Use of Methylene Blue in Acquired Methemoglobinemia: Prospective Observational Registry (metHb)

Complete Title: Use of Methylene Blue in Acquired Methemoglobinemia: Prospective Observational Registry (metHb)

Short Title: Acquired Methemoglobinemia Observational Registry
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Sponsor Name: Hospital Quality Foundation
Address: 450 Shrewsbury Plaza #111 Shrewsbury, NJ 07702

Study Principal Investigator: Charles V. Pollack, Jr, MA, MD, FACEP, FESC, FAHA
Office Address: 450 Shrewsbury Plaza #111 Shrewsbury, NJ 07702
Phone: 610-329-2986
Fax: 888-959-8345
Email: cvp@metHb.org

Conducted as a collaborative prospective registry with support from Provepharm Inc.

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Protocol Synopsis

Study Title: **Use of Methylene Blue in Acquired Methemoglobinemia: Prospective Observational Registry (metHb)**

Protocol Number: HQF-METHB-2018001

Study Type: Phase IV (post-approval, observational)

Sponsor: Hospital Quality Foundation

Study Principal Investigator: Charles V. Pollack, Jr, MA, MD, FACEP, FESC, FAHA

Study Rationale

This prospective, observational registry aims to collect real-world data regarding the safety and efficacy of ProvayBlue® (methylene blue 0.5%) used according to normal standard of care for the treatment of acquired methemoglobinemia. Methylene blue has been used for decades as a rescue medication for the treatment of methemoglobinemia, a rare and potentially life-threatening condition in which elevated levels of methemoglobin impede the delivery of oxygen from blood to body tissues. However, consistent prospective data about the safety and efficacy of this medication are sparse, simply because of the rarity of the disorder. ProvayBlue® received accelerated FDA approval for treatment of acquired methemoglobinemia in 2016. This large, prospective, multi-center observational registry has been initiated to gain more information on the use of methylene blue in the treatment of acquired methemoglobinemia.

Study Objectives:

**Primary Objective:** To confirm that ProvayBlue® is efficacious in patients with acquired methemoglobinemia

**Key Secondary Objectives:** To confirm the safety and tolerability of ProvayBlue® in patients with acquired methemoglobinemia

Study Design / Methods:

Prospective, multi-center, observational registry. De-identified (safe-harbor compliant) patient data will be collected by chart review. When possible, brief post-discharge follow-up data will be collected by telephonic interview, survey, or chart review.

Study Centers: Up to 50 acute care facilities in the United States (US)

Subject 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).

Number of Subjects

- At least ten (10) subjects who are treated for acquired methemoglobinemia that is symptomatic (eg, exhibiting sleepiness, cyanosis, dizziness, etc) or with measured methemoglobin levels > 30% (the “Primary Treated Group”).
● Up to ninety (90) additional patients who are treated for acquired methemoglobinemia with measured methemoglobin levels ≤ 30% (the “Secondary Treated Group”).

Inclusion and Exclusion Criteria

● Inclusion Criteria
  ○ Adult or pediatric patients diagnosed with acquired methemoglobinemia and receiving treatment with ProvayBlue® (methylene blue 0.5%) as per the treating physician’s diagnosis and the acute care facility’s standard of care
  ○ Those acquired methemoglobinemia patients whose diagnosis is aided by measurement of methemoglobin and whose ongoing treatment is guided by re-measurement of methemoglobin ~1h post-treatment with ProvayBlue® in accordance with the US FDA Label prescribing information

● Exclusion Criteria
  ○ Refusal of consent (in those subjects approached for consent where required by local institutional procedures)
  ○ Treatment of methemoglobinemia with another methylene blue product

Efficacy Evaluations

● Time to reduction and magnitude of reduction in methemoglobin after administration of ProvayBlue™ for treatment of acquired methemoglobinemia.
● Time to normalization of the respiratory rate, heart rate, and blood pressure after administration of ProvayBlue™ for treatment of acquired methemoglobinemia.

Safety Evaluations

● Prevalence and nature of any adverse events occurring within 10 days of the administration of ProvayBlue™.

Study Duration

● Each enrolled subject will be followed through the index hospitalization or 10 days post-treatment, whichever is longer.
● The entire study is expected to last 2 years after the commencement of enrollment.

Study Phases and Procedures

● Screening and enrollment
  ○ Identify candidate cases through EMR alerts or clinician reports
  ○ Screen through chart review and enroll eligible subjects
● Principal data collection on index hospitalization
  ○ Data collection on index hospitalization by chart review
● Follow-up data collection
  ○ The study will track adverse events occurring up to 10 days after last treatment with ProvayBlue®
○ Sites should collect follow-up data following local institutional practice and IRB requirements.
○ All means of follow-up data collection, including but not limited to chart review, telephonic interview, and in-person interview are specifically permissible, as is data collection under a waiver of consent or with verbal or written consent according to IRB guidance.

1. Background and Rationale

1.1. Rationale for the Registry
This prospective, observational registry aims to collect real-world data regarding the safety and efficacy of ProvayBlue® (methylene blue 0.5%) used according to normal standard of care for the treatment of acquired methemoglobinemia. Methylene blue has been used for decades as a rescue medication for the treatment of methemoglobinemia, a rare and potentially life-threatening condition in which elevated levels of methemoglobin impede the delivery of oxygen from blood to body tissues. However, consistent prospective data about the safety and efficacy of this medication are sparse, simply because of the rarity of the disorder. ProvayBlue® received accelerated FDA approval for treatment of acquired methemoglobinemia in 2016. This large, prospective, multi-center observational registry has been initiated to gain more information on the use of methylene blue in the treatment of acquired methemoglobinemia.

1.2. Acquired Methemoglobinemia
Methemoglobinemia is characterized by a reduced ability of the blood to deliver oxygen to body tissues, 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).

Methemoglobinemia results from an increase in blood concentration of an altered form of hemoglobin, methemoglobin, 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 oxygen. This change in conformation of the methemoglobin structure reinforces the oxygen affinity of the remaining ferrous hems, increasing the impairment of oxygen delivery to tissues (Hersh 2004; Lunenfeld and Kane 2004; Beutler 2005; Kane et al. 2007; Umbreit 2007).

Most cases of acquired methemoglobinemia reported in the adult population are related to topical anesthetics (particularly benzocaine), the use of the antibiotic dapsone, aniline products, or nitrates or nitrites (from food, water, chemical exposure, or medicines). Clinical symptoms of methemoglobinemia vary according to the level of methemoglobin 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 methemoglobin as shown in the table below.

### Table 1: Signs and Symptoms related to blood levels of methemoglobin (Do Nascimento, 2008)

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

### 1.3. 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). Methylene Blue facilitates the rapid conversion of methemoglobin into normal hemoglobin and rapid improvement of methemoglobinemia symptoms. The broad use of MB for the treatment of methemoglobinemia is documented by case reports available in the literature and is the current standard of care. ProvayBlue™ (methylene blue 0.5%) is the only MB product currently approved by the US FDA.

### 2. Study Objectives

#### 2.1. Study Objectives

The objective of the study is to confirm that ProvayBlue™ is safe and efficacious when used in real-world practice in accordance with its FDA approval for treatment of patients with acquired methemoglobinemia.

#### 2.2. Primary Efficacy Endpoint

- Time to reduction and magnitude of reduction in methemoglobin after administration of ProvayBlue™ for treatment of acquired methemoglobinemia.

#### 2.3. Secondary Endpoints

- Time to normalization of the respiratory rate, heart rate, and blood pressure after administration of ProvayBlue™ for treatment of acquired methemoglobinemia.
- Prevalence and nature of any adverse events occurring within 10 days of the administration of ProvayBlue™.

2.4. Other Endpoints
- Prevalence of acquired methemoglobinemia cases by suspected causal agent
- Resolution of methemoglobinemia-related symptoms during the index hospitalization

3. Study Design
This is a prospective, multi-center, observational registry to confirm the safety and efficacy of ProvayBlue™ used in real-world practice in accordance with its FDA approval for treatment of patients with acquired methemoglobinemia. This study is purely observational and specifically does not involve any changes in treatment from normal standard of care. Patient data will be collected by chart review, and in patients who consent to be contacted, post-discharge follow-up data will be collected by brief survey. Only de-identified (safe-harbor compliant) data will be collected, and all aspects of the study will be conducted in compliance with Good Clinical Practice.

4. Study Subject Selection
This study seeks to enroll patients who present in a hospital / urgent care setting diagnosed with acquired methemoglobinemia and who are treated with ProvayBlue™ used in accordance with its FDA approval. Methemoglobinemia is an ultra-rare condition and thus capturing data on all qualified cases is a key priority. The study population may include pediatric and adult patients (males and females of all ages are included).

4.1. Study Groups and Numbers of Subjects
- “Primary Treated Group”: At least ten (10) subjects who are diagnosed and treated for acquired methemoglobinemia that is symptomatic (eg, exhibiting sleepiness, cyanosis, dizziness, or other symptoms that are judged to be clinically related to methemoglobinemia) or with measured methemoglobin levels > 30%.
- “Secondary Treated Group”: Up to ninety (90) additional patients who are treated for acquired methemoglobinemia with measured methemoglobin levels ≤ 30%.

4.2. Inclusion Criteria
- Adult or pediatric patients diagnosed with acquired methemoglobinemia and receiving treatment with ProvayBlue® (methylene blue 0.5%) as per the treating physician’s diagnosis and the acute care facility’s standard of care
● Those acquired methemoglobinemia patients whose diagnosis is aided by measurement of methemoglobin and whose ongoing treatment is guided by re-measurement of methemoglobin ~1h post-treatment with ProvayBlue® in accordance with the US FDA Label prescribing information

4.3. Exclusion Criteria
● Refusal of consent (in those subjects approached for consent where required by local institutional procedures)
● Treatment of methemoglobinemia with another methylene blue product

5. Study Procedures

5.1. Study-Specific Training
All study personnel will participate in a study-specific remote site initiation and data entry training session provide by the sponsor.

5.2. Recruitment
Each site will be responsible for recruitment of qualified subjects. It is anticipated that recruitment will be most efficiently accomplished by programming EMR-based alerts to identify qualified cases as allowable at individual sites, and then conducting initial chart review to confirm eligibility.

5.3. Data Collection Schedule

5.3.1. Principal Data Collection
Principal data collection will be conducted by chart review and will be focused on data from the index hospitalization. It is anticipated that this principal data collection will be conducted under a waiver of consent.

5.3.1. Follow-up Data Collection
The study will track adverse events occurring up to 10 days after last treatment with ProvayBlue®. Follow-up data collection to assess patient status 10 days after treatment should follow local institutional practice and IRB requirements. All means of follow-up data collection, including but not limited to chart review, telephonic interview, and in-person interview are specifically permissible, as is data collection under a waiver of consent or with verbal or written consent according to IRB guidance.

5.4. Treatment of Methemoglobinemia
Treatment of acquired methemoglobinemia with ProvayBlue® should follow the treating physician’s diagnosis and the acute care facility’s standard of care. For convenience only,
summary points on the treatment of acquired methemoglobinemia with ProvayBlue® are repeated here. These summary points are based on the FDA Label prescribing information for ProvayBlue®, which is attached as Appendix 1 and should be consulted for definitive guidance.

5.4.1. Considerations before the use of ProvayBlue® for acquired methemoglobinemia

If a diagnosis of methemoglobinemia is confirmed either by laboratory findings or by symptomatology, a complete history should be taken to:

1. Attempt to determine the cause of the methemoglobinemia
2. Ensure that the patient is not taking selective serotonin reuptake inhibitors (SSRIs), serotonin and norepinephrine reuptake inhibitors (SNRIs), or monoamine oxidase inhibitors (as these drugs have been associated with serotonin syndrome when used concomitantly with or within 72 hours of methylene blue)
3. Note that the dosing volume of ProvayBlue® (methylen blue, 0.5%) will be twice that of unapproved, 1% methylene blue products. Additionally, ProvayBlue® is hypotonic and may be diluted in 50 ml of 5% Dextrose in Water (D5W) and administered as an iv infusion. Dilution of the product in 0.9% sodium chloride (normal saline) should be avoided as it reduces the solubility of methylene blue and may result in precipitation. Do not use the product if precipitation occurs.

5.4.2. Use of ProvayBlue® for acquired methemoglobinemia

If treatment with methylene blue is warranted:

1. Administer ProvayBlue® 1mg/kg intravenously over 5-30 minutes, making sure to note the time
   a. Monitor vital signs, symptomatology, and methemoglobin levels for resolution over one hour (specifically including respiration rate, heart rate, diastolic blood pressure, and systolic blood pressure)
   b. One hour after treatment, re-assess methemoglobin levels and any relevant signs and symptoms of methemoglobinemia to inform next steps
2. If methemoglobin levels remain high or unsatisfactory or symptoms do not improve after one hour, administer a second dose of ProvayBlue® 1mg/kg intravenously, making sure to note the time
   a. Monitor vital signs, symptomatology, and methemoglobin levels for resolution over one hour (specifically including respiration rate, heart rate, diastolic blood pressure, and systolic blood pressure)
   b. One hour after treatment, re-assess methemoglobin levels and any relevant signs and symptoms of methemoglobinemia to inform next steps
3. If methemoglobin levels remain high or unsatisfactory or symptoms do not improve after 1 hour after a second dose of ProvayBlue®, consider initiation of alternative interventions for the treatment of methemoglobinemia.
4. Prior to discharge from the facility
   a. Assess clinical symptomatology related to methemoglobinemia
   b. Draw a blood sample for hematology (including methemoglobin) and biochemistry.
   c. Ensure that all concomitant therapies are listed in the patient’s records, and note any adverse events that occurred during the course of the patient’s treatment.
6. Safety Reporting

- For clarity, safety reporting in **methHb** focuses on SAEs that may be related to the use of ProvayBlue®, and not on any prescribed drugs that may be causally related to acquired methemoglobinemia.
- In cases where study data indicates the occurrence of a serious adverse event after the use of ProvayBlue®, Site Investigators will be required to report these adverse events and their assessment of any causal relationship to the manufacturer’s representative within 24 hours. Details on these reporting requirements can be found in the attached Safety Reporting Plan (Appendix 2).
- Safety reporting related to regulated drugs that may be causally associated with acquired methemoglobinemia is not governed by this protocol, and should follow normal Site procedures.
- Safety reporting related to the use of ProvayBlue® in patients who are not enrolled in **methHb** or in enrolled subjects outside of the study period (10 days post-treatment) is not governed by this protocol, and should follow normal Site procedures. Such reporting may include ongoing tracking of SAEs that have not resolved within the study period in enrolled subjects.

7. Data Collection and Management

7.1. Data Collection Logistics

Subject data will be captured via secure web-based eDCFs using an FDA 21 CFR-part-11 compliant EDC system. No PHI or PII will be collected in the study data set, which will be specifically restricted to safe-harbor compliant data (with dates of service recorded as study days, for example). Access to the eDCFs will be restricted to staff receiving specific study training, as well as certified training related to HIPAA.

7.2. Data to be Collected

- **Principal Data Collection (Index Hospitalization), Overview of Key Data**
  - Qualifying arterial blood gas and co-oximetry data pre-treatment (with methylene blue)
  - All arterial blood gas and co-oximetry data after treatment with methylene blue
  - Any signs and symptoms judged to be related methemoglobinemia
  - Details of methylene blue use to treat methemoglobinemia
  - Details of any other drugs used to treat methemoglobinemia
  - Details of any non-drug therapies used to treat methemoglobinemia
  - Listing of prior meds, focusing on those most likely to precipitate methemoglobinemia
○ Listing of concomitant diseases / medical history
○ All lab values obtained 24h before first and 48h after last methylene blue treatment
○ All vital signs obtained 24h before first and 24h after last MB treatment
○ Any ECG data from the period 24h before first and 48h after last MB treatment
○ Disposition from hospital and ICD-10 codes at disposition

- **Follow-up Data (10d post-treatment), Overview of Key Data**
  ○ Vital status and any AEs or SAEs occurring within 10 days post-treatment with methylene blue

- **Facility Census Data**
  ○ On a quarterly basis, sites will provide hospital / acute care facility census data (patient counts only) on patient populations of interest to provide the denominator for prevalence calculations. These data will be transmitted and recorded outside of the eDCFs.

### 7.3. Confidentiality

Only unique patient numbers assigned by the Sponsor in the electronic data collection forms (eDCFs) will identify patients in the registry. Study data collected in the eDCFs will be fully de-identified by the safe-harbor standard. A patient enrollment log linking these numbers with local medical record numbers and other PHI should be maintained at the research site in a secure fashion.

### 7.4. Data Quality and Monitoring

The electronic database into which study data will be entered will have programmatic quality/edit checks to detect data errors. The study Principal Investigator, data manager, and study manager will monitor the database on an ongoing basis to assess site volumes, site data quality, and completeness of entries. Data from the subgroup of at least ten (10) severe acquired methemoglobinemia cases will be validated by source review using PHI/PII-redacted source records provided by sites.

### 7.5. Study Records Retention

The Sponsor will retain the study data for a minimum of two years following the final publication of study findings, data submission to FDA, or discontinuation of the registry. The site Principal Investigator will retain the patient enrollment log, IRB approvals, ICDs, and other regulatory binder contents for a minimum of two years following the final publication of study findings, data submission to FDA, or discontinuation of the registry, or for a shorter period upon written notification by the Sponsor.
8. Statistical Considerations

8.1. Sample Size
Because this is an ultra-orphan indication, the enrollment targets of 10 severe cases and up to 90 non-severe cases are driven simply by FDA requirements.

8.2. Groups for Analyses
- Efficacy Evaluations
  - The Primary Treated Group: the subgroup of subjects with severe methemoglobinemia (measured methemoglobin levels > 30% or symptomatic methemoglobinemia)
    - Stratified by contraindications in the use of methylene blue, as relevant
    - Stratified by suspected causative agent, as possible
  - All qualifying patients stratified by suspected causative agent, and known contraindications and warnings related to the use of methylene blue
- Safety Evaluations
  - All qualifying patients stratified by suspected causative agent, and known contraindications and warnings related to the use of methylene blue

8.3. Statistical Methods
Descriptive statistics will be used to summarize the primary and the secondary endpoints and other data collected in the study. Continuous variables will be summarized using the number of patients reflected in the calculation (n), mean, standard deviation (std), median, interquartile range, minimum, and maximum. Categorical data will be summarized using frequencies and percentages. If applicable, 95% confidence intervals of means, medians, and/or percentages will be provided to demonstrate the precision of the findings.

8.4. Interim Analyses
No interim analyses are planned.

9. Ethical Considerations

9.1. Overview and Process
This study will be conducted in compliance with the protocol, HQF standard operating procedures and/or guidelines where applicable, US FDA regulations, the ICH GCP guidelines, and the Declaration of Helsinki.

9.2. Institutional Review Board
This protocol and informed consent form (if required) shall be submitted to the IRB identified with this responsibility at the research facility, unless specifically exempted as a quality improvement (QI) project. Notification in writing of approval must come from the IRB chairman or secretary, to the investigator, either as a letter or as a copy of the appropriate section of the IRB meeting minutes where this protocol was discussed. The investigator will not participate in the decision. If the investigator is an IRB member, the written approval must indicate such non-participation. The investigator will submit status reports to the IRB at least annually (when applicable). The IRB must be notified by the investigator in writing of the interruption and/or completion of the study; the investigator must promptly report to the IRB all changes in research (protocol amendments) and will not make such changes without IRB approval except where necessary to eliminate apparent immediate hazards to human patients. In these cases, the IRB must be notified within five days of the change. The investigator will promptly report to the IRB all unanticipated problems involving risk to patients or others. The investigator is required to maintain an accurate and complete record of all written correspondence to and received from the IRB and must agree to share all such documents and reports with HQF.

10. Informed Consent / Assent and HIPAA Authorization

10.1. Overview and Informed Consent / Assent
metHb is a minimal-risk observational study, with data collection restricted to a de-identified safe-harbor compliant dataset. metHb aims to characterize the prevalence and outcomes of an ultra-rare condition, and the feasibility of these scientific aims will be compromised if subjects are excluded because of the inability to obtain consent / assent. It is anticipated that data collection by anonymized passive chart review will qualify for a waiver of all consent / assent. At institutions where patient follow-up is part of standard of care or quality improvement programs, follow-up data collection may be completed by anonymized passive chart review. Sites are specifically permitted to use all convenient means to collect follow-up data according to their local practice. In all cases, sites must follow IRB guidance on any requirements for consent / assent.

10.2. HIPAA Authorization
metHb will collect a de-identified safe-harbor compliant dataset. No HIPAA Authorization is anticipated.
11. References

12. Investigator Agreement

I have read and understand the protocol including its appendices and agree that it contains all the ethical, legal and scientific information necessary to conduct this study. I will personally conduct the study as described.

I will provide copies of the protocol to all physicians, nurses and other professional personnel responsible to me who will participate in the registry study. I will discuss the protocol with them to assure myself that they are sufficiently informed. I am aware that this protocol must be approved by the Institutional Review Board (IRB) responsible for such matters in the clinical study facility prior to commencement of this registry study, unless specifically exempted. I understand that this IRB-approved protocol may be submitted to the Food and Drug Administration (FDA) and other regulatory authorities by Hospital Quality Foundation (HQF), as appropriate. I agree that clinical data entered on Data Collection Forms by my institution may be utilized by HQF in various ways such as for submission to governmental regulatory authorities and/or in combination with clinical data gathered from other research sites, whenever applicable. I agree to provide HQF monitors access, with adequate notice, to redacted medical records for patients entered in the registry study as may be needed for data quality monitoring.

_________________________________
SITE INVESTIGATOR

_________________________________
DATE
APPENDIX 1: PROVAYBLUE™ FDA LABEL

Please refer to http://methb.org/label for the definitive version of the US FDA Label, which may be amended from time to time. A current version (as of May 1, 2018) of the FDA Label is attached for convenience.
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

WARNING: SEROTONIN SYNDROME WITH CONCOMITANT USE OF SEROTONERGIC DRUGS
See full prescribing information for complete boxed warning.

PROVAYBLUE® may cause serious or fatal serotonergic syndrome when used in combination with serotonergic drugs. Avoid concomitant use (5.1, 7.1).

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)

DOSE 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

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: 12/2017

FULL PRESCRIBING INFORMATION: CONTENTS*

WARNING: SEROTONIN SYNDROME WITH CONCOMITANT USE OF SEROTONERGIC DRUGS
1 INDICATIONS AND USAGE
2 DOSAGE AND ADMINISTRATION
2.1 Dosage and Administration
2.2 Preparation and Storage
3 DOSAGE FORMS AND STRENGTHS
4 CONTRAINDICATIONS
5 WARNINGS AND PRECAUTIONS
5.1 Serotonin Syndrome with Concomitant Use of Serotonergic Drugs
5.2 Hypersensitivity
5.3 Lack of Effectiveness
5.4 Hemolytic Anemia
5.5 Interference with In Vivo Monitoring Devices
5.6 Effects on Ability to Drive and Operate Machinery
5.7 Interference with Laboratory Tests
6 ADVERSE REACTIONS
6.1 Clinical Trials Experience
7 DRUG INTERACTIONS
7.1 Serotonergic Drugs
7.2 Agents Affecting Cytochrome P450 Enzymes
8 USE IN SPECIFIC POPULATIONS
8.1 Pregnancy
8.2 Lactation
8.4 Pediatric Use
8.5 Geriatric Use
8.6 Renal Impairment
8.7 Hepatic Impairment
10 OVERDOSAGE
11 DESCRIPTION
12 CLINICAL PHARMACOLOGY
12.1 Mechanism of Action
12.2 Pharmacodynamics
12.3 Pharmacokinetics
13 NONCLINICAL TOXICOLOGY
13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility
14 CLINICAL STUDIES
14.1 Treatment of Acquired Methemoglobinemia
16 HOW SUPPLIED/STORAGE AND HANDLING
17 PATIENT COUNSELING INFORMATION

*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 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 sulfia 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.
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>
<thead>
<tr>
<th>Table 1. Adverse Reactions Following Infusion of PROVAYBLUE® 2 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adverse Reaction</td>
</tr>
<tr>
<td>Pain in extremity</td>
</tr>
<tr>
<td>Chromaturia</td>
</tr>
<tr>
<td>Dysgeusia</td>
</tr>
<tr>
<td>Feeling hot</td>
</tr>
<tr>
<td>Dizziness</td>
</tr>
<tr>
<td>Hyperhidrosis</td>
</tr>
<tr>
<td>Nausea</td>
</tr>
<tr>
<td>Skin discoloration</td>
</tr>
<tr>
<td>Headache</td>
</tr>
<tr>
<td>Musculoskeletal pain</td>
</tr>
<tr>
<td>Paresthesia oral</td>
</tr>
<tr>
<td>Paresthesia</td>
</tr>
<tr>
<td>Infusion site pain</td>
</tr>
<tr>
<td>Feeling cold</td>
</tr>
<tr>
<td>Pallor</td>
</tr>
<tr>
<td>Dermatitis contact</td>
</tr>
<tr>
<td>Syncope</td>
</tr>
<tr>
<td>Influenza like illness</td>
</tr>
<tr>
<td>Pruritus</td>
</tr>
<tr>
<td>Anxiety</td>
</tr>
<tr>
<td>Decreased appetite</td>
</tr>
<tr>
<td>Chest discomfort</td>
</tr>
<tr>
<td>Back pain</td>
</tr>
<tr>
<td>Cold sweat</td>
</tr>
<tr>
<td>Dizziness postural</td>
</tr>
<tr>
<td>Muscle spasms</td>
</tr>
<tr>
<td>Presyncope</td>
</tr>
</tbody>
</table>
Table 1. Adverse Reactions Following Infusion of PROVAYBLUE® 2 mg/kg

<table>
<thead>
<tr>
<th>Adverse Reaction</th>
<th>Any Grade TEAE (n=82)</th>
<th>Moderate-Severe TEAE (n=82)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vomiting</td>
<td>2 2%</td>
<td>1 1%</td>
</tr>
<tr>
<td>Arthralgia</td>
<td>2 2%</td>
<td>1 1%</td>
</tr>
<tr>
<td>Chills</td>
<td>2 2%</td>
<td>0</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>2 2%</td>
<td>0</td>
</tr>
<tr>
<td>Discomfort</td>
<td>2 2%</td>
<td>0</td>
</tr>
<tr>
<td>Dyspnea</td>
<td>2 2%</td>
<td>0</td>
</tr>
<tr>
<td>Erythema</td>
<td>2 2%</td>
<td>0</td>
</tr>
<tr>
<td>Hypoesthesia oral</td>
<td>2 2%</td>
<td>0</td>
</tr>
<tr>
<td>Infusion site discomfort</td>
<td>2 2%</td>
<td>0</td>
</tr>
<tr>
<td>Limb discomfort</td>
<td>2 2%</td>
<td>0</td>
</tr>
<tr>
<td>Oral discomfort</td>
<td>2 2%</td>
<td>0</td>
</tr>
<tr>
<td>Catheter site pain</td>
<td>2 2%</td>
<td>0</td>
</tr>
<tr>
<td>Ecchymosis</td>
<td>2 2%</td>
<td>0</td>
</tr>
</tbody>
</table>

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 toxici ties 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 overdose (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 C_{16}H_{18}ClN_{3}S and its molecular weight is 319.86 g/mol. Its structural formula is:

![Methylen Blue Structural Formula](image)

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-enzymatic 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 marrow 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)].

Manufactured for:
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, Inc.
Shirley, NY 11967
Questions? : 1-800-734-9236
12/2017 [controlled part number code]
APPENDIX 2: SAFETY REPORTING PLAN

Study Title: Use of Methylene Blue in Acquired Methemoglobinemia: Prospective Observational Registry (metHb)
Protocol Number: HQF-METHB-2018001
Study Type: Phase IV (post-approval, observational)
Sponsor: Hospital Quality Foundation
Study Principal Investigator: Charles V. Pollack, Jr, MA, MD, FACEP, FESC, FAHA

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.
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.

Definition of a Serious Adverse Events (SAE)
An SAE is defined as any untoward medical occurrence that:

- Results in death;
- Is life-threatening;
- Requires inpatient hospitalization or prolongation of existing hospitalization;
- Results in persistent or significant disability/incapacity;
- 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.

Recording and Reporting of Adverse Events (AEs) in the eDCFs
All AEs, as defined above, encountered during the study as well as any SAEs will be reported in the appropriate section of the eDCFs. Information will include the following:

- Duration of the AE (onset date and time and resolution date);
- Relationship to ProvayBlue®
- Time relationship to ProvayBlue® dosing time;
- Assessment of whether AE is an infusion site reaction;
- Severity
- Outcome of the AE;
- Concomitant treatment dispensed (or other action taken);
- Action taken with respect to the ProvayBlue®
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 eDCFs. The Investigator is to make his/her own assessment of each SAE to be recorded on the eDCFs 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 below:

### 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®; |
|              | Time sequence from taking ProvayBlue® is reasonable; |
|              | Event stops upon stopping ProvayBlue®, event returns upon restarting ProvayBlue®; |

Definition of Severity of Adverse Events (AEs)

Severity of any AE will be recorded in the eDCFs. Severity of any AE will be graded according to the National Cancer Institute - Common Terminology Criteria for Adverse Event (Version 4.03) as reported in the following Table.
Definition of Severity of Adverse Events (AEs)

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

National Cancer Institute - Common Terminology Criteria for Adverse Event 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.

Serious Adverse Event (SAE) Reporting Procedure

The Investigator must report (by fax, telephone or email) all initial and follow-up SAE reports to Regulis’ Pharmacovigilance Department within 24 hours of awareness of an SAE, copying the study PI (Dr. Pollack) on all correspondence.

Study Contact for Reporting Serious Adverse Events

Regulis Consulting Ltd.
Miranda Tasko (EU QPPV)
safety@regulis.com
EU & US: SAE Fax: +44 (0)1442 890903
Tel: +44 (0)1442 890909

- 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 eDCFs.
- Blank copies of the SAE forms will be distributed at the time of Site initiation.
- 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 eDCF pages or EMR records, 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.

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.