Provepharm Inc.

DATA SUMMARY PLAN
NCT#03395223

Protocol Number:	**PVP-2016003**
Protocol Title:	**Open Label Clinical Study to Evaluate the Safety and Efficacy of ProvayBlue™ (Methylene Blue) for the Treatment of Acquired Methemoglobinemia**
Date of Protocol	**Final Version 3, 10 August 2017**
Protocol Number:	**HQF-METHB-2018001**
Protocol Title:	**Use of Methylene Blue in Acquired Methemoglobinemia: Prospective Observational Registry (metHb)**
Date of Protocol	**13 April 2018**
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LIST OF ABBREVIATIONS AND PHRASES

Abbreviation	Definition
AE	adverse event
CRF	case report form
DSP	Data Summary Plan
ECG	electrocardiogram
ICH	International Conference on Harmonization
metHb	methemoglobin
PCHG	percent change from baseline
PMR	Post-Marketing Requirement
PVP	Provepharm, Inc.
TEAE	treatment-emergent adverse event
VS	vital signs

1. INTRODUCTION AND SCOPE

ProvayBlue™ (methylene blue) was approved under the accelerated approval regulation, 21 CFR 314.510. Provepharm was required to conduct "further adequate and well-controlled studies" to verify and describe the clinical safety and benefit of the agent in therapeutic use for the treatment of acquired methemoglobinemia. To fulfill this requirement, Provepharm has initiated 2 studies and enrollment to both studies are closed at this time.

The first study, protocol PVP-2016003, titled "Open Label Clinical Study to Evaluate the Safety and Efficacy of ProvayBlue™ (Methylene Blue) for the Treatment of Acquired Methemoglobinemia," was initiated 19 October 2017; the study had enrolled a total of 7 patients when the enrollment was closed on 31 August 2020. This study is referred to hereafter as the Interventional Study. Because of the slow pace of enrollment in this study, doubtless due to the paucity of patients with this ultra-rare disease available for assessment, a stand-alone database, as commonly utilized with clinical trials, has not been setup to capture the data; rather, all clinical data were captured on a paper case report form (CRF).

The second study, protocol HQF-METHB-2018001, titled "Use of Methylene Blue in Acquired Methemoglobinemia: Prospective Observational Registry (metHb)," started May 2018, and enrolled 24 patients before closure on 31 August 2020. This study is referred to hereafter as the Observational Study. The Observational Study was expected to last 2 years after the commencement of enrollment in up to approximately 50 hospital systems. This Observational Study was designed to support low-burden screening and data collection, and to enroll real-world cases of acquired methemoglobinemia treated with ProvayBlue™ without restriction; thus, study sites were more amenable to participate in this program than in the Interventional Study. When enrollment was closed, approximately 90 individual hospitals were approved to enroll patients into the Observational Study; however, only 24 patients were enrolled, again owing to the rarity of the clinical presentation of interest. An electronic database has been set up to house data from both studies.

Provepharm plans to combine the data from both studies in its report to FDA to satisfy the company's first Post-Marketing Requirement (PMR-*3065-1)*. This Data Summary Plan (DSP) is intended to provide a technical, detailed elaboration of the methods utilized to pool those 2 studies into a single database and to prepare an integrated summary of the data without inferential statistics. It is anticipated that the pooled results will be presented as a series of individual patient narratives with limited statistical analysis, befitting the observational nature of most of the data. The objective of this DSP is to assure that the methodologies to be used for summary are complete, accurate, and consistent.

In the development of this DSP, the following documents were used:

- Protocol PVP-2016003: final protocol dated 10 August 2017; CRF dated 04 December 2017

- Protocol HQF-METHB-2018001: final protocol dated 13 April 2019; CRF dated 04 May 2018
    - For the purpose of combining with data from Protocol PVP-2016003, the original CRF dated 04 May 2018 was updated on 27 October 2020

The principles in the following guidance documents have been followed in preparation of this DSP:

- International Conference on Harmonization (ICH) E3 (1995): Structure and Content of Clinical Study Reports
- ICH E6 (1996): Guideline for Good Clinical Practice
- ICH E9 (1998): Statistical Principles for Clinical Trials

In the event that a discrepancy is found between the descriptions in the statistical section of the protocols and this document, the description in this document supersedes the descriptions in the statistical section of the protocols.

A teleconference was held on 27 April 2020 between the sponsor and the agency. A post-meeting information package was submitted on 01 May 2020, in which metHb data collected from 13 patients (3 from PVP-2016003 and 10 from HQF-METHB-2018001) were summarized and presented to the agency. On 09 August 2020, the agency confirmed that the combined database was acceptable to be utilized to fulfill the post marketing requirement No. 1 (PMR1). This DSP was finalized after incorporation of the details of the meeting discussion and subsequent post-meeting communications between the sponsor and the agency.

2. OVERVIEW OF STUDY OBJECTIVES AND ASSESSMENTS

2.1. Study Objectives and Endpoints

An overview of study objectives and study endpoints is provided in Table 1. The Observational Study was designed to mirror the objectives, endpoints, and data collection elements of the Interventional Study, with modifications to (1) reflect the nature of observational data, where the specific timing of procedures and measurements is driven by institutional standard care, not a study protocol, and (2) to streamline the protocol and study procedures where possible.

Table 1: Overview of Study Objectives and Endpoints

Protocol Number	PVP-2016003 (Interventional Study)	HQF-METHB-2018001 (Observational Study)
Primary Objective	To confirm that ProvayBlue™ after a single administration is efficacious in patients with acquired methemoglobinemia	To confirm that ProvayBlue™ 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.	To confirm the safety and tolerability of ProvayBlue™ in patients with acquired methemoglobinemia
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).	Patients who are diagnosed in any care setting with acquired methemoglobinemia. The population may include pediatric and adult patients (males and females of all ages are included).

Protocol Number	PVP-2016003 (Interventional Study)	HQF-METHB-2018001 (Observational Study)
		Primary Treated Group: At least ten (10) patients who are treated for acquired methemoglobinemia that is symptomatic (eg, exhibiting sleepiness, cyanosis, dizziness) and/or with measured methemoglobin levels >30% **Secondary Treated Group:** Up to ninety (90) additional asymptomatic patients who are treated for acquired methemoglobinemia with measured methemoglobin levels ≤30%
Safety Assessments	• Physical examination; • Vital signs; • Hematology and biochemistry • Electrocardiogram (ECG); • Adverse events (AEs).	Prevalence and nature of any adverse events occurring within 10 days of the administration of ProvayBlue™
Efficacy Endpoints	• 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™.	• 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 • Prevalence and nature of any adverse events occurring within 10 days

Protocol Number	PVP-2016003 (Interventional Study)	HQF-METHB-2018001 (Observational Study)
		of the administration of ProvayBlue™ • Prevalence of acquired methemoglobinemia cases by suspected causal agent • Resolution of methemoglobinemia-related symptoms during the index hospitalization
PK Endpoint	PK blood draws were planned and plasma concentrations of methylene blue and azure B were to be determined. However, PK data were not collected due to poor PK blood sample handling. Therefore, the PK endpoint will not be assessed.	None

2.2. Data Collection

2.2.1. HQF-METHB-2018001

Protocol HQF-METHB-2018001 is designed to be a low-burden minimal-risk **Observational Study**; data collection largely is by passive chart review, with follow-up by telephone or postal mail and/or using medical records where applicable. At some participating study sites, follow-up of acquired methemoglobinemia patients is standard care or part of quality improvement efforts, and some study sites are operating under a full waiver of consent / assent, based on local IRB approvals.

To minimize risk and qualify for the most streamlined study procedures, the Observational Study is electronic source (paper forms are not required) and the study data are de-identified to the HIPAA Safe-Harbor standard. Notably, the Safe-Harbor method involves collection of relative "study days" (eg, "Study Day 1, 12:45") rather than absolute "calendar days" (eg, December 31, 2019, 12:45). Significantly, the "study day" safe-harbor format does not restrict any of the proposed analyses. In our proposed analyses, a pseudo enrollment date was created for each patient when the patient's case was registered in the database.

To support intuitive and consistent presentation of the data, Study Day 1 Hour 0 is defined as the day and time of the completion of the first methylene blue infusion, and the timing of all study

measurements is presented as the relative time elapsed from this reference point. Thus, a negative relative time indicates that an event/assessment occurred prior to the first infusion completion time, whereas a positive relative time indicates that an event/assessment occurred after the first infusion conclusion time.

To gain understanding about all real-world cases of acquired methemoglobinemia treated with ProvayBlue™, and to encourage more sites to participate by making the enrollment criteria less restrictive, enrollment of a "Secondary Treated Group" was included in the Observational Study. That is, this study groups enrolled patients into two subgroups: Primary cases or Secondary cases.

All primary cases must fulfill the following requirements:

1. Baseline metHb ≥30%, OR
2. Documented signs or symptoms of methemoglobinemia with subsequent assay of methemoglobin levels above the institutional upper limit of normal (ULN). For these patients, response to the question 'Were there any signs or symptoms judged to be clinically associated with the acquired methemoglobinemia?' must be a 'Yes'

Data from all Primary cases are fully monitored; inconsistencies are to be queried.

The Secondary Treated Group provides relatively sparse data on non-severe (asymptomatic with methemoglobin levels <30%) acquired methemoglobinemia cases that were nonetheless treated per clinical judgment with ProvayBlue™. Aside from verification of the inclusion criteria, data in these cases are not monitored.

2.2.1. PVP-2016003

Protocol PVP-2016003 is an open-label, uncontrolled Phase 4 **Interventional Study** of 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). Eligible patients will receive a single dose of study drug (typically 1 mg/kg of ProvayBlue™) treatment to treat acquired methemoglobinemia resulting in a direct health benefit. Patients will be followed post treatment; safety, efficacy, and PK data will be collected following a predefined data collection schedule (Table 2).

To create a harmonized electronic database, data collected from this study will be entered into the electronic data collection system used for the Observational Study, and the absolute dates of service recorded in this study will be converted to safe-harbor compliant "study days" before data are entered into the database for analyses. In instances where a specific procedure/data collection time was not initially recorded on the paper CRF, a data query will be generated to obtain the specific time from the site. All patients from this protocol are considered as Primary cases.

The original CRFs for Protocol PVP-2016003 requested posttreatment AE tracking, but the Study Procedure Chart indicates that AEs are tracked pretreatment. To be consistent with the Observational Study, any AEs collected prior to study drug treatment will be identified as NOT treatment-emergent events. Non-treatment-emergent AEs will be included in the data listing but will be excluded from data summary (see Section 6.2 for additional details).

Table 2: Study PVP-2016003 Procedures

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[l]	X									
Methemoglobin	X		X[f]	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 followed 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 (°C or °F), 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.

3. DATA POOLING CONSIDERATIONS

A Data Integration Plan (DIP) will provide the details of methods to combine the 2 studies into a single database. The following provides the key elements of the DIP.

3.1. Database Structure

It is the intention of Provepharm to enter selected, pertinent CRF data elements from the Interventional Study into the database serving the Observational Study for the purpose of preparing the data summary for PMR-3065-1.

It is therefore expected that certain procedures will have to take place before the CRF data elements from the Interventional Study can be entered into the electronic database. Those procedures include but are not limited to converting the absolute calendar dates from the Investigational Study CRFs to the relative study days used in the database. The integrated datasets will mimic the SDTM structure and naming convention whenever possible.

In study PVP-2016003, PK blood draws were planned and plasma concentration were to be determined. However, PK data are not collected due to poor PK blood sample handling. Therefore, the corresponding dataset (PC) will not be created.

In a situation where a data field that is planned in one protocol but not in the other protocol, the missing data will be marked as 'Not Done' in the database. For instance, the Observational - Study (HQF-METHB-2018001) collects "Patient setting before first presentation", "Diagnosed by", "Diagnosis setting". These 3 questions are not included in the Interventional Study. Hence, data value will be set to 'Not Done' in 'Patient setting before first presentation' dataset XD for patients from the Interventional Study (PVP--2016003).

Table 3: Expected SDTM Domains for the Pooled Database

Name of Data Collection Elements/Form	SDTM Domain
Demographics (age, sex, race, ethnicity)	DM
Weight and height at enrollment	VS
Patient setting before first presentation	XD
Past medical history	MH
Acquired methemoglobinemia diagnosis, including diagnosis ICD-10 Code	XD
Methemoglobinemia assessment and treatment before receiving study drug	XD
Methemoglobinemia signs and symptoms	CE
Co-oximetry before and after ProvayBlue™ treatment	LB
Arterial blood gas	LB
ProvayBlue™ administration	EX
Other treatment for methemoglobinemia	CM
Vital signs	VS
ECG	EG
Hematology, biochemistry, and other labs	LB
Medications and procedures	CM, PR
Hospital course	HO
Adverse events	AE
Follow-up overview and questionnaires	QS

3.2. Analysis Population

The following analysis sets will be identified for data summary.

Enrolled Analysis Set: All patients who sign the informed consent or otherwise meet all local enrollment criteria for the study are considered to have enrolled.

Safety Analysis Set: The safety set will include all enrolled patients who are treated with at least 1 ProvayBlue™ infusion dose. The safety analysis set will be used for safety data summary. Specifically, the safety population will include all treated patients from the Interventional Study and the Observational Study.

Efficacy Analysis Set: The efficacy analysis set includes all safety analysis set patients who have at least 1 metHb assessment before and after ProvayBlue™ infusion and who are Primary cases. This analysis set will be used for efficacy endpoint evaluation.

3.3. Endpoints

3.3.1. Efficacy Endpoints

The efficacy data summary will include the following endpoints:

1) Change from baseline in metHb after the first dose of ProvayBlue™ infusion.

   The primary efficacy will be the number (%) patients with ≥50% reduction in metHb based on the first available posttreatment assessment.

   - The following are secondary endpoints to support the primary endpoint.
     i. Change in metHb posttreatment over time
     ii. Time when 50% reduction in metHb is observed for the first time.

2) Change from baseline in **vital signs (blood pressure, respiratory rate, and heart rate)** after the first dose of ProvayBlue™ infusion.

   - The secondary efficacy will be the number (%) of patients with concomitant normalization of vital signs within 2 hours, inclusive, after the end of infusion of the first dose of ProvayBlue™
   - Time (hours) when the first incidence of normal vital signs was observed in patients who had abnormal baseline vital signs

3) In addition, 13 prespecified clinical signs and symptoms associated with methemoglobinemia and their intensity (as National Cancer Institute Common Terminology Criteria for Adverse Events [NCI-CTCAE] grade) before and after ProvayBlue™ treatment are collected. The presence/absence of those signs and symptoms will be treated as an 'other' efficacy endpoint since decrease and/or resolution of those signs and symptoms may be considered supportive of efficacy.

3.3.2. Relative Time of Efficacy Endpoints

A relative time frame will be used to display and to summarize efficacy assessments.

The stop time of the first ProvayBlue™ infusion will be used to derive relative time (hours) for each post infusion metHb and vital signs assessment. After all efforts are made to ensure the capture of infusion start and stop time, when the stop time is missing, the following imputation rule will be used to impute the missing stop time.

Missing stop time will be imputed with the median infusion duration of the efficacy population. Mathematically, this imputation rule can be expressed as

Stop time=start time + median infusion duration

Where the median infusion duration is derived from all patients in the efficacy population.

Relative time of an assessment (hours) = Time of assessment – Time of stop time of 1st Infusion.

3.3.3. Safety Endpoints

The following safety endpoints will be summarized:

- Prevalence and nature of treatment-emergent adverse events (TEAEs), defined as all AEs with onset date **after the first dose of ProvayBlue™ infusion and within 10 days of the last dose of ProvayBlue™ infusion**.
- Results of clinical laboratory tests before and after ProvayBlue™ treatment.
- Results of ECG before and after ProvayBlue™ treatment.

3.4. Test Hypothesis and *P* Value Justification

Not applicable since no inferential statistics will be provided for this PMR. Whenever it is appropriate, 2-sided 95% confidence intervals will be provided for estimates.

3.5. Procedures for Handling Missing Data

No missing data imputation will be performed for safety parameters. However, patients with missing infusion stop time will be assigned an imputed stop time in order to derive the efficacy endpoints (See Section 3.3.2 for details).

3.6. Analysis Center

Both studies are multicenter studies; hence, investigative center will be identified as part of the unique patient identification. Data summaries will not be prepared by center unless data warrant.

3.7. Definitions and Derived Variables

3.7.1. Baseline

Baseline will be defined as the last measurement before the start of the first infusion. Baseline records will be marked for all efficacy and safety (laboratory, ECG, vital signs, and clinical signs and symptoms) records in the database.

There was a situation where a baseline metHb measurement had the exact time of the first dose infusion start time; the data were queried and confirmed.

3.7.2. Patient Groups by Methemoglobinemia Suspected Causative Agent

Patients are grouped in 2 subsets based on their methemoglobinemia suspected causative agent: Group 1 includes all patients that metHb was associated with Dapsone use, and Group 2 includes all non-Dapsone-caused metHb. The differentiation is primarily based on

- the frequency with which Dapsone is expected to be identified as the causative agent for inducing methemoglobinemia, and
- the long half-life of Dapsone and its metabolite dapsone hydroxylamine, which likely results in ongoing production of metHb through the acute treatment phase, in contrast to short-acting inciting agents such as amyl nitrate, where normal physiologic conversion of metHb to hemoglobin may begin shortly after exposure.

Patient group will be used for data summary tables.

3.7.3. Patient Groups by Methemoglobinemia Severity

In the Observational Study, all enrolled patients are classified as either a Primary case or a Secondary case (See Section 2.2.1). The patients marked as Primary cases are more severe cases and the data are monitored, whereas the patients marked as Secondary cases are less severe and, aside from verification of the inclusion criteria, data in these cases are not monitored.

All patients from the Interventional Study are classified as Primary cases.

Secondary cases are excluded from the efficacy analysis set (the efficacy analysis set includes the Primary cases that have baseline and at least 1 postbaseline metHb measurement, See Section 3.2). Although all patients are included in the safety analysis set, due to the difference in data monitoring between the Primary and Secondary cases, the safety data will be analyzed twice: one set will include patients from the Primary cases, and the second set will include all patients.

Patient type (Primary vs Secondary) will be clearly flagged in the individual patient data listings as well as the profile figures.

4. DATA SUMMARIES

It is anticipated that the pooled results will be presented as a series of individual patient descriptive narratives with limited statistical analysis. The narratives will also identify issues in individual patient past medical history, exposures, and comorbidities that may impact the consideration and interpretation of "normal" vital signs and related parameters.

When appropriate, summary tables will also be prepared that will include descriptive statistics, such as sample size, mean, standard deviation, minimum, median, and maximum for continuous variables and number (%) of patients for categorical variables. Data summaries will provide descriptive statistics for all patients, patients with possible causative agent of Dapsone, and patients with non-Dapsone causative agents.

All data in the pooled database will have a patient level data listing.

4.1. Disposition

A summary table (**Table 14.1.1**) will provide frequency counts for all patients enrolled, patients in the safety analysis set, patients in the efficacy analysis set, and 10-day posttreatment status (alive, deceased, unknown, lost to follow-up). A summary of disposition will be provided overall and by protocol (PVP-2016003 vs HQF-METHB-2018001).

Disposition data will be provided as a data listing (Listing 16.2.1).

4.2. Demographics and Baseline Characteristics

The demographic summary will include descriptive statistics for age, sex, race, ethnicity, weight, height, and body mass index (BMI) at baseline.

Baseline characteristics and patient population characteristics will also include the following study information:

1) Patient setting before first presentation
2) Acquired methemoglobinemia diagnosis:
    a. Diagnosis by
    b. Diagnosis setting
    c. Suspected causative agent
    d. Exposure to agent
3) Methemoglobinemia assessments and treatment at baseline:
    a. Any signs and symptoms judged to be clinically associated with the acquired methemoglobinemia (yes/no)?
    b. Are co-oximetry results available from the index hospitalization (yes/no)?

c. Are arterial blood gas results available from the index hospitalization (yes/no)?

d. Baseline metHb level

e. Was ProvayBlue™ administered before enrollment to the study (yes /no)?

f. Were any other treatments employed to mitigate the acquired methemoglobinemia (yes/no)?

Demographics and baseline characteristics will be tabulated for the safety analysis set (**Tables 14.1.2.1 and 14.1.2.2**) and efficacy analysis set (**Table 14.1.2.3**) with no formal inferential tests. Demographic data will be provided as a data listing (Listing 16.2.2).

4.3. Medical History

All medical history data captured will be listed without any formal summaries (Listing 16.2.3).

4.4. Protocol Deviations

Any clinically relevant protocol deviation will be identified and described in the clinical study report without any formal summary.

4.5. Treatment Compliance

Not applicable.

4.6. Prior and Concomitant Medications

All prior and concomitant medications will be listed without any formal summaries (Listing 16.2.6).

5. EFFICACY ANALYSIS

Efficacy data listings by individual patient will be prepared for all efficacy endpoints, including the observed value and derived variables (Listing 16.2.8, Listing 16.2.9, Listing 16.2.13). Efficacy analysis will be based on the patients included in the efficacy analysis set.

5.1. Treatment Effect on metHb

MetHb measurements can be obtained via co-oximetry from either arterial or venous blood gas specimens. Data will be pooled and sorted by time of measurement taken for analysis. The following parameters will be derived to aid discussion of treatment effect on the metHb reduction.

5.1.1. MetHb Level at 1-hour Post Dosing

The timing of postdosing metHb level assays is inconsistent in clinical practice and may not be obtained until hours after the conclusion of first dose infusion in the emergency department. It should be noted that, consistent with such practice, the Observational Study does not have a prespecified schedule for metHb postdosing collection. MetHb level at 1-hour postdosing will be derived using linear interpolation. This method will be referred to as backward linear interpolation if the first postdosing metHb was after 1 hour, or forward linear interpolation if the first postdosing metHb was before 1 hour. This method will be mathematically expressed as follows.

Step 1: calculate rate of metHb change per minutes)

= (metHb_1 - baseline) / time of metHb_1 in minutes

Step 2:

metHb_1hr = baseline + 60*[(metHb_1 - baseline) / (time of metHb_1 in minutes)]

where

- baseline = baseline metHb defined as the last metHb before the first dose of study drug)
- metHb_1 = first post treatment metHb observation
- constant (60) converted the time units from minutes to hours

For example, a patient has metHb of 74.40 at baseline and 15.70 at 70 minutes postdosing. The estimated metHb_1hr is 24.08571.

Percent change from baseline (PCHG) at 1-hour post dosing can be estimated as follows.

PCHG (%) at 1-hour post dosing = 100*[(metHb_1-baseline) / time of metHb_1 in minutes]/baseline

The PCHG for the above example is -67.63%.

PCHG at 1-hour =100* {[60*(15.7-74.4)/70] / 74.4}

Due to the mathematical property of this derivation, a patient could never achieve 50% reduction at 1-hour post dosing if the first postdosing metHb was taken after 2 hours. Hence, this derivation will be applied only to those patients who had first postdosing metHb before 2 hours.

5.1.2. Time When metHb Level Reached 50% Reduction

To estimate the time when a 50% reduction would be obtained, the following linear interpolation will be applied.

T50 (minutes after first dose) = (baseline/2) / [60*(baseline -metHb_1) / time of metHb_1 in minutes]

T50 for the above example is 44 minutes.

T50 = (74/2) / {[(74.4-15.7)/70] / 74.4}=37.2/0.838571428 = 44.36

The units for T50 will be converted to hours by T50 /60 in the dataset.

Due to the mathematical property of this derivation, a patient could never achieve 50% reduction at 1-hour post dosing if the first postdosing metHb was taken after 2 hours. Hence, this derivation will be applied only to those patients who had first postdosing metHb before 2 hours.

5.1.3. Summary of metHb

The treatment effect on metHb will be evaluated based on change in metHb from baseline. Because of the wide range in assessment times, the summary table will include only 2 time points: Baseline (last metHb before treatment) and predicted metHb at 1-hour posttreatment. The table will provide descriptive statistics for the observed metHb level, change from baseline, and percent change from baseline, including sample size, mean, standard deviation, and 2-sided 95% confidence intervals for the mean, minimum, median, and maximum. Number (%) patients with ≥50% metHb reduction 1 hour posttreatment will be identified. (**Table 14.2.1.1**). This table will also include predicted time when metHb 50% reduction would occur and time when the first metHb is obtained.

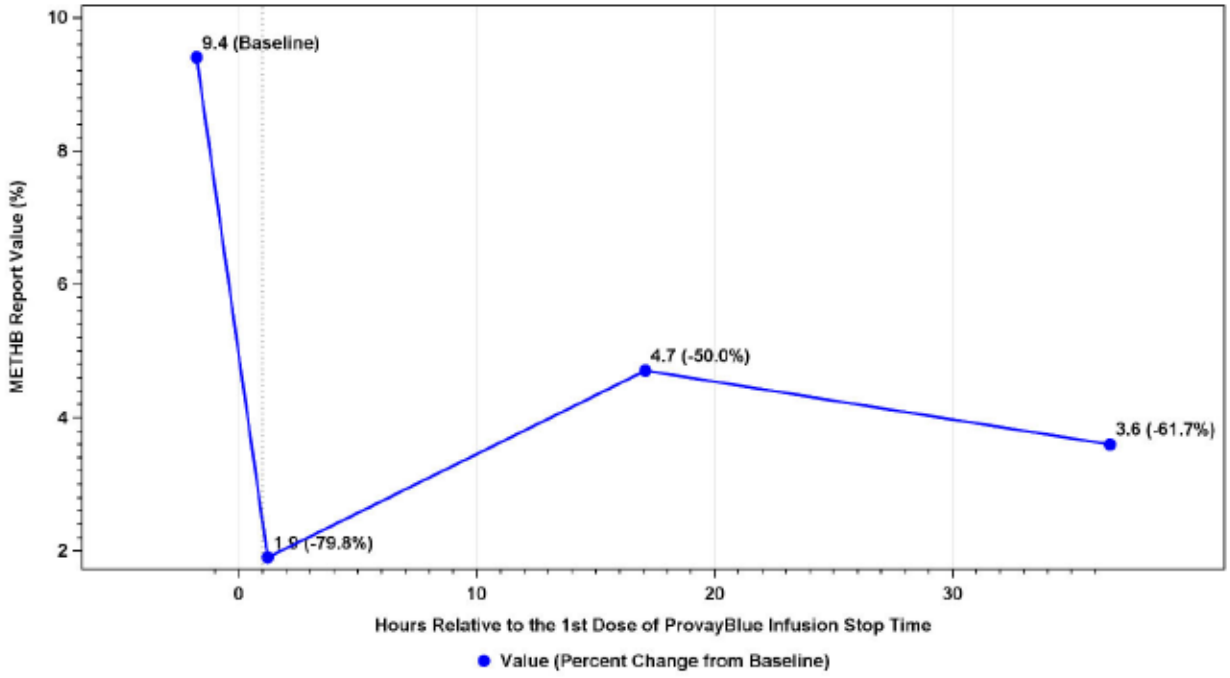
A second summary table (**Table 14.2.1.2**) will provide metHb level descriptive summary at baseline and metHb level of the first follow-up posttreatment for all patients in the efficacy analysis set. In this table, the level of metHb at the first follow-up and time when the first follow-up metHb is taken will be summarized.

5.1.4. Individual Profile of metHb

Individual profiles of metHb over time will be prepared for each patient. All metHb assessments will be included. The profile will flag if the patient is a Primary case or a Secondary case.

The change from baseline in metHb will also be graphically presented, showing the change from baseline by time (hours) for each patient (**Figure 14.2.1.3**) as illustrated in Figure 1.

Figure 1: Example of Individual Profile of metHb Before and After Treatment



Hour 0 is when the first dose of ProvayBlue™ infusion is completed and the dotted vertical reference line is 1 hour after the end of infusion.

5.2. Change in Vital Signs

Vital signs change from baseline, including blood pressure, respiratory rate, and heart rate, will be derived for each patient. Baseline is the last vital sign measurement before a patient receives the first infusion of ProvayBlue™.

5.2.1. Median Vital Signs within 2-Hour Post Treatment

Vital signs measurements, including systolic blood pressure, diastolic blood pressure, heart rate and respiratory rate, were taken at various time points and frequency. In order to synchronize the measurements, all vital signs measures taken within 2 hours will be pooled within each individual patient dataset to derive the median for the patient.

5.2.2. Vital Signs Normal/Abnormal Assessments

Change from baseline in **vital signs (blood pressure, respiratory rate, and heart rate)** after the first dose of ProvayBlue ™ infusion will be evaluated.

- The secondary efficacy will be the number (%) of patients with concomitant normalization of vital signs (as per the clinical context) within 2 hours, inclusive, after the end of infusion of the first dose of ProvayBlue ™
- Time (hours) when the first incidence of normal VS is observed in patients who have abnormal baseline vital signs

The normal ranges used to define normal blood pressure are provided in Table 4. However, in the individual patient narratives, vital signs will be contextualized for comorbidity and other pertinent issues. For example, a patient with chronic obstructive pulmonary disease likely has an elevated respiratory rate even when not suffering for acquired methemoglobinemia; a "return to normal" for such a patent would be clinically expected to differ from "return to normal" for a patient without COPD.

Table 4: Vital Signs Normal Ranges

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 to 15)	110-131	64-83	12-20	60-70
Adults (≥ 16 years)	90-140	60-90	12-20	50-100

All assessments (with and without assessment time) will be included in the data listing (Listing 16.2.9).

5.2.3. Summaries of Vital Signs Change from Baseline

A summary table will include VS measurement and change from baseline by relative time frame with descriptive statistics of sample size, mean, standard deviation, 2-sided 95% confidence intervals, quartiles (25% tile, 50% tile [median], and 75% tile), minimum, and maximum. The table will also include number (%) patients whose vital signs are within the normal range (**Table 14.2.2.1**). Number (%) patients who had at least 1 incidence of normal vital signs within the 2 hours following the completion of first dose ProvayBlue™ infusion will also be presented.

Change in SpO_2 will be assessed in 3 categories, based on ±2% rule as follows

1) Increased if the difference (postbaseline – baseline) is >2
2) No Change if the difference (postbaseline – baseline) is within ±2
3) Decreased if the difference (postbaseline – baseline) is <2

5.2.4. Individual Profiles of Vital Signs

The change from baseline in vital signs will also be graphically presented, showing the change by time (hours) for each patient (**Figure 14.2.2.2**), as illustrated in Figure 2: Example of Individual Profile of Blood Pressure Before and After Treatment and Figure 3. The profile will flag if the patient is a Primary case or a Secondary case.

Figure 2: Example of Individual Profile of Blood Pressure Before and After Treatment

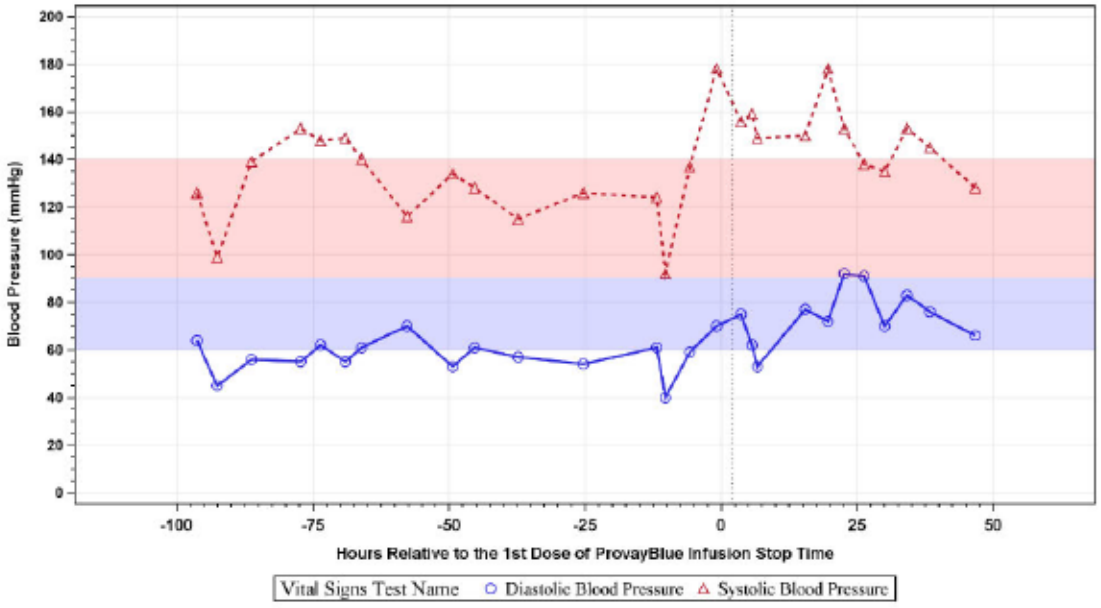
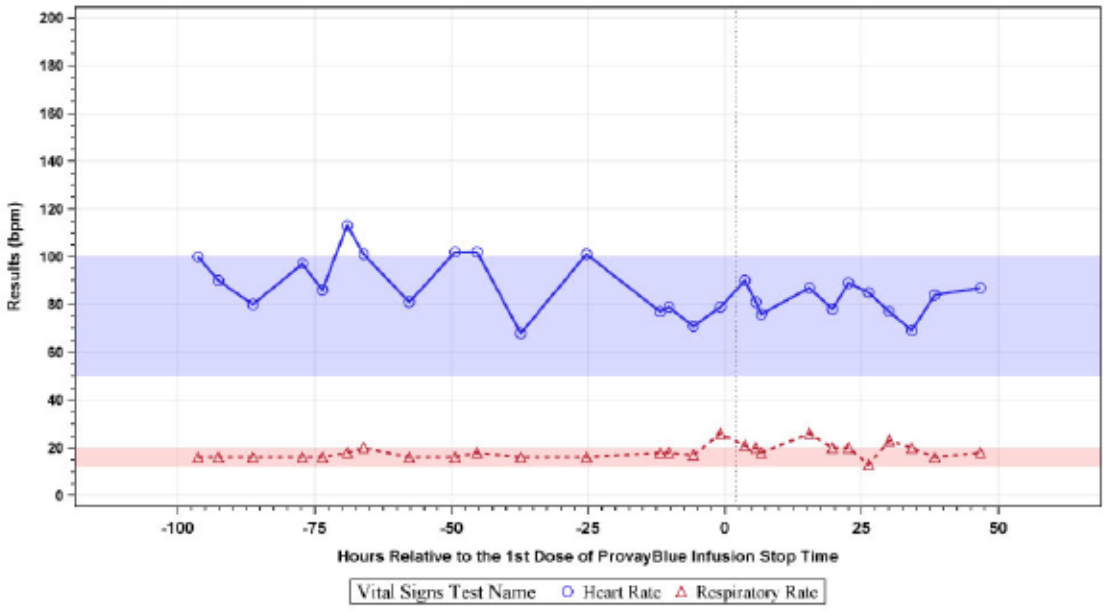


Figure 3: Example of Individual Profile of Respiratory and Heart Rate Before and After Treatment



5.3. Methemoglobinemia Signs and Symptoms

The following 13 signs and symptoms that may be associated with methemoglobinemia will be collected. Decrease and/or resolution of these symptoms may be considered supportive of efficacy. This assessment is applicable only for the Primary case.

1. Grayish-blue skin discoloration
2. Cyanosis
3. Dyspnea
4. Headache
5. Fatigue
6. Weakness
7. Dizziness
8. Syncope
9. Coma
10. Seizures

11. Depressed CNS
12. Arrhythmias
13. Metabolic acidosis

Because the terms 'Grayish-blue skin discoloration' and 'Cyanosis' are essentially the same medically speaking, these 2 terms will be collapsed when the analysis dataset is created from the CRF database. If both boxes on the CRF are checked with identical time, the record with highest intensity grade will be flagged to be used for data summary; all records will be displayed in data listing.

Other noted signs and symptoms will also be collected (as 'Other'). Verbatim text of 'Other' signs and symptoms will be reviewed, and similar symptoms will be manually grouped into a group term when the analysis dataset is built. The grouped term will be labeled as the Modified term in the analysis dataset. Further, it is understood that some of these signs and symptoms may be inconsistently detected (such as cyanosis in a darkly pigmented patient) or inconsistently classified/severity-rated (such as "depressed CNS").

Data collection will include severity assessment per NCI-CTCAE grade and time of observations. In some incidences, a symptom could be reported without severity. Those incidences will be displayed as 'Y,' indicating that the symptom is present with unknown grade.

Observations from signs and symptoms assessments will be grouped into the following time periods:

- Before Treatment
- Posttreatment: ≤24 hours
- Posttreatment: 24-48 hours
- 'Posttreatment: 48-72 hours
- Posttreatment: 72-96 hours
- Posttreatment: 96-120 hours
- Posttreatment: >120 hours

5.3.1. Summary of Signs and Symptoms

Summary tables (**Table 14.3.3.1.1, Table 14.3.3.1.2**) will be prepared to provide number (%) of patients who had a given sign/symptom present. The percentage will be calculated using 2 different denominators:

- Reported: the denominator will be the number of patients who had assessment within each time period

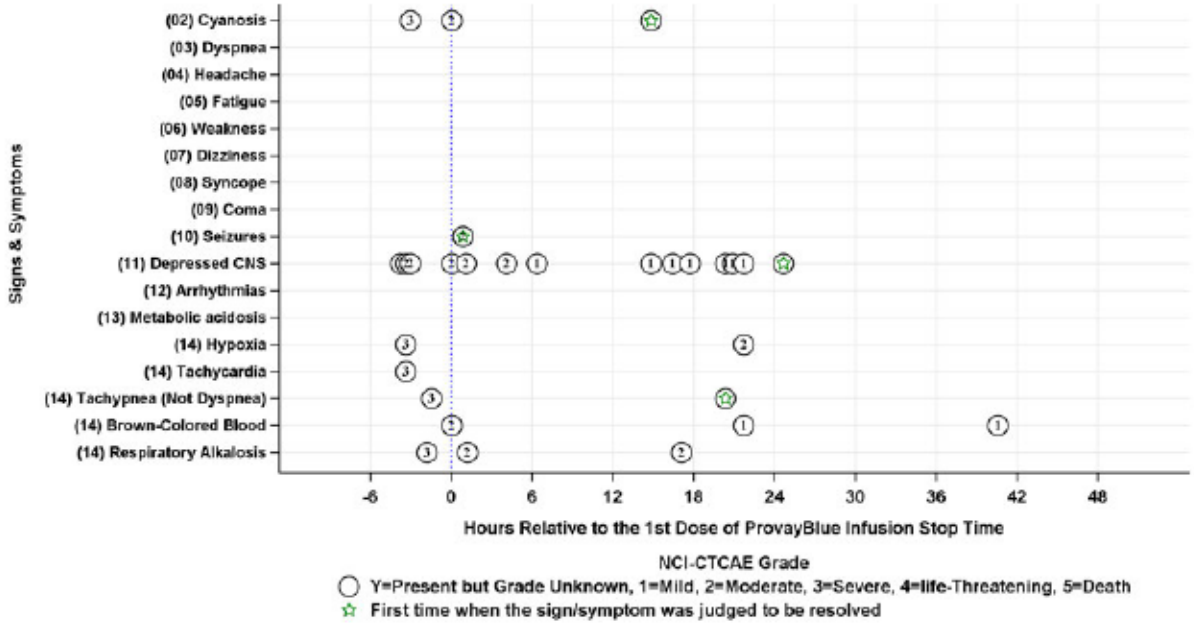
- All Patients: the denominator will be the total number of patients in each patient group in the table.

5.3.2. Individual Profiles of Signs and Symptoms

Each symptom and its severity will be displayed via scatter bubble plots for each patient (**Figure 14.3.3.2**), showing the observed grade and relative time (X) to the first infusion. The first time when a symptom is judged to be resolved will also be displayed. Data with assessment time will be included in the graphic and all data (with and without time) will be included in the listing. This figure is illustrated in Figure 4.

All 12 prespecified signs and symptoms will be identified in the profile Y-axis, the sign/symptom NCI-CTCAE grade will be displayed if presented at a given time (X-axis). Any other symptoms will also be displayed using grouped term which will be reviewed and finalized based on verbatim term before database lock.

Figure 4: Example of Individual Profile of Methemoglobinemia Signs and Symptoms Before and After Treatment



6. SAFETY AND TOLERABILITY EVALUATIONS

Safety data summaries will be based on the safety analysis set. However, the data will be tabulated twice: the first table will include Primary cases only and the second table will include all patients. Safety data listing by individual patient will be prepared for all safety endpoints.

6.1. Total Exposure

Number of doses received and infusion duration will be tabulated (**Table 14.3.1.1 and Table 14.3.1.2**). Please note that any patient without a recorded infusion stop time will have a missing infusion duration in this summary, although the missing infusion stop time is to be imputed in order to derive the relative time frame for efficacy endpoints (see Section 3.3.2).

6.2. Adverse Events

Adverse events reported after the first dose of ProvayBlue™ infusion start time and within 10 days after the last dose of ProvayBlue™ infusion will be considered treatment-emergent adverse events (TEAEs). Any AEs that do not have onset time will be included as TEAEs if reported. Patients who did not have any AEs will be verified via source documentation.

The Medical Dictionary for Regulatory Activities (Version 23) will be used to classify all AEs with respect to system organ class and preferred term. All adverse events will be listed.

The following summary tables will be produced for the TEAEs. All data summaries will provide number (%) patients as well as total number of events in each category by patient group. Each summary will be produced in 2 sets: one includes the Primary cases and one includes all patients in the safety analysis set.

1. a topline summary of TEAEs (**Table 14.3.2.1.1, Table 14.3.2.1.2**)
2. a summary table by preferred term in descending order of total incidence (**Table 14.3.2.2.1, Table 14.3.2.2.2**)
3. a summary table by system organ class and preferred term (**Table 14.3.2.3.1, Table 14.3.2.3.2**)
4. a detailed summary table by system organ class, preferred term and severity (**Table 14.3.2.4.1, Table 14.3.2.4.2**)
5. a detailed summary table by system organ class, preferred term and relationship (**Table 14.3.2.5.1, Table 14.3.2.5.2**)
6. a table of serious TEAEs by system organ class and preferred term (**Table 14.3.2.6.1, Table 14.3.2.6.2**)

6.3. Other Safety Parameters

6.3.1. Changes in Other Blood Gas Tests

In addition to metHb, results from 11 other tests obtained via blood gas analysis with or without co-oximetry are collected (**Table 5**). Individual profiles of the co-oximetry results will be prepared (**Figure 14.3.4**), showing the observed value (Y) and relative time (X) to the first infusion. Data with assessment time will be included in the graphic display; all data (with and without assessment time) will be included in the data listing (Listing 16.2.12).

Table 5: Blood Gas Parameters of Interest

Parameter (Units)		Data Panel	
		Arterial Blood Gas	Co-Oximetry
1	Total Hemoglobin (g/dL)	Yes	Yes
2	SO_2/SaO_2 (%)	No	Yes
3	C2CT (%)	No	Yes
4	OxyHb (%)	Yes	Yes
5	deoxyHb (%)	No	Yes
6	COHb (%)	No	Yes
7	pH	Yes	Yes
8	PaO_2	Yes	No
9	$PaCO_2$	Yes	No
10	HCO_3	Yes	No
11	SaO_2	Yes	No

Abbreviations: SO_2 = oxygen saturation; SaO_2 = arterial oxygen saturation; O2CT = oxygen content; OxyHb = oxygenated hemoglobin; deoxyHb = deoxyhemoglobin; COHb = carboxyhemoglobin; pH = hydrogen ions (H+) in blood; PaO_2 = partial pressure of oxygen; $PaCO_2$ = partial pressure of carbon dioxide; HCO_3 = Bicarbonate.

6.3.2. Changes in Laboratory Tests

Selected safety laboratory tests of interest will be collected (see Table 6). All laboratory results were from local laboratory. Although other laboratory tests outside this list could be collected, for the purpose of the data summary, the results of laboratory tests collected in 'Other Labs' will only be available in the listing (Listing 16.2.11.3).

Table 6: Safety Laboratory Tests of Interest

Hematology	Biochemistry
Hemoglobin	Glucose
Platelet Count	Sodium
Red Blood Cell Count	Potassium
White Blood Cell Count	Chloride
	Urea
	Calcium
	Inorganic Phosphorus
	Creatinine

Laboratory results will be graphically displayed by patient, showing the observed value (Y) and relative time (X) after the first dose of ProvayBlue™ Infusion (**Figure 14.2.5 for hematology, Figure 14.3.6 for Chemistry**).

6.3.3. Changes in 12-Lead ECG

Results of ECG are to be collected before and after ProvayBlue™ treatment, including PQ interval, QRS duration, QT interval, QTc interval and overall interpretation (normal; abnormal not clinically significant; abnormal clinically significant).

Number (%) patients in each results interpretation category at each time point will be tabulated at each planned time point (**Table 14.3.7.1, Table 14.3.7.2**). The PQ interval, QRS duration, QT interval, and QTc interval will not be tabulated, but a data listing will be provided (Listing 16.2.14).

7. ABLE OF CONTENTS OF PLANNED DATA SUMMARIES

7.1. Summary Tables

Number	Type	Title	Analysis Set
14.1.1	Table	Patient Enrollment and Analysis Set and Post Treatment Follow-up Status	Enrolled Analysis Set
14.1.2.1	Table	Demographics and Baseline Characteristics	Safety Analysis Set - Primary Cases
14.1.2.2	Table	Demographics and Baseline Characteristics	Safety Analysis Set - All Patients
14.1.2.3	Table	Demographics and Baseline Characteristics	Efficacy Analysis Set
14.2.1.1	Table	Predicted MetHb Level 1 Hour Post First Infusion in Patients with First MetHB Drawn Within 2 Hours of Dosing	Efficacy Analysis Set
14.2.1.2	Table	Summary of 1st Follow-up MetHb Level Regardless When the Assessment Was Taken	Efficacy Analysis Set
14.2.1.3	Figure	Individual Profile of Change from Baseline in MetHb Before and After Treatment	Safety Analysis Set - All Patients
14.2.2.1	Table	Summary of Vital Signs at Baseline and Within 2 Hours Post First Infusion	Efficacy Analysis Set
14.2.2.2	Figure	Individual Profile of Change from Baseline in Vital Signs Before and After Treatment	Safety Analysis Set - All Patients
14.2.3.1	Table	Summary of Methemoglobinemia Signs and Symptoms Before and After Treatment	Efficacy Analysis Set
14.2.3.2	Figure	Scatter Plots Showing Individual Profile of Methemoglobinemia Signs and Symptoms Before and After Treatment	Efficacy Analysis Set
14.3.1.1	Table	Study Drug Infusions	Safety Analysis Set - Primary Cases
14.3.1.2	Table	Study Drug Infusions	Safety Analysis Set - All Patients
14.3.2.1.1	Table	Topline Summary of Treatment Emergent Adverse Event	Safety Analysis Set - Primary Cases
14.3.2.1.2	Table	Topline Summary of Treatment Emergent Adverse Event	Safety Analysis Set - All Patients
14.3.2.2.1	Table	All TEAE by Preferred Term Displayed the Most Commonly Reported Event First	Safety Analysis Set - Primary Cases
14.3.2.2.2	Table	All TEAE by Preferred Term Displayed the Most Commonly Reported Event First	Safety Analysis Set - All Patients
14.3.2.3.1	Table	All TEAE by SOC and Preferred Term	Safety Analysis Set - Primary Cases
14.3.2.3.2	Table	All TEAE by SOC and Preferred Term	Safety Analysis Set - All Patients
14.3.2.4.1	Table	All TEAE by SOC, Preferred Term and Intensity	Safety Analysis Set - Primary Cases
14.3.2.4.2	Table	All TEAE by SOC, Preferred Term and Intensity	Safety Analysis Set - All Patients
14.3.2.5.1	Table	All TEAE by SOC, Preferred Term and Relationship to Study Drug	Safety Analysis Set - Primary Cases
14.3.2.5.2	Table	All TEAE by SOC, Preferred Term and Relationship to Study Drug	Safety Analysis Set - All Patients
14.3.2.6.1	Table	All Serious TEAE by SOC and Preferred Term	Safety Analysis Set - Primary Cases

14.3.2.6.2	Table	All Serious TEAE by SOC and Preferred Term	Safety Analysis Set - All Patients
14.3.3	Figure	Individual Profile of Blood Gas Results Before and After Treatment	Safety Analysis Set - All Patients
14.3.4	Figure	Individual Profile of Hematology Test Results Before and After Treatment	Safety Analysis Set - All Patients
14.3.5	Figure	Individual Profile of Biochemistry Test Results Before and After Treatment	Safety Analysis Set - All Patients
14.3.6.1	Table	Summary of 12-Lead ECG Results by Interpretation Category and Timepoint	Safety Analysis Set - Primary Cases
14.3.6.2	Table	Summary of 12-Lead ECG Results by Interpretation Category and Timepoint	Safety Analysis Set - All Patients

7.2. Data Listings

Number	Title	Analysis Set
16.2.1	Disposition and Analysis Population	ITT Analysis Set
16.2.2	Demographics	Safety Analysis Set - All Patients
16.2.3	Medical History	Safety Analysis Set - All Patients
16.2.4	Methemoglobinemia Assessment and Diagnosis	Safety Analysis Set - All Patients
16.2.5	Study Drug Administration	Safety Analysis Set - All Patients
16.2.6	Prior and Concomitant Medications	Safety Analysis Set - All Patients
16.2.7	Other Procedures During Study	Safety Analysis Set - All Patients
16.2.8	MetHb Derived Efficacy Parameters	Safety Analysis Set - All Patients
16.2.9	Vital Signs and Change from Baseline	Safety Analysis Set - All Patients
16.2.10	All Adverse Events Reported in the Study	Safety Analysis Set - All Patients
16.2.11.1	Study Identified Hematology Test Results	Safety Analysis Set - All Patients
16.2.11.2	Study Identified Biochemistry Test Results	Safety Analysis Set - All Patients
16.2.11.3	Results from Other Lab Tests Collected	Safety Analysis Set - All Patients
16.2.12	Blood Gas Parameters Results	Safety Analysis Set - All Patients
16.2.13	Methemoglobinemia Signs and Symptoms	Safety Analysis Set - All Patients
16.2.14	12-Lead ECG Results	Safety Analysis Set - All Patients
16.2.15	Follow-up	Safety Analysis Set - All Patients
16.2.16	Listing of Hospital Course	Safety Analysis Set - All Patients