, including potentially fatal serotonin syndrome. Although the mechanism is not clearly, understood, literature reports suggest inhibition of MAO by methylene blue may be involved. In addition, in vitro studies cannot rule out the potential involvement of CYP 2D6 inhibition by methylene blue. If the intravenous use of PROVAYBLUE® cannot be avoided in patients treated with serotonergic medicinal products, choose the lowest possible dose and observe closely the patient for CNS effects for up to 4 hours after administration *[see Warning and Precautions (5.1) ,Clinical Pharmacology (12.3)]*.

7.2 Agents Metabolized by Cytochrome P450 Enzymes

Methylene blue inhibits a range of CYP isozymes in vitro, including 1A2, 2B6, 2C8, 2C9, 2C19, 2D6 and 3A4/5. This interaction could be more pronounced with narrow therapeutic index drugs that are metabolized by one of these enzymes (e.g, digoxin, warfarin, phenytoin, alfentanil, cyclosporine, dihydroergotamine, ergotamine, fentanyl, pimozide, quinidine, sirolimus and tacrolimus). However, the clinical relevance of these in vitro interactions is unknown.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Risk Summary

PROVAYBLUE® may cause fetal harm when administered to a pregnant woman. Intra-amniotic injection of pregnant women with a methylene blue class product during the second trimester was associated with neonatal intestinal atresia and fetal death. Methylene blue produced adverse developmental outcomes in rats and rabbits when administered orally during organogenesis at doses at least 32 and 16 times, respectively, the clinical dose of 1 mg/kg *[see Data]*. Advise pregnant women of the potential risk to a fetus.

In the U.S. general population, the estimated background risks of major birth defects and miscarriage in clinically recognized pregnancies are 2-4% and 15-20%, respectively.

Clinical Considerations

Fetal/neonatal adverse reactions

Intra-amniotic injection of a methylene blue class product hours to days prior to birth can result hyperbilirubinemia, hemolytic anemia, skin staining, methemoglobinemia, respiratory distress and photosensitivity in the newborn. Following administration of PROVAYBLUE® to a pregnant woman at term, observe the newborn for these adverse reactions and institute supportive care.

Data

Animal Data

Methylene blue was administered orally to pregnant rats at doses of 50 to 350 mg/kg/day, during the period of organogenesis. Maternal and embryofetal toxicities were observed at all doses of methylene blue, and were most evident at the 200 and 350 mg/kg/day doses. Maternal toxicity consisted of increased spleen weight. Embryo-fetal toxicities included reduced fetal weight, post-implantation loss, edema, and malformations including enlarged lateral ventricles. The dose of 200 mg/kg (1200 mg/m2) in rats is approximately 32 times a clinical dose of 1 mg/kg based on body surface area.

Methylene blue was administered orally to pregnant rabbits at doses of 50, 100, or 150 mg/kg/day, during the period of organogenesis. Maternal death was observed at the methylene blue dose of 100 mg/kg. Embryofetal toxicities included spontaneous abortion at all dose levels and a malformation (umbilical hernia) at the 100 and 150 mg/kg/day doses. The dose of 50 mg/kg (600 mg/m2) in rabbits is approximately 16 times a clinical dose of 1 mg/kg based on body surface area.

8.2 Lactation

Risk Summary

There is no information regarding the presence of methylene blue in human milk, the effects on the breastfed infant, or the effects on milk production. Because of the potential for serious adverse reactions, including genotoxicity discontinue breast-feeding during and for up to 8 days after treatment with PROVAYBLUE® *[see Clinical Pharmacology (12.3)]*.

8.4 Pediatric Use

The safety and effectiveness of PROVAYBLUE® have been established in pediatric patients. Use of PROVAYBLUE® is supported by two retrospective case series that included 2 pediatric patients treated with PROVAYBLUE® and 12 treated with another methylene blue class product. The case series included pediatric patients in the following age groups: 3 neonates (less than 1 month), 4 infants (1 month up to less than 2 years), 4 children (2 years up to less than 12 years), and 3 adolescents (12 years to less than 17 years). The efficacy outcomes were consistent across pediatric and adult patients in both case series *[see Clinical Studies (14)]*.

8.5 Geriatric Use

The retrospective case series included 3 patients age 65 years and over treated with PROVAYBLUE® (or a bioequivalent formulation) and 5 treated with another methylene blue class product. The efficacy outcomes were consistent across adult and elderly patients in both case series *[see Clinical Studies (14)]*. This drug is known to be substantially excreted by the kidney, so the risk of adverse reactions to this drug may be greater in patients with impaired renal function. Because elderly patients are more likely to have decreased renal function, treatment of methemoglobinemia in these patients should use the lowest number of doses needed to achieve a response *[see Dosage and Administration (2)]*.

8.6 Renal Impairment

Approximately 40% of methylene blue is excreted by the kidneys. Patients with any renal impairment should be monitored for toxicities and potential drug interactions for an extended period of time following treatment with PROVAYBLUE®.

8.7 Hepatic Impairment

Methylene blue is extensively metabolized in the liver. Monitor patients with any hepatic impairment for toxicities and potential drug interactions for an extended period of time following treatment with PROVAYBLUE®.

10 OVERDOSAGE

Hypotension, wheezing and reduced oxygenation have been reported in patients who received methylene blue class products in single doses of 3 mg/kg or more.

Administration of large intravenous doses (cumulative dose ≥ 7 mg/kg) of a methylene blue class product caused nausea, vomiting, precordial pain, dyspnea, tachypnea, chest tightness, tachycardia, apprehension, tremor, mydriasis, blue staining of the urine, the skin and mucous membranes, abdominal pain, dizziness, paresthesia, headache, confusion, mild methemoglobinemia (up to 7%) and electrocardiogram changes (T-wave flattening or inversion). These effects lasted 2-12 hours following administration.

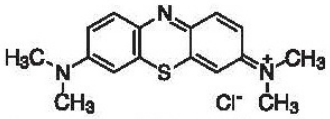
A severe overdosage (single dose of 20 mg/kg or more) of a methylene blue class product caused severe intravascular hemolysis, hyperbilirubinemia and death.

In case of overdose of PROVAYBLUE®, maintain the patient under observation until signs and symptoms have resolved, monitor for cardiopulmonary, hematologic and neurologic toxicities, and institute supportive measures as necessary.

11 DESCRIPTION

PROVAYBLUE® is an oxidation-reduction agent. PROVAYBLUE® (methylene blue) is a sterile solution intended for intravenous administration. Each PROVAYBLUE®, 10 mL ampule contains 50 mg Proveblue® methylene blue and water for injection q.s. Each mL of solution contains 5 mg methylene blue and water for injection q.s.

Methylene blue is 3,7-bis(dimethylamino)phenothiazin-5-ium, chloride. The molecular formula of methylene blue is C16H18ClN3S and its molecular weight is 319.86 g/mol. Its structural formula is:



PROVAYBLUE® is a clear dark blue solution with a pH value between 3.0 and 4.5. The osmolality is between 10 and 15 mOsm/kg.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Methylene blue is a water soluble thiazine dye that promotes a non-enyzmatic redox conversion of metHb to hemoglobin. In situ, methylene blue is first converted to leucomethylene blue (LMB) via NADPH reductase. It is the LMB molecule which then reduces the ferric iron of metHb to the ferrous state of normal hemoglobin.

12.2 Pharmacodynamics

Low concentrations of methylene blue speeds up the in vivo conversion of methemoglobin to hemoglobin. Methylene blue has been observed to stain tissues selectively. The exposure-response or –safety relationship for methylene is unknown.

Cardiac Electrophysiology

The results of a thorough QT study demonstrated PROVAYBLUE® at an intravenous dose of 2 mg/kg as a 5-minute intravenous infusion had no effect on the QT, PR or QRS intervals.

12.3 Pharmacokinetics

The mean (CV%) Cmax and AUC of methylene blue 2,917 ng/mL (39%) and 13977 ng.hr/mL (21%) following a 2 mg/kg dose administered as a 5-minute intravenous infusion.

Distribution

The mean± standard deviation steady state volume of distribution of a 2 mg/kg dose of PROVAYBLUE® was 255 L ± 58. The mean plasma protein binding of methylene blue is approximately 94% in vitro. Methylene blue exhibits concentration-dependent partitioning into blood cells in vitro. The blood-to-plasma ratio was 5.1±2.8 at 5 minutes from the start of a 2 mg/kg dose administered as a 5-minute intravenous infusion and reached a plateau of 0.6 at 4 hours in a clinical study. Methylene Blue is a substrate for the P-glycoprotein (P-gp, ABCB1) transporter, but not for BCRP or OCT2 in vitro.

Elimination

Methylene blue has a half-life of approximately 24 hours.

Metabolism

Methylene blue is metabolized by CYPs 1A2, 2C19 and 2D6 in vitro; however, the predominant in vitro pathway appears to be UGT-mediated conjugation by multiple UGT enzymes, including UGT1A4 and UGT1A9.

Azure B, which is a minor impurity in methylene blue, is also formed in humans as a metabolite of methylene blue, with an overall drug/metabolite AUC ratio of greater than 6:1. Azure B has 8-fold lower potency than methylene blue.

Excretion

Approximately 40% of methylene blue is excreted in to the urine unchanged.

Drug Interaction Studies

The clinical relevance of in vitro inhibition or induction of the metabolizing enzymes and transporter systems described below is unknown, but it cannot be excluded that the systemic exposure of medicinal products being substrates for these enzymes or transporter systems may be affected with concomitant administration with PROVAYBLUE® Injection.

Cytochrome P450

Methylene blue inhibits CYP isozymes 1A2, 2B6, 2C8, 2C9, 2C19, 2D6 and 3A4/5 in vitro. Possible time-dependent inhibition of CYP2C9, CYP2D6 and CYP3A4/5 (testosterone as substrate) was also observed in vitro. Methylene blue induces CYP1A2, but does not induce CYP2B6 or CYP3A4 in vitro.

Glucuronosyltransferase

Methylene blue inhibits UGT1A9 and UGT1A4 in vitro, but did not significantly inhibit UGTs 1A1, 1A3, 1A6, 2B7 or 2B15.

Transporter Interactions

Methylene blue is both a substrate for and an inhibitor of P-gp, but is not a substrate for BCRP or OCT2 in vitro. Methylene blue is not a significant inhibitor of BCRP, OAT1, OAT3, OAT1B1 or OAT1B3 in vitro. Methylene blue inhibits OCT2, MATE1 and MATE2-K in vitro. The OCT2/MATE pathway for renal transport is reported to play a significant role in the elimination of several substances, including metformin, cimetidine, acyclovir and creatinine

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

In a two-year carcinogenicity study, rats were administered oral doses of methylene blue at 5, 25, or 50 mg/kg. Methylene blue caused pancreatic islet adenomas or carcinomas (combined) in male rats. In a two-year year carcinogenicity study, mice were administered oral doses of methylene blue at 2.5, 12.5, or 25 mg/kg. There were no drug-related neoplastic findings in mice.

Methylene blue was genotoxic in gene mutation assays in bacteria (Ames test), and in an in vitro sister chromatid exchange test and an in vitro chromosomal aberration test in Chinese hamster ovary (CHO) cells. Methylene blue was negative for micronucleus induction in bone narrow or peripheral blood collected from mice treated with methylene blue.

Fertility studies with methylene blue have not been conducted. In vitro, methylene blue reduced motility of human sperm in a concentration dependent manner.

14 CLINICAL STUDIES

14.1 Treatment of Acquired Methemoglobinemia

The efficacy of PROVAYBLUE® was assessed on the basis of a methemoglobin decrease of at least 50% within 1 hour after intravenous administration of 1 – 2 mg/kg PROVAYBLUE® (or a bioequivalent formulation) in 6 patients identified by retrospective chart review or literature search. The 6 patients included 3 males and 3 females of median age 54 years (range, 6 days to 69 years). The median methemoglobin level at baseline was 37% (range, 11% to 47%). All 6 (100%) patients had a decrease in methemoglobin by at least 50% within 1 hour after treatment.

An additional 41 cases of treatment of methemoglobinemia with a methylene blue class product were identified in the published literature. These cases included 24 males and 17 females of median age 33 years (range, 9 days to 80 years). The median methemoglobin level at baseline was 40% (range, 10% to 98%). Of these 41 patients, 37 (90%) had a methemoglobin decrease of at least 50% within 1 hour after intravenous administration of the methylene blue class product.

In a combined analysis of all 47 patients treated intravenously with PROVAYBLUE® (or a bioequivalent formulation) or with another methylene blue class product, there was no difference in response rate by dose. The methemoglobin decreased by at least 50% within 1 hour of infusion for 15/17 (88%) of patients treated with 1 mg/kg, 12/13 (92%) treated with 2 mg/kg and 16/17 (94%) treated with a different dose or for those whose dose was not reported.

16 HOW SUPPLIED/STORAGE AND HANDLING

PROVAYBLUE® is supplied in 10 mL single-dose ampules. Each 10 mL ampule contains 50 mg of methylene blue as a clear dark blue solution. A box contains five ampules placed in a tray.

Box of 5 ampules: NDC 0517-0374-05

Storage:

Store at 20°C to 25°C (68°F to 77°F). *[See USP Controlled Room Temperature]*

Any unused product or waste material should be disposed of in accordance with local practice.

Do not refrigerate or freeze.

Keep the ampule in the original package to protect from light.

17 PATIENT COUNSELING INFORMATION

Serotonin Syndrome

Advise patients of the possibility of serotonin syndrome, especially with concomitant use of serotonergic agents such as medications to treat depression and migraines. Advise patients to seek immediate medical attention if the following symptoms occur after treatment with PROVAYBLUE®: changes in mental status, autonomic instability, or neuromuscular symptoms with or without gastrointestinal symptoms *[see Warnings and Precautions (5.1)]*.

Pregnancy

Advise pregnant women of the potential risk to the fetus with the use of PROVAYBLUE® during pregnancy *[see Use in Specific populations (8.1)]*.

Breastfeeding

Advise patients to discontinue breast-feeding for up to 8 days after treatment with PROVAYBLUE® *[see Use in Specific populations (8.2)]*.

Driving and Using Machines

Advise patients to avoid driving and use of machines during treatment with PROVAYBLUE®. Driving can be affected as a result of a confusional state, dizziness and possible eye disturbances *[see Warnings and Precautions (5.6)]*.

Phototoxicity

Advise patients to take protective measures against exposure to light, because phototoxicity may occur after administration of methylene blue *[see Adverse Reactions (6.1)]*.

Skin and Body Fluid Blue Discoloration

Advise patients that PROVAYBLUE® may cause a blue discoloration of the skin and body fluids *[see Adverse Reactions (6.1)]*.

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PROVEPHARM SAS
22 rue Marc Donadille
13013 Marseille, France

Manufactured by:
CENEXI
52 rue Marcel et Jacques Gaucher
94120 Fontenay sous Bois, FRANCE

Distributed by:
American Regent
Shirley, NY- 11967
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APPENDIX 2: STUDY FLOW CHART

Data collection/ Assessments	SCREENING/ ENROLLMENT	TREATMENT (Hours after patient presentation)					DISCHARGE	FOLLOW-UP VISIT (Day 5-10) [b]	FOLLOW-UP CALL (Day 10-15)[b]	WITH-DRAWAL
		0	1	2	3	24 [a]				
Informed consent[c]	X									
Demographics	X									
Medical history, incl. all concomitant disease(s)	X									
Hospital admission data[d]	X									
Methemoglobinemia diagnosis/clinical symptoms	X[e]		X[f]	X[f]	X[f]	X[f]	X[f]	X[f]		X[f]
Inclusion and exclusion criteria	X									
Physical exam[g]	X (incl. weight)						X	X		
Vital signs[h]	X[i]		X[j]	X[j]	X[j]	X	X	X		X
Pregnancy test[t]	X									
Methemoglobin	X		X[l]	X[m]	X[n]	X	X			X
Hematology[k]	X		X	X	X[n]	X	X	X		X
Biochemistry[k]	X		X	X	X[n]	X	X	X		X
12-Lead ECG[o]	X					X	X			
ProvayBlue™ Dose 1 administration[p]		X								
ProvayBlue™ Dose 2 administration[p]			X							
Additional ProvayBlue™ doses administration[p]				X	X					
PK sample[q]			X	X	X[n]	X	X			
Concomitant medications/ medical procedures[r]	←----------X----------→									
Adverse events[s]	←----------X----------→									

a. 24 hours after the end of the infusion of ProvayBlue™ Dose 1.

b. Patients will be follow-up with a visit to take place 5 to 10 days following the last ProvayBlue™ administration and a telephone call to take place 10 to 15 days following the last ProvayBlue™ administration.

c. Due to the nature of the disease indication, the patient's treatment with ProvayBlue™ as per standard of care can take place prior to or after PICD signature. Data collection (retrospective and prospective) for this study and study specific assessments will only take place after PICD signature.

d. Date, time and initial reason for hospitalization.

e. Methemoglobinemia diagnosis (including name of toxic agents responsible for methemoglobinemia if available, clinical symptoms related to methemoglobinemia [e.g. sleepiness, cyanosis, dizziness, etc.], methemoglobinemia level at presentation in hospital).

f. Clinical symptoms related to methemoglobinemia and severity (e.g. sleepiness, cyanosis, dizziness, etc.).

g. Physical examinations during the study will be based on patient standard of care as appropriate to determine general condition. Weight will be recorded at screening.

h. Vital signs will include: Systolic (SBP) and diastolic (DBP) blood pressure (mmHg), heart rate (beats per minute [bmp]), body temperature (ºC or ºF), respiration rate (breaths per minute), oxygen saturation by co-oximetry.

i. If the patient is treated prior to consent, all available pre-treatment vital sign information should be retrieved where possible;

j. Within 1 and 2 hours after the end of each ProvayBlue™ infusion (i.e. both Dose 1, Dose 2 and any additional dose if applicable).

k. Additional (optional) parameters (for example blood gas) will be collected based on available data from patient standard of care.

l. Within 1 hour after the end of ProvayBlue™ infusion.

m. If applicable (if the metHb level is acceptable within 1 hour after the end of Dose 1 ProvayBlue™ infusion, the repeat dose of ProvayBlue™ will not be needed).

n. If applicable, in case of administration of additional doses of ProvayBlue at the discretion of the Investigator

o. Collection of ECG data is mandatory at admission and approximately at 24 hours after the end of the last ProvayBlue™ infusion or just prior to discharge from the clinic if earlier than 24 hours. The collection of additional data will be based on available 12-lead ECG recordings performed as per patient standard of care as appropriate to determine general condition.

p. ProvayBlue™ will be administered IV at a dose of 1 mg/kg over 5-30 minutes. If the methemoglobin level remains greater than 30% or if clinical signs and symptoms persist, a repeat dose of 1 mg/kg may be given 1 hour after the first dose. Depending on the clinical status of the patient, additional doses may be administered at the discretion of the Investigator. The data on these additional doses will be recorded in the study CRF.

q. PK blood samples are to be drawn at the discretion of the Investigator. The suggested schedule of PK blood sampling is:
   - Approximately 1 hour after the end of each ProvayBlue™ infusion (i.e. Dose 1, Dose 2 and any additional dose if applicable);
   - Prior to Dose 2 (or any additional dose) ProvayBlue™ infusion (if applicable);
   - Approximately 24 hours post the end of the last ProvayBlue™ infusion, or just prior to discharge from the clinic if earlier than 24 hours.

r. Collection of concomitant medications/medical procedures from PICD signature or the start of ProvayBlue™ treatment (whichever occurs sooner) until 10 to 15 days after the last administration of ProvayBlue™;

s. Collection of AEs that may have occurred since the start of ProvayBlue™ treatment until 10 to 15 days after the last ProvayBlue™ dosing.

t. All females of childbearing potential will be submitted to a pregnancy test (both urine and serum unless one is not possible) at Screening.

APPENDIX 3: ESTIMATES OF 1% OF THE PATIENT'S BLOOD VOLUME

Weight in Kilograms	Weight in Pounds	Total Blood Volume mL	1% Total Blood Volume mL	Weight in Kilograms	Weight in Pounds	Total Blood Volume mL	1% Total Blood Volume mL
1	2.2	105	2.2	31 - 35	68 - 77	2480 - 2800	62 - 70
2	4.4	210	4.4	36 - 40	79 - 88	2880 - 3200	72 – 80
3	6.6	240	6.6	41 – 45	90 - 99	3280 - 3600	82 – 90
4	8.8	320	8.8	46 – 50	101 – 110	3680 - 4000	92 –100
5	11	400	11	51 – 55	112 - 121	4080 - 4400	102 –110
6	13.2	480	13.2	56 – 60	123 - 132	4480 - 4800	112 - 120
7	15.4	560	15.4	61 – 65	134 - 143	4880 - 5200	122 – 130
8	17.6	640	17.6	66 – 70	145 – 154	5280 - 5600	132 – 140
9	19.8	720	19.8	71 – 75	156 - 165	5680 - 6000	142 – 150
10	22	800	22	76 – 80	167 - 176	6080 - 6400	152 – 160
11 –15	24 - 33	880 - 1200	24 - 33	81 – 85	178 - 187	6480 - 6800	162 – 170
16 – 20	35 – 44	1280 - 1600	35 – 44	86 – 90	189 - 198	6840 - 7200	172 – 180
21 – 25	46 – 55	1680 - 2000	46 – 55	91 – 95	200 - 209	7280 - 7600	182 – 190
26 - 30	57 – 66	2080- 2400	57 – 66	96 - 100	211 - 220	7680 - 8000	192 - 200

Source: Usher et al, 1963