CLINICAL STUDY PROTOCOL

(AURORA)

A Randomized, Double-blind, Placebo-Controlled Study of Bitopertin to Evaluate the Safety, Tolerability, Efficacy, and Protoporphyrin IX (PPIX) Concentrations in Participants with Erythropoietic Protoporphyria (EPP)

Protocol Number:	DISC-1459-201
Investigational Medicinal Product:	DISC-1459/bitopertin
IND Number:	157419
Sponsor:	Disc Medicine, Inc. 321 Arsenal Street, Suite 101 Watertown, MA 02472, USA
Date of Protocol and Version:	02 March 2022, v 1.0 24 June 2022, v2.0 11 August 2022, v3.0

SIGNATURE PAGE

Protocol Title:	(AURORA) A Randomized, Double-blind, Placebo-Controlled Study of Bitopertin to Evaluate the Safety, Tolerability, Efficacy, and Protoporphyrin IX (PPIX) Concentrations in Participants with Erythropoietic Protoporphyria (EPP)
Protocol Number:	DISC-1459-201
Date of Protocol and Version:	11 August 2022, v3.0

Protocol DISC-1459-201, Version 3.0, was approved by:

Sponsor Signatory:

Signature: DocuSigned by: PPD
Signing Reason: I approve this document
Signing Time: 12-Aug-2022 | 11:10:40 AM PDT
6212F1AE038B4DDE8741956C95204FE9

Date: 12-Aug-2022

INVESTIGATOR'S SIGNATURE

Protocol Title:	(AURORA) A Randomized, Double-blind, Placebo-Controlled Study of Bitopertin to Evaluate the Safety, Tolerability, Efficacy, and Protoporphyrin IX (PPIX) Concentrations in Participants with Erythropoietic Protoporphyria (EPP)
Protocol Number:	DISC-1459-201
Date of Protocol and Version:	11 August 2022, v3.0

I have read this clinical study protocol and agree to conduct this study in accordance with all stipulations of the protocol and in accordance with the ethical principles that have their origin in the Declaration of Helsinki, and that are consistent with International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), all applicable local Good Clinical Practice (GCP) guidelines, and the applicable regulatory requirement(s).

Principal Investigator:

Print/Type Name: _____

Signature: _____

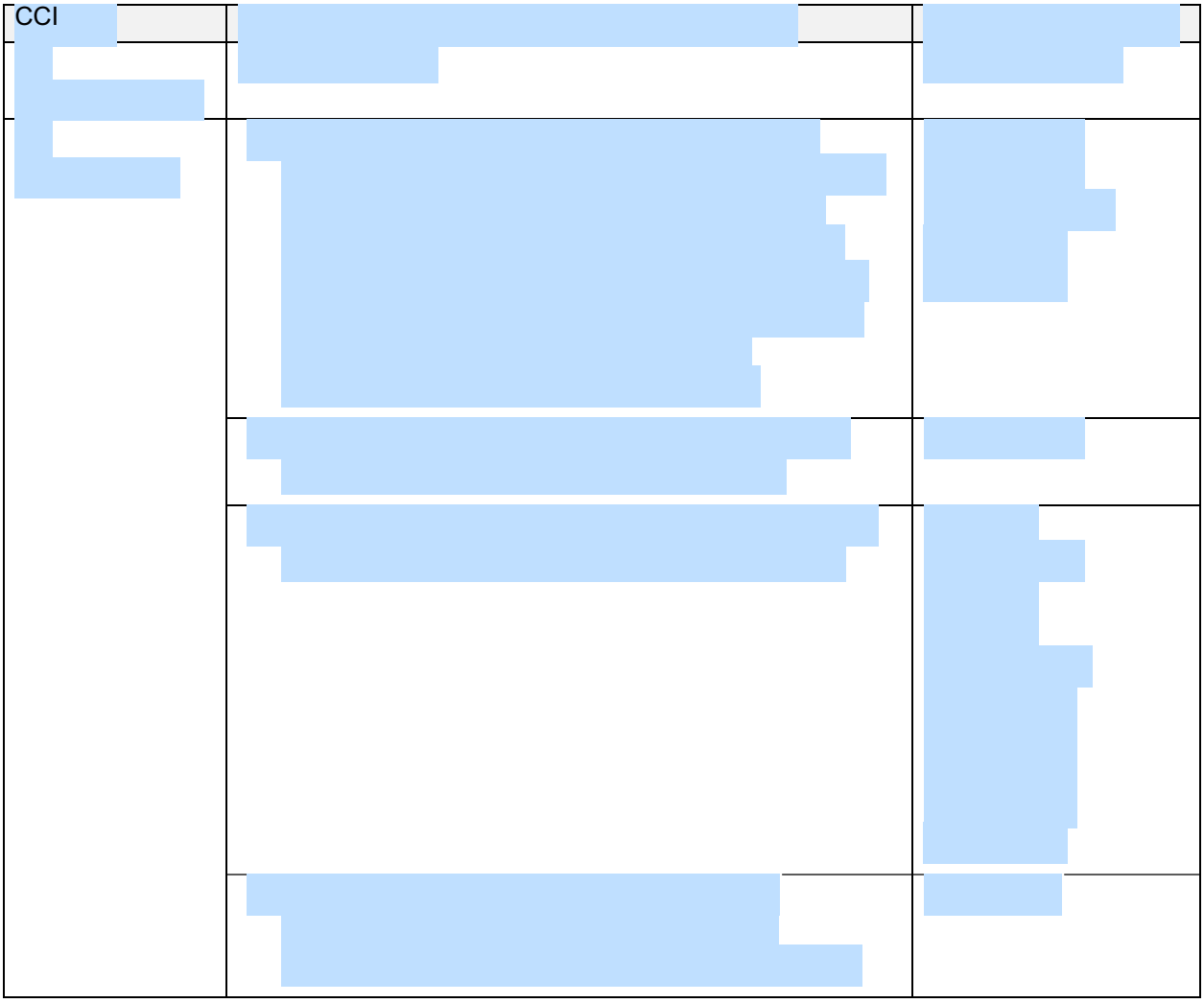
Date: _____

Table of Contents

List of Tables

List of Figures

SUMMARY OF CHANGES



Version	Description and Rationale for Amendment	Applicable Sections
	5. CCI • CCI • CCI	CCI
	6. CCI	

Version	Description and Rationale for Amendment	Applicable Sections
	CCI	
3.0 *11 August 2022*	CCI	

1. SYNOPSIS

Protocol Number: DISC-1459-201

Title:
(AURORA) A Randomized, Double-blind, Placebo-Controlled Study of Bitopertin to Evaluate the Safety, Tolerability, Efficacy, and Protoporphyrin IX (PPIX) Concentrations in Participants with Erythropoietic Protoporphyria (EPP)

Investigational Product Name: DISC-1459 (Bitopertin)

Sponsor: Disc Medicine

Study Centers: Approximately 10 in the United States (US)

Development Phase: 2

Study Objectives:

Primary Objective:

- To assess changes in PPIX concentrations in response to bitopertin treatment

Secondary Objectives:

- To characterize the effect of bitopertin on daily daylight tolerance
- To assess the safety and tolerability of bitopertin at two dose levels
- To characterize the relationship between bitopertin dose level and key biological indicators of mechanism engagement
- To evaluate bitopertin pharmacokinetics (PK)

Exploratory Objectives:

- CCI

Protocol Number: DISC-1459-201

Study Endpoints:

Primary:

- Percent change from baseline in whole blood metal-free PPIX levels

Key Secondary:

- Total hours of sunlight exposure to skin on days with no pain from 1000 to 1800 hours (10:00 AM to 6:00 PM)

Secondary:

- A two-week average daily sunlight exposure time (minutes) to first prodromal symptom (e.g., burning, tingling, itching, or stinging) associated with sunlight exposure between 1 hour post -sunrise and 1 hour pre-sunset.
- Pain intensity of phototoxic reactions according to a Likert scale (0-10)
- Safety and tolerability of bitopertin, as assessed by the incidence of treatment-emergent adverse events (TEAEs)
- Erythrocyte metal-free PPIX concentrations
- Plasma and whole blood total PPIX concentrations
- Plasma bitopertin concentrations

Pharmacokinetic:

The following parameters will be determined from plasma PK sampling (if data permits):

- Maximum observed drug concentration (C_{max})
- Observed time of the maximum drug concentration (T_{max})
- Area under the concentration-time curve from time 0 to 24 hours post-dose on Day 1 (AUC_{0-24})

Exploratory:

- CCI

Protocol Number: DISC-1459-201

CCI

Study Design:

This is a Phase 2, multi-center, double-blind, placebo-controlled, parallel group study of bitopertin to evaluate the safety, tolerability, efficacy, and PPIX concentration change in participants with EPP. Participants will be screened for study eligibility within CCI days before Baseline (Day 1). All participants will undergo light tolerance assessment during Screening that includes a diary assessment and wearing a light dosimeter.

Up to 75 participants aged 18 and older are planned to be enrolled. Participants who are determined to be eligible based on Screening assessments will be randomized upon confirmation of eligibility or on Day 1 in a 1:1:1 equal allocation ratio amongst the following three treatment groups:

- Placebo (N=25) administered CCI
- Bitopertin 20 mg (N=25) administered CCI
- Bitopertin 60 mg (N=25) administered CCI

Randomization will be stratified by sunlight exposure time to prodromal symptom (<30 minutes or ≥30 minutes), as assessed during a 2-week run-in period.

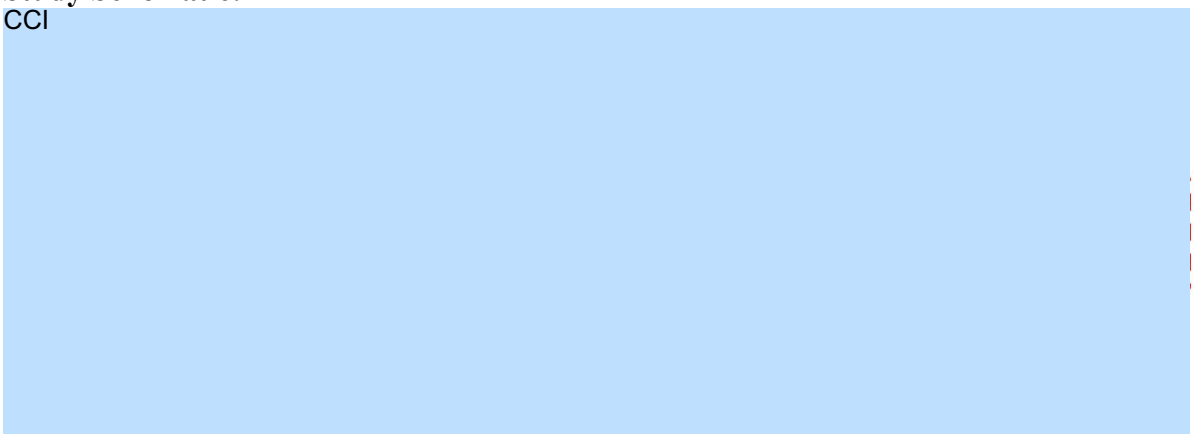
On Day 1, participants will begin study drug according to their random assignment and will be evaluated during a 120-day treatment period thereafter. Participants will be required to wear light dosimeters daily and maintain a daily sun exposure diary throughout the study.

After completion of the 120-day treatment period, including assessment of light tolerance, and End-of-Study (EOS) visit, CCI The study schema follows.

Protocol Number: DISC-1459-201

Study Schematic:

CCI



Study Treatments:

Investigational Product, Dose, and Route of Administration:

Bitopertin (chemical name: (*S*)-[4-(3-Fluoro-5-trifluoromethyl-pyridin-2-yl)-piperazin-1-yl]-[5-methanesulfonyl-2-(2,2,2-trifluoro-1-methyl-ethoxy)-phenyl]-methanone) is presented in 2 CCI .

Participants randomized to receive bitopertin will receive:

- CCI once daily (QD) for 120 days (17 weeks) OR
- CCI QD for 120 days (17 weeks).

Reference Therapy, Dose, and Route of Administration:

Placebo consists of the inactive ingredients, formed into tablets matching the size and appearance of the CCI .

Participants randomized to receive placebo will receive:

- Two placebo tablets QD for 120 days (17 weeks)

Duration of Individual Study Participation:

Individual participation will be up to 5 months, consisting of:

- Screening Period: up to C CI days

Protocol Number: DISC-1459-201

- Treatment Period: up to CCI days (CCI)

After completion of treatment phase, participants may continue on open-label bitopertin (20 mg QD).

Independent Data Monitoring Committee:

An Independent Data Monitoring Committee (IDMC) will aid in the monitoring of unblinded, accumulating study data to ensure the ongoing safety of trial participants. The IDMC membership will be composed of external medical experts with relevant clinical experience. The IDMC will have the right to recommend stopping the study at any time due to the concerns for the safety of the participants.

Number of Participants:

Planned: Approximately 75 adults (≥18 years) will be enrolled for an anticipated 69 adults completing 4 months of treatment.

Inclusion criteria:

Participants must meet all of the following criteria to be eligible for enrollment in the study:

1. Aged 18 years or older at the time of signing the informed consent form (ICF).
2. Diagnosis of EPP, based on medical history by ferrochelatase (FECH) genotyping or by biochemical porphyrin analysis.
3. Body weight ≥50 kg.
4. Washout of at least 2 months prior to Screening of afamelanotide and dersimelagon, if applicable.
5. Aspartate aminotransferase (AST) and alanine transaminase (ALT) <2′ upper limit of normal (ULN) and total bilirubin <ULN (unless documented Gilbert syndrome) at Screening. Albumin >lower limit of normal (LLN).
6. If male with female sexual partner(s) of childbearing potential, agrees he and partner will use one of the following acceptable methods of birth control during the study and for 30 days after the last study drug dose:
    - abstinence
    - stable hormonal contraceptive
    - barrier method (e.g., condom [male or female] or diaphragm)
    - intrauterine device, in place for at least 3 months
    - surgically sterile by hysterectomy, bilateral oophorectomy, or bilateral tubal ligation

Protocol Number: DISC-1459-201

7. If female of childbearing potential, defined as prior menarche, no hysterectomy, no bilateral oophorectomy, not postmenopausal (at least 12 months natural, spontaneous amenorrhea), must commit to one of the following methods of acceptable birth control during the study and for 30 days after the last study drug dose:
    - abstinence
    - stable hormonal contraceptive in conjunction with a barrier method (e.g., condom [male or female] or diaphragm)
    - intrauterine device, in place for at least 3 months
8. Negative pregnancy test (females of childbearing potential) at Screening (Days CCI AND Baseline (Day 1), prior to dosing.
9. Able to understand the study aims, procedures, and requirements, and provide written informed consent.
10. Able to comply with all study procedures.

Exclusion Criteria:

Participants meeting any of the following criteria are not eligible for study enrollment:

Medical History:

1. Major surgery within 8 weeks before Screening or incomplete recovery from any previous surgery.
2. Other than EPP, an inherited or acquired red cell disease associated with anemia.
3. A history or known allergic reaction to any investigational product excipients or history of anaphylaxis to any food or drug.
4. History of liver transplantation.
5. History of alcohol dependence or excessive alcohol consumption, as assessed by the Investigator.
6. Human immunodeficiency virus (HIV), active Hepatitis B, or C. A positive hepatitis result should be discussed between the Investigator and Sponsor prior to enrollment.
7. CCI
8. Other medical or psychiatric condition or laboratory finding not specifically noted above that, in the judgment of the Investigator or Sponsor, would put the participant at unacceptable risk or otherwise preclude the participant from participating in the study

Protocol Number: DISC-1459-201

9. Condition or concomitant medication that would confound the ability to interpret clinical, clinical laboratory, or participant diary data, including a major psychiatric condition that has had an exacerbation or required hospitalization in the last 6 months.

Treatment History:

10. Concurrent or planned treatment with afamelanotide or dersimelagon during the study period.
11. Treatment with opioids for any period >7 days in the 2 months prior to screening or anticipated to require opioid use for >7 days at any point during the study.
12. New treatment for anemia, including initiation of iron supplementation, in the 2 months prior to Screening.
13. Current or planned use of any drugs or herbal remedies known to be strong inhibitors or inducers of CYP3A4 enzymes for CCI days prior to the first dose and throughout the study.
14. Current or planned treatment with anti-psychotic medication.

Laboratory Exclusions:

15. CCI .

Miscellaneous:

16. If female, pregnant or breastfeeding.
17. Participation in any other clinical protocol or investigational trial that involves administration of experimental therapy and/or therapeutic devices within 30 days of Screening.
18. Grapefruit/Seville orange and products containing them for 14 days prior to first dose and throughout the study.

Statistical Methods:

***Sample Size**:*

Approximately 75 participants will be randomized and allocated in a 1:1:1 ratio to receive bitopertin 20 mg QD, bitopertin 60 mg QD, or placebo.

The primary efficacy endpoint is percent change from baseline in whole blood metal-free PPIX level at Day CCI Means of percent change from baseline values will be compared for each bitopertin dose group and placebo. Assuming the difference in mean percent change in PPIX between bitopertin and placebo is 30% with a corresponding pooled SD of 35%, a sample size of 23 participants per group provides 80% power to detect a difference between each bitopertin dose group and placebo using a two-sample t-test with a 2-sided alpha=0.05. Assuming an 8% dropout rate, a total of 75 participants will be randomized.

The key secondary efficacy endpoint is total hours of sunlight exposure to skin on days with no pain from 1000 to 1800 hours (10:00 AM to 6:00 PM) over 120 days. Means of total hours of sunlight exposure on days with no pain will be compared for each bitopertin dose group and placebo. Assuming the difference in means between bitopertin and placebo is 55 hours with a corresponding pooled SD of 67 hours, a sample size of 25 participants per group provides 80% power to detect a difference between each bitopertin dose group and placebo using a two-sample t-test with a 2-sided alpha=0.05.

Analysis Sets:

- Intent-to-Treat Analysis Set: To be included in the Intent-to-Treat (ITT) analysis set, participants must be randomized to study drug.

  All analyses of the ITT analysis set will be based on each participant's randomized treatment. If a participant is randomized according to the incorrect stratification, the participant will be analyzed under the randomized treatment for the stratum recorded in the randomization system. All efficacy analyses will be based on the ITT analysis set.
- Full Analysis Set: To be included in the Full Analysis Set (FAS), participants must be randomized and take at least one dose of double-blind study drug, and have a baseline measurement and at least one postbaseline measurement of the primary efficacy variable or key secondary efficacy variable.

  All analyses of the FAS will be based on each participant's randomized treatment. If a participant is randomized according to the incorrect stratification, the participant will be analyzed under the randomized treatment for the stratum recorded in the randomization system. The FAS will be used for a sensitivity analysis of the primary and key secondary efficacy endpoints.
- Per Protocol Analysis Set: The Per Protocol (PP) Analysis Set will include all participants in the FAS who meet the study eligibility requirements and have no major protocol deviations that affect the validity of the efficacy measurements. The PP Analysis Set will be used for a sensitivity analysis of the primary and key secondary efficacy endpoints. The criteria for inclusion in the PP Analysis Set will be finalized prior to study unblinding and detailed in the Statistical Analysis Plan (SAP).

- Safety Analysis Set: All participants who are randomized and take at least one dose of double-blind study drug will be included in the Safety Analysis Set. Safety analyses will be based on the study drug that was dispensed to each participant.

Primary Efficacy Endpoint Analysis:

For the primary endpoint of percent change from baseline in whole blood metal-free PPIX level, a mixed model repeated measures (MMRM) analysis of covariance (ANCOVA) model will be fitted using all data as observed. The dependent variable is the percent change from baseline whole blood metal-free PPIX level for all post-baseline assessments for each participant. The model will include fixed effects for treatment, sunlight exposure time to prodromal symptom randomization stratification factor , baseline metal-free PPIX level, visit (Days CCI), and visit-by-treatment interaction and a random effect for the participant.

The primary efficacy analysis will be based on comparisons of bitopertin and placebo at the Day CCI visit. From the MMRM model, the Day CCI pairwise differences between least square (LS) means of bitopertin treatment groups and placebo will be presented along with the corresponding 95% CIs and 2-sided p-values. This analysis will be repeated for the FAS and PP Analysis Set.

Key Secondary Endpoint Analysis:

The key secondary endpoint for this study is total hours of sunlight exposure on days with no pain summed over the entire treatment period from randomization to Day CCI An ANOVA model will be used for the ITT analysis set with effects for randomized treatment group and sunlight exposure time to prodromal symptom randomization stratification factor. Pairwise differences between LS means of bitopertin treatment groups and placebo will be presented along with the corresponding 95% CIs and 2-sided p-values.

Since a highly skewed distribution for this endpoint is expected, a Kruskal-Wallis test will be used to test the treatment effect as a sensitivity analysis. The 2-sided p-values will be presented. The Hodges–Lehmann estimates of the median differences between the bitopertin treatment groups and placebo, and their 95% CIs will also be presented.

Safety Analysis:

All safety analyses will be based on the Safety Analysis Set.

Safety data will include AEs, physical examination results, vital signs, CCI , and clinical laboratory measurements. Observed data will be listed by participant and summarized using descriptive statistics by treatment group.

AEs that begin after the first administration of study drug, or existing AEs that worsen after the first dose of study drug will be considered TEAEs. The number and percentage of participants reporting TEAEs will be summarized for each treatment group by Medical Dictionary for Regulatory Activities (MedDRA) system organ class and preferred term, by severity, and by relationship to study drug (drug-related TEAEs versus TEAEs not related to study drug). Drug-related TEAEs will be considered those to be at least possibly related to

Protocol Number: DISC-1459-201

study drug based on the Investigator's assessment. The number and percentage of participants reporting SAEs, and the number and percentage of participants reporting AEs leading to study drug discontinuation will also be summarized for each treatment group by MedDRA system organ class and preferred term.

Clinical laboratory parameters and vital signs will be measured at baseline and post-baseline visits. Each continuous variable will be summarized as changes from baseline by treatment group.

Laboratory and vital signs data will also be classified as low, normal, or high relative to the parameter's reference range. Laboratory and vital signs abnormalities for each treatment group will be summarized using shift tables.

2. LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation or Special Term	Definition or Explanation
AE	adverse event
AESI	adverse event of special interest
ALT	alanine transaminase
ANCOVA	analysis of covariance
ANOVA	analysis of variance
AST	aspartate aminotransferase
AUC	area under the curve
$AUC_{0-\tau}$	area under the curve over a dosing interval
BUN	blood urea nitrogen
CFR	Code of Federal Regulations
CHr	reticulocyte hemoglobin
CI	confidence interval
CIOMS	Council for International Organizations of Medical Sciences
CO_2	carbon dioxide
C_{max}	maximum concentration
CNS	central nervous system
CSR	clinical study report
CTCAE	Common Terminology Criteria for Adverse Events
CYP	Cytochrome P450
DLT	dose-limiting toxicity
EC_{50}	half-maximal effective concentration
CCI	
eCRF	electronic case report form
EOS	end of study
EPP	erythropoietic protoporphyria
FAS	full analysis set

Abbreviation or Special Term	**Definition or Explanation**
FDA	US Food and Drug Administration
FECH	ferrochelatase
GCP	good clinical practice
GGT	gamma-glutamyl transferase
GlyT1	glycine neurotransmitter transporter 1
GMP	good manufacturing practice
Hb	hemoglobin
HBC	hepatitis C virus
HBV	hepatitis B surface antigen
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human immunodeficiency virus
ICF	informed consent form
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
IDMC	Independent Data Monitoring Committee
IEC	Independent Ethics Committee
IRB	Institutional Review Board
ITT	intent-to-treat
LLN	lower limit of normal
MCH	mean corpuscular hemoglobin
MCHC	mean corpuscular hemoglobin concentration
MCV	mean corpuscular volume
MedDRA	Medical Dictionary for Regulatory Activities
MMRM	Mixed model repeated measures
MTD	maximum tolerated dose
NCI	National Cancer Institute
OCD	Obsessive Compulsive Disorder
PD	pharmacodynamic

Abbreviation or Special Term	**Definition or Explanation**
CCI	
PK	pharmacokinetics
PP	per protocol
PPIX	protoporphyrin IX
PRO	patient reported outcome
CCI	
QD	once daily
QOL	quality of life
RBC	red blood cell
SAE	serious adverse event
SAP	Statistical Analysis Plan
SAS	Statistical Analysis Software
SD	standard deviation
TEAE	treatment-emergent adverse event
T_{max}	time to maximum drug concentration
ULN	upper limit of normal
WHO-DD	World Health Organization Drug Dictionary
XLP	X-linked protoporphyria

3. INTRODUCTION: BACKGROUND AND SCIENTIFIC RATIONALE

3.1. Background

3.1.1. Bitopertin

Bitopertin selectively inhibits glycine neurotransmitter transporter 1 (GlyT1), a transporter that influences the intracellular and extracellular levels of glycine. Glycine plays a critical role in several biological functions with one of these functions being heme biosynthesis in the erythroblast. Inhibition of GlyT1 leads to reduced intracellular glycine levels affecting the biosynthesis of heme. Since GlyT1 is also expressed in other tissues, for example in the central nervous system (CNS), GlyT1 inhibition affects the extracellular (synaptic) levels of glycine that acts as a neurotransmitter.

The initial hypothesis for developing bitopertin for a CNS indication was that this mode of action might have a positive effect in patients with schizophrenia and obsessive-compulsive disorder (OCD). However, after extensive development the clinical programs in these indications were terminated due to lack of efficacy.

One of the consistent side effects observed in all clinical studies was the reversible, dose-dependent reduction in hemoglobin (Hb) levels in healthy volunteers as well as in psychiatric patients treated with bitopertin. The GlyT1 protein is also expressed by the erythroid lineage, in all precursors of red blood cells (RBCs), including reticulocytes. Glycine is an important amino acid for the first biochemical step in heme synthesis. Inhibiting glycine transport into developing RBCs, erythroid cells produce less heme, leading to Hb level reduction in healthy participants.

In a study of 69 healthy volunteers treated for 4 months with 0, 10, 30, or 60 mg bitopertin daily, the reduction in Hb was dose-dependent and mild, with maximal decreases of ~2 g/dL with chronic dosing at 60 mg once daily (QD). Decreases in reticulocyte mean corpuscular volume (MCV) and reticulocyte hemoglobin (CHr) at 2 weeks were predictive of whole blood Hb decreases that stabilized at approximately 4 months into chronic dosing. The effect of Hb reduction with bitopertin is anticipated to be attenuated in people with erythropoietic protoporphyria (EPP) because excess (protoporphyrin IX) PPIX substrate should always be available to ferrochelatase (FECH), even in the context of reduced flux in the heme synthesis pathway due to GlyT1 inhibition.

3.1.2. Erythropoietic Protoporphyria (EPP)

In most cases, EPP is caused by a mutation of FECH, which is the final enzyme in the heme biosynthesis cycle, adding Fe+2 with PPIX to make heme. Approximately 5-10% of cases are caused by a gain of function mutation in ALAS2, the first enzyme in the heme biosynthesis cycle. The ALAS2 mutation subtype of EPP is also known as X-linked protoporphyria (XLP). In patients with EPP, PPIX significantly accumulates and causes elevated PPIX concentration in RBCs, plasma, liver, and skin.(1) (2) Insufficient FECH enzyme activity results in increased accumulation of protoporphyrin that lacks Fe+2 or other metals, particularly zinc (i.e., metal-free

protoporphyrin). In EPP, the main source of production of increased red cell and plasma protoporphyrin is bone marrow reticulocytes, as red cells are major sources of heme production in the body. Conditions that increase erythropoiesis may result in increased formation of protoporphyrins by the bone marrow.(3) PPIX can be actively transported out of RBCs into plasma or enter plasma after RBC membrane damage. PPIX absorbs visible light and releases its energy to oxygen, which may create free radicals that result in skin damages when the patients with EPP are exposed to light.(2) This reactivity is the basis for the acute, painful, non-blistering photosensitivity and liver disease.(4) (5)

EPP typically presents in early childhood with immediate pain and crying upon exposure to bright sunlight. It is seasonal in nature with symptoms principally occurring in the spring and summer season. EPP is a lifelong disease, and repeated phototoxic reactions eventually lead to thickening of the skin and wax-like scarring on the face. In a small number of patients, the accumulation of PPIX in the liver leads to cirrhosis and liver failure. Onset in adulthood is rare, but an acquired form has been identified, in which clones of cells with mutated FECH expand in the setting of the myelodysplastic or myeloproliferative syndrome.(6)

There are several lines of evidence that link PPIX concentrations with the degree of phototoxicity in people with EPP. First, there is epidemiologic evidence that lower sunlight tolerance is associated with higher blood PPIX concentrations.(2) Second, women with EPP who become pregnant have a reduction in PPIX of 40-50% (for unknown reasons), and this reduction is associated with a remission of phototoxicity in many cases.(7) Third, in an interventional study of extracorporeal photoinactivation, a procedure that temporarily reduced blood PPIX by approximately 30%, light tolerance increased 14-fold.(6) Thus, a treatment that causes reduction in PPIX is anticipated to lead to increased sunlight tolerance.

3.2. Profile of Bitopertin and Previous Experience

3.2.1. Non-clinical Pharmacology

A functional assay using a recombinant human transporter demonstrated that bitopertin potently inhibited GlyT1b mediated glycine uptake with a half-maximal effective concentration (EC_{50}) of 0.025 µM and selectivity was greater than 1000-fold selectivity compared to human recombinant GlyT2 mediated glycine uptake. Ex vivo glycine uptake study has demonstrated that bitopertin can reduce glycine uptake into RBCs from rat, non-human primate, and humans in a dose-dependent manner.

In vitro studies using K562-EPP and human $CD34^+$-EPP cellular models demonstrated bitopertin can reduce the PPIX accumulation in erythroid cells in a dose-dependent manner and without affecting heme formation. The PPIX-lowering EC_{50} for bitopertin in K562-EPP cellular models ranges from 3 to 10 nM. The PPIX-lowering EC_{50} for bitopertin in $CD34^+$-EPP cellular models ranges from 23 to 33 nM.

The efficacy of bitopertin to treat EPP was further evaluated in female *Fech*^{m1Pas} EPP and male *Alas2*$^{Q548X/Y}$ XLP mouse models. At 8 weeks, PPIX levels had decreased in the

Fech^{m1Pas}/*Fech*^{m1Pas} and *Alas2*$^{Q548X/Y}$ animals receiving bitopertin with a mean reduction of 45% and 73%, respectively, compared to the control group. Importantly, no changes in hemoglobin levels were observed, indicating bitopertin can reduce PPIX accumulation without significant impact on erythropoiesis at the current dosing regimen.

3.2.2. Non-clinical Pharmacokinetics and Metabolism

In mice, rats, and cynomolgus monkeys, bitopertin showed 78% (rats) absolute oral bioavailability and a high plasma protein binding (free fraction 2-3%). The in vitro hepatic metabolism of bitopertin in hepatocytes and microsomal incubations was qualitatively similar across species (rat, cynomolgus monkey and human), with the two primary metabolites being formed via CYP3A4.

In rat, drug-related material was rapidly distributed into nearly all tissues, with the highest concentration found in the liver. Excretion of bitopertin and its metabolites in rat and cynomolgus was rapid and complete, and occurred mainly via bile into feces (~92% of dose). The parent molecule bitopertin accounted for less than 5% of the drug related material excreted into rat bile and was detected only in trace amounts in urine.

In human liver microsomes, bitopertin showed little inhibition of cytochrome P450s (CYPs) 1A2, 2C8, 2C9, 2C19, 2D6 or 3A4/5 activities at concentrations up to 9 μM. Drug-drug interactions due to inhibition of CYP-based metabolism of a co-medication are therefore not expected. Bitopertin showed no potential to induce the activity of CYP450 isoenzymes CYP3A4, CYP2C9 and CYP1A2 in human hepatocytes and no signs of reduced exposure after multiple dosing in rats and cynomolgus have been detected. Metabolism mediated by CYP3A4 is the major elimination pathway for bitopertin. Co-medications, which are CYP3A4 inhibitors or inducers, will have an influence on the elimination of bitopertin since CYP3A4 is the major metabolizing enzyme.

3.2.3. Non-clinical Toxicology

In repeat-dose studies, the main targets for bitopertin-related systemic toxicity were identified as the CNS and the RBC system. Incidence, severity and onset of the CNS-related effects were dose-dependent (hypersalivation; hypoactivity and tremor; posture anomalies; reduced body temperature) and they were generally transient overnight or reversible upon cessation of treatment. Effects on the RBC system (mainly increased reticulocyte and RBC counts and decreased hemoglobin concentration, mean corpuscular volume and mean corpuscular hemoglobin) were generally reversible and not accompanied by any relevant bone marrow toxicity. The hematological effects noted following 44 days (rat) and 79 days (monkey) of dosing did not show further significant worsening until the end of the entire 26-week dosing period (plateau). There were also no indications for hemolysis and no relevant changes in white blood cells (WBC) or lymphoid tissues. The side effects (on the CNS and the RBC system) identified in non-clinical safety studies are related to the pharmacological target (i.e., GLYT1).

In safety pharmacology studies, bitopertin did not induce pro-convulsant effects or catalepsy and no pro-arrhythmic potential was determined in vitro (hERG channel and isolated heart tests) or in

vivo (single-dose telemetry and repeat-dose monkey toxicity studies). The telemetry study indicated a mild hypertensive effect at high doses.

Following repeated dosing in toxicological studies, comparatively low heart rate was seen. In the 6-week toxicology study this was also coupled with long QTcF in male monkeys at dose levels also associated with behavioral changes. In single dose studies in rats, high doses decreased the respiratory rate associated with compensatory increases of expiration time and tidal volume, delayed gastric emptying and decreased intestinal motility.

No signs of a genotoxic potential were identified and bitopertin was not classified as an irritant or skin sensitizer.

Bitopertin had no effect on reproductive performance and fertility and showed no effects on sperm motility or head count in the rat and no effect on sexual organ weights and testicular size, and no histopathological findings in sexual organs in the rat and the monkey.

Potential effects of bitopertin on embryo-fetal development were investigated in the rat and rabbit. Bitopertin did not prove to be teratogenic (i.e., dysmorphogenic) in any species.

3.2.4. Clinical Experience with Bitopertin

Bitopertin has been investigated by F. Hoffmann La-Roche in 26 clinical pharmacology studies in healthy volunteers, in two Phase II studies in schizophrenia, and one long-term treatment study in Japanese patients and in five global Phase III studies in schizophrenia. One Phase 3 study, plus one long-term extension treatment study, were carried out in Japanese patients and one Phase II study in OCD.

The clinical safety of bitopertin has been assessed in adult healthy volunteers, patients with renal or hepatic impairment, and patients with psychiatric disorders. Cumulatively, 4334 participants have received bitopertin in clinical trials where the product was the investigational drug.

Single dose studies escalated to 240 mg bitopertin, and the maximum tolerated dose (MTD) was 240 mg. In multiple dose studies that escalated up to 180 mg, the MTD was considered to be 120 mg. The most common dose-dependent adverse event (AE) in healthy volunteers was dizziness. Headache and somnolence were the other identified AEs. In the Phase 2 programs in schizophrenia, obsessive-compulsive disorder and β-thalassemia, daily doses up to 90 mg bitopertin were well tolerated. In healthy volunteer studies, reversible blurred vision was observed in some participants, especially at doses of 60 mg and above. CCI

Hb levels gradually decreased from baseline in a dose-dependent manner in participants receiving bitopertin in line with the mechanism of action of the study drug. This decline was small in magnitude and had a gradual onset. Few participants (<1%) met the protocol withdrawal criteria for decreased hemoglobin and were discontinued from study drug. As observed in the cellular and mouse models of EPP discussed earlier, such an effect is not expected in participants with EPP; even though bitopertin is anticipated to reduce PPIX concentrations, there is still

expected to be a significant excess of PPIX available to serve as substrate to make adequate heme.

No effects on vital signs, visual function assessments including electroretinogram parameters and electrocardiogram parameters were seen.

In the drug-drug interaction study, 400 mg ketoconazole QD for 17 days increased the maximum concentration (C_{max}) of single doses of 10 mg bitopertin by approximately 20% (exposure ratio: 1.21, 90% confidence interval (CI) 1.12-1.32) from 77.0 ± 20.2 ng/mL to 94.7 ± 20.7 ng/mL and the area under the curve $(AUC)_{0-312}$ increased by a factor of approximately 4.2 (90% CI 3.5-5.0) from 2450 ± 1050 ng×h/mL to 9770 ± 1520 ng×h/mL. The half-life increased from approximately 2.5 days to approximately 20 days. Therefore, strong CYP3A4 inhibitors and inducers will be excluded from use in all clinical studies.

In a prior randomized, double blind safety and PK study in 67 healthy volunteers in which patients were randomized to 120 days of treatment with placebo or bitopertin (10, 30 or 60 mg daily) 3 participants in the 60 mg group discontinued within the first month of the study with a constellation of depression plus at least one of the following: dizziness, visual impairment, headache, malaise. Two participants also withdrew each from the placebo, 10 mg, and 30 mg arms.

3.3. Study Rationale

Bitopertin is a GlyT1 inhibitor, and erythroid precursors utilize GlyT1 as a glycine source for the first step in heme synthesis. By reducing the availability of glycine via inhibition of GlyT1, it is hypothesized that a decrease in the flux of heme synthesis intermediates will decrease the formation of PPIX. Because PPIX is the photoreactive compound that leads to phototoxicity, the reduction of PPIX is anticipated to translate into an improved sunlight tolerance.

3.4. Rationale for Dose Selection

The rationale for dose selection considers the extensive clinical safety profile demonstrated in the bitopertin development program, clinical pharmacokinetic (PK) information, PK/pharmacodynamic (PD) relationship, and indicators of bitopertin concentration predictive of PD effects in heme synthesis, which aim to at maximize the prospect of benefit in people with EPP.

Generally, bitopertin PK in patient populations were similar to healthy volunteers. Single oral doses up to 240 mg showed a slightly less than proportional increase in AUC and C_{max} with dose, whereas multiple doses up to 180 mg showed a linear and dose proportional increase in the respective parameters ($AUC_{0-t,ss}$ and $C_{max,ss}$ respectively). Overall, the half-life estimate of bitopertin is around 2 days, supporting once daily dosing regimen. There was relevant accumulation after daily repeat dosing. Area under the curve over a dosing interval ($AUC_{0-\tau}$) increased from Day 1 to Day 10 about 2.7 to 3.8-fold with a tendency for greater accumulation with higher doses. Attainment of steady state was estimated to be between 6 and 8 days. The PK

characteristics indicate that 20 mg and 60 mg doses of bitopertin will be in the linear PK range and result in meaningfully different exposures of approximately 3-fold.

The pharmacological effect of bitopertin was observed in an ex-vivo assay in circulating blood. The relationship between total bitopertin plasma concentrations and inhibition of the GlyT1 in RBCs showed that a near maximal effect would be achieved at a 60 mg dose exposure and 83% inhibition is achieved at a 20 mg dose exposure.

3.5. Benefit-Risk Assessment

3.5.1. Risk Monitoring and Mitigation

The monitoring requirements were designed to protect the safety of individual participants.

To evaluate and mitigate the potential risks of bitopertin, the following safety measures and monitoring requirements have been implemented (Table 1).

Table 1: Risk Mitigation and Monitoring

Organ	Potential Risk	Proposed Mitigation and Monitoring
Liver	Bitopertin did not cause liver toxicity in prior clinical studies, but EPP is associated with hepatic impairment and participants with EPP may be an at-risk population.	Serial monitoring of AST, ALT, bilirubin, albumin, and alkaline phosphatase. DLT for Grade 3 clinical lab elevations, considered by the Investigator to be treatment-related with at least possible imputability.
Blood	Bitopertin causes a mild anemia in people with normal heme synthesis pathway enzymes, although anemia is not expected based on cell and animal model data of EPP.	Serial monitoring of reticulocyte and complete blood counts. DLT for Hb drop of 2 g/dL from lowest pre-dose Hb value. Hold and/or reduce dose for developing anemia.
CCI	CCI	• CCI • CCI • CCI
CCI		

AST=aspartate aminotransferase; ALT=alanine aminotransferase; DLT=dose-limiting toxicity

4. STUDY OBJECTIVES AND ENDPOINTS

4.1. Objectives

4.1.1. Primary Objective

- To assess changes in PPIX concentration in response to bitopertin treatment

4.1.2. Secondary Objectives

- To characterize the effect of bitopertin on daily daylight tolerance
- To assess the safety and tolerability of bitopertin at two dose levels
- To characterize the relationship between bitopertin dose level and key biological indicators of mechanism engagement
- To evaluate bitopertin PK

4.1.3. Exploratory Objectives

- CCI

4.2. Endpoints

4.2.1. Primary Endpoint

- Percent change from baseline in whole blood metal-free PPIX levels

4.2.2. Key Secondary Endpoint

- Total hours of sunlight exposure to skin on days with no pain from 1000 to 1800 hours (10:00 AM to 6:00 PM)

4.2.3. Secondary Endpoints

- A two-week average daily sunlight exposure time (minutes) to first prodromal symptom (burning, tingling, itching, or stinging) associated with sunlight exposure between 1 hour post-sunrise and 1 hour pre-sunset.
- Pain intensity of phototoxic reactions according to a Likert scale (0-10)

- Safety and tolerability of bitopertin, as assessed by the incidence of treatment-emergent adverse events (TEAEs)
- Erythrocyte metal-free PPIX concentrations
- Plasma and whole blood total PPIX concentrations
- Plasma bitopertin concentrations

4.2.4. Pharmacokinetic Endpoints

- C_{max}
- Observed time of the maximum drug concentration (T_{max})
- AUC from time 0 to 24 hours post-dose on Day 1 (AUC_{0-24})

4.2.5. Exploratory Endpoints

- CCI

5. INVESTIGATIONAL PLAN

5.1. Study Overview and Plan

This is a Phase 2, multi-center, double-blind, placebo-controlled, parallel group study of bitopertin to evaluate the safety, tolerability, efficacy, and PPIX concentration change in participants with EPP.

Participants will be screened for study eligibility within CCI days before Baseline (Day 1). All participants will undergo light tolerance assessment during Screening that includes a diary assessment of historical light tolerance as well as maximal sunlight tolerance time until phototoxic prodrome during one day per week over a 2-week run-in period. Patient reported recall of maximal average sunlight tolerance time until phototoxic prodrome may be used in the event that sufficient sun exposure to elicit prodrome cannot be achieved. Participants will also wear a light dosimeter during this Screening light tolerance assessment.

Up to 75 participants aged 18 and older are planned to be enrolled. Participants who are determined to be eligible based on Screening assessments will be randomized on Day 1 in a 1:1:1 equal allocation ratio amongst the following three treatment groups:

Placebo (N=25) administered as CCI

Bitopertin 20 mg (N=25) administered as CCI

Bitopertin 60 mg (N=25) administered as CCI .

Randomization will be stratified by sunlight exposure time to prodromal symptom (<30 minutes or ≥30 minutes), as assessed during a 2-week screening period.

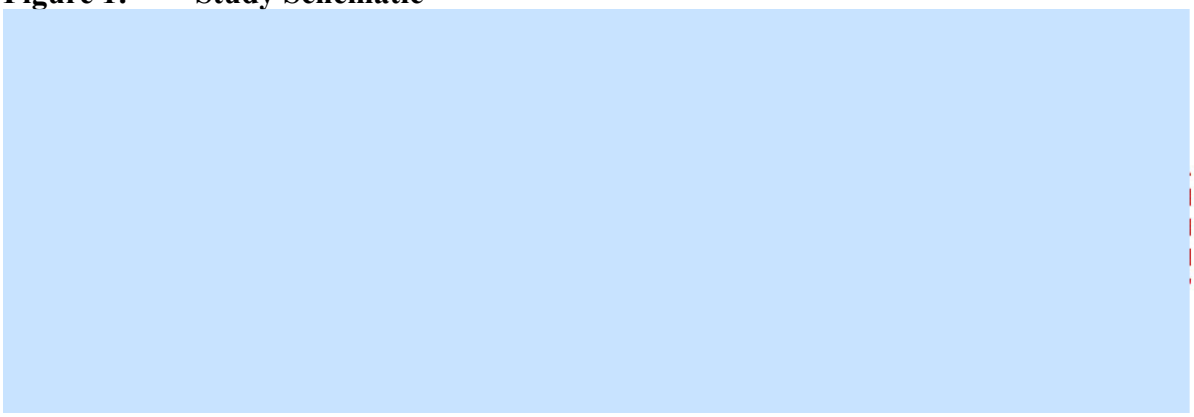
On Day 1, participants will begin study drug according to their random assignment and will be evaluated during a 120-day treatment period thereafter. Participants will be CCI .

An Independent Data Monitoring Committee (IDMC) will aid in the monitoring of unblinded, accumulating study data to ensure the ongoing safety of trial participants. The IDMC will have the right to recommend stopping the study at any time due to the concerns for the safety of the participants.

After completion of the 120-day treatment period, including assessment of light tolerance, and End-of-Study (EOS) visit, participants may CCI . Study visits will be scheduled every CCI weeks.

Figure 1 depicts the study schema.

Figure 1: Study Schematic



5.2. Independent Data Monitoring Committee

The IDMC will aid in the monitoring of unblinded, accumulating study data to ensure the ongoing safety of trial participants. The IDMC membership will be composed of external medical experts with relevant clinical experience. The IDMC will have the right to recommend stopping the study or discontinuing a treatment arm at any time due to the concerns for the safety of the participants.

The IDMC will evaluate for study stopping in any instance when 2 participants need study treatment interruption due to CTCAE Grade 3 or above psychiatric, neurological or eye AEs.

5.3. Overall Participant Study Duration and Follow-Up

The overall study duration for participants is up to 7 months as described below:

Screening: up to CCI days

Treatment Period: CCI days

- The EOS visit (CCI) occurs the day after the final double-blind dose (CCI).

Additional, open-label treatment may occur on study after the EOS visit, for up to CCI .

6. SELECTION OF PARTICIPANTS

6.1. Inclusion Criteria

Participants are eligible for the study if all of the following criteria apply:

1. Aged 18 years or older at the time of signing the informed consent form (ICF).
2. Diagnosis of EPP, based on medical history by FECH genotyping or by biochemical porphyrin analysis.
3. Body weight ≥50 kg.
4. Washout of at least 2 months prior to Screening of afamelanotide and dersimelagon, if applicable.
5. Aspartate aminotransferase (AST) and alanine transaminase (ALT) <2× upper limit of normal (ULN) and total bilirubin <ULN (unless documented Gilbert syndrome) at Screening. Albumin >lower limit of normal (LLN).
6. If male with female sexual partner(s) of childbearing potential, agrees he and partner will use one of the following acceptable methods of birth control during the study and for 30 days after the last study drug dose:
    - abstinence
    - stable hormonal contraceptive
    - barrier method (e.g., condom [male or female] or diaphragm)
    - intrauterine device, in place for at least 3 months
    - surgically sterile by hysterectomy, bilateral oophorectomy, or bilateral tubal ligation
7. If female of childbearing potential, defined as prior menarche, no hysterectomy, no bilateral oophorectomy, not postmenopausal (at least 12 months natural, spontaneous amenorrhea), must commit to one of the following methods of acceptable birth control during the study and for 30 days after the last study drug dose:
    - abstinence
    - stable hormonal contraceptive in conjunction with a barrier method (e.g., condom [male or female] or diaphragm)
    - intrauterine device, in place for at least 3 months
8. Negative pregnancy test (females of childbearing potential) at Screening (Days CCI to C CI) AND Baseline (Day 1), prior to dosing.
9. Able to understand the study aims, procedures, and requirements, and provide written informed consent.
10. Able to comply with all study procedures.

6.2. Exclusion Criteria

Participants are excluded from the study if any of the following criteria apply:

Medical History:

1. Major surgery within 8 weeks before Screening or incomplete recovery from any previous surgery.
2. Other than EPP, an inherited or acquired red cell disease associated with anemia.
3. A history or known allergic reaction to any investigational product excipients or history of anaphylaxis to any food or drug.
4. History of liver transplantation.
5. History of alcohol dependence or excessive alcohol consumption, as assessed by the Investigator.
6. Human immunodeficiency virus (HIV), active Hepatitis B, or C. A positive hepatitis result, should be discussed between the Investigator and Sponsor prior to enrollment.
7. CCI
8. Other medical or psychiatric condition or laboratory finding not specifically noted above that, in the judgment of the Investigator or Sponsor, would put the participant at unacceptable risk or otherwise preclude the participant from participating in the study
9. Condition or concomitant medication that would confound the ability to interpret clinical, clinical laboratory, or participant diary data, including a major psychiatric condition that has had an exacerbation or required hospitalization in the last 6 months.

Treatment History:

10. Concurrent or planned treatment with afamelanotide or dersimelagon during the study period.
11. Treatment with opioids for any period >7 days in the 2 months prior to screening or anticipated to require opioid use for >7 days at any point during the study.
12. New treatment for anemia, including initiation of iron supplementation, in the 2 months prior to Screening.
13. Current or planned use of any drugs or herbal remedies known to be strong inhibitors or inducers of CYP3A4 enzymes for C CI days prior to the first dose and throughout the study (see Appendix 1).
14. Current or planned treatment with anti-psychotic medication.

Laboratory Exclusions:

15. CCI .

Miscellaneous:

16. If female, pregnant or breastfeeding.

17. Participation in any other clinical protocol or investigational trial that involves administration of experimental therapy and/or therapeutic devices within 30 days of Screening.
18. Grapefruit/Seville orange and products containing them for 14 days prior to first dose and throughout the study.

6.3. Handling of Screen Failures

A screen failure is defined as a candidate who consents to study participation but does not get randomized to study drug. Candidates determined to be screen failures must be captured in the clinical database by completion of the appropriate electronic case report form (eCRF) pages. Please refer to the eCRF guidelines for additional instructions.

The following information should be collected in the candidate's source documents and eCRF page(s): the date the ICF was signed, demographics, the reason(s) for ineligibility, and the Investigator's signature for the eCRF pages. Serious adverse events (SAEs) experienced by screen failure candidates will be collected from the date of signing consent to the day the candidate is confirmed as a screen failure (see Section 10.18.5 for information on SAE reporting). Relevant information will also be recorded on the Screening Log.

Screen failures will be allowed to rescreen once, ≥[C] weeks after the initial screen failure. Partial re-screening for Hb, AST, ALT, and bilirubin entry criteria will be allowed once within the screening period.

7. TREATMENT OF PARTICIPANTS

7.1. Enrollment

7.1.1. Screening and Enrollment

75 adult (≥18 years) participants will be enrolled. All participants will be screened for study eligibility within CCI days of randomization (Day 1).

7.1.2. Randomization

Adult participants will be randomized on Day 1 in a 1:1:1 allocation ratio to one of the following three treatment groups:

- Placebo (N=25) administered as CCI
- Bitopertin 20 mg once daily (N=25) administered as CCI
- Bitopertin 60 mg once daily (N=25) administered as CCI

Randomization will be stratified by average sunlight exposure time to prodromal symptom (<30 minutes or ≥30 minutes), as assessed during a 2-week run-in period.

7.2. Blinding

Study drug and dose group allocation will be blinded to all participants and study personnel.

7.3. Study Drug Administration

Study drug dosing will begin on Day 1 and continue over a CCI -day treatment period. Tablets will be taken orally, once daily, in the morning on an empty stomach, at least 30 minutes prior to food. If a participant inadvertently eats before taking study drug, it is acceptable to take study drug 2 hours after the food ingestion.

7.4. Modification of Dose and/or Treatment Schedule

If a participant becomes intolerant of study drug, as assessed by the Investigator, study drug may be withheld and subsequently restarted following consultation between the Investigator and Sponsor. CCI

f symptoms resolve, treatment may be re-introduced CCI). Then, if after 2 weeks symptoms have not recurred, the full dose will be resumed. If the symptoms recur and are not tolerated, then subject may have the dose reduced again or be withdrawn from the study at the discretion of the investigator.

CCI

CCI Then, if after 2 weeks symptoms have not recurred, the full dose will be resumed. If the symptoms recur to any degree severity after reintroduction of drug, then subject may have the dose reduced again or be withdrawn from the study at the discretion of the investigator.

7.5. Overdose

An overdose is defined as any dose of study drug greater than the intended dose.

A known or suspected overdose must be reported to the study team immediately, and medical monitoring is recommended. There is no known antidote for bitopertin overdose. Based on the available clinical and non-clinical data for bitopertin, all reported TEAEs have been reversible.

7.6. Discontinuation of Study Drug

Study drug administration must be discontinued if any of the following events occur:

- Hypersensitivity or suspected allergic reaction to the study drug.
- Withdrawal of consent.
- At the discretion of the Investigator or Sponsor for situations including, but not limited to, a clinically significant event, non-compliance with treatments or procedures to the extent that study integrity is compromised, or prohibited medication exposure.
- A medical emergency that requires permanent discontinuation of study drug.
- CCI
- Participant becomes pregnant.
- CCI .
- Return of signs or symptoms of an AE after reintroduction of study drug, as described in Section 7.4

Participants should remain in the study and follow the schedule of assessments, even if they meet study drug discontinuation criteria.

The reason for discontinuation of study drug must be recorded in the participant's eCRF.

Participants who prematurely discontinue study drug should be encouraged to continue in the study and continue protocol-required clinic visits, tests, and assessments.

7.7. Withdrawal of Participants from Study

Participants must be withdrawn from the study for any of the following reasons:

- As determined by the Investigator in conjunction with the Sponsor, participant does not sufficiently adhere to study drug or protocol requirements.
- Participant withdraws consent.
- Participant is unwilling or unable to comply with the protocol to an extent that undermines the integrity of the study.
- At the discretion of the Investigator or Sponsor for medical reasons.
- Participant is lost to follow-up.
- Return of signs or symptoms of an AE after reintroduction of study drug, as described in Section 7.4

The reason for study withdrawal is to be documented in the participant's eCRF.

Participants who prematurely withdraw from the study during the treatment period should be encouraged to complete the study assessments for the EOS visit at the time of withdrawal.

Participants who prematurely withdraw from the follow-up period should be encouraged to complete the appropriate follow-up assessments at the time of withdrawal.

A participant will be declared lost to follow-up if reasonable attempts by the site have been made to contact the participant at least three times using any of the following methods: telephone calls, e-mails, text messages. At least one time should be an attempt to contact by telephone.

7.8. Study Stopping Rules

Enrollment or treatment in this study may be paused at any time for safety reasons. This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause.

CCI

Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to the Investigator and regulatory authorities.

If the study is prematurely terminated or suspended, the Investigator will promptly inform the institutional review board (IRB)/independent ethics committee (IEC) and will provide the reason(s) for the termination or suspension.

Circumstances that may warrant study termination or suspension include, but are not limited to:

- Determination of unexpected, clinically significant, or unacceptable risk to participants
- Sponsor decision

7.9. Replacement of Participants

Participants who withdraw or are withdrawn will not be replaced.

7.10. CCI

After completion of the 120-day treatment period, including assessment of light tolerance, and EOS visit CCI

7.11. End of Study

The end of the study is defined as completion of the last participant's last procedure shown in the randomized, double-blind, placebo-controlled phase of the study as detailed in the Schedule of Assessments (Appendix 2). CCI

8. CONCOMITANT THERAPY AND PROCEDURES

8.1. Concomitant Therapy

Any medication or vaccine (including over-the-counter or prescription medicines, vitamins, and/or herbal supplements) that the participant reports from C Cl days before signing the ICF up to the end-of-study visit must be recorded on the eCRF along with:

Reason for use

Dates of administration including start and end dates

Dosage information including dose and frequency

Prior medications are those received up to C Cl days before signing of the ICF to the first dose of study drug and concomitant medications are those received after the first dose.

If the use of any concomitant treatment becomes necessary (e.g., for treatment of an AE), the treatment and administration details must be recorded in the source documents and the eCRF. The Medical Monitor must be notified of all prohibited medications administered to any participant, in order to assess the participant's eligibility to continue in the study.

8.2. Allowed Concomitant Therapy

Participants are allowed to continue taking any stable dose of prescription or over-the-counter medications during the study other than those listed in Section 8.3 at the discretion of the Investigator.

8.3. Disallowed Concomitant Therapy

Prohibited medications during the study are listed in the exclusion criteria for enrollment (Section 6.2). The following concomitant medications are specifically excluded during the course of the study:

Any known strong inhibitor of CYP3A4 (see Appendix 1) taken within C Cl days prior to the first dose of study drug (Day 1)

Any known strong inducer of CYP3A4 (see Appendix 1) taken within C Cl days prior to the first dose of study drug (Day 1)

Products containing grapefruit or Seville orange, or grapefruit/Seville orange juice are not to be consumed from 14 days prior to the first dose of study drug and throughout the study

Afamelanotide

Dersimelagon

New or discontinued iron replacement therapy (oral or intravenous). Stable or chronic replacement acceptable.

Chronic opioids (>7 days)

Another investigational drug or device

8.4. Concomitant Procedures

Any procedure, including transfusion, that the participant reports from C Cl days before signing the ICF up to the end-of-study visit must be recorded on the eCRF along with:

- Reason for procedure(s)
- Dates of procedure(s)

9. DESCRIPTION OF STUDY DRUG

Bitopertin (chemical name: (S)-[4-(3-Fluoro-5-trifluoromethyl-pyridin-2-yl)-piperazin-1-yl]-[5-methanesulfonyl-2-(2,2,2-trifluoro-1-methyl-ethoxy)-phenyl]-methanone) is presented in CCI Further details are presented in the Investigator's Brochure.

9.1. Study Drug Preparation and Dispensing

Film-coated tablets at the dosage strength of C CI mg and C CI mg of bitopertin will be used. No special drug preparation is required.

Dispensing of bitopertin will be carefully recorded on appropriate study drug accountability forms and will be verified by the study monitor at each monitoring visit.

More detailed information is located in the Pharmacy Manual for the study.

9.2. Study Drug Storage

The Sponsor reserves the right to inspect the study drug storage area before and during the study. A written record will be made of the storage condition of the study materials and retained according to institutional operating procedures

Bitopertin will be stored by the site study personnel or pharmacist according to the Pharmacy Manual. Bitopertin should be stored in a locked area with access limited to designated personnel.

The recommended storage condition for bitopertin drug product is at 15-30˚C. Temperature monitoring is required at the storage location to ensure that the study drug is maintained within an established temperature range. The Investigator or site designee is responsible for ensuring that the temperature is monitored throughout the duration of the study and that records are maintained. Please refer to the study Pharmacy Manual for additional storage instructions.

9.3. Study Drug Labeling

Bitopertin will be labeled in accordance with text that is in full regulatory compliance. Study drug labels will not bear any statement that is false or misleading in any manner or represents that the study drug is safe or effective for the purposes for which it is being investigated.

The Pharmacy Manual for the study will have more detailed information.

9.4. Study Drug Handling and Disposal

Bitopertin accountability and traceability are ultimately the responsibility of the Investigator and Sponsor. However, this responsibility may be delegated to suitably qualified personnel who have had appropriate study-specific training and whose names have been appropriately listed on the Delegation of Responsibility Log for this task.

Following completion and verification of accountability logs, all unused and used study drug must be destroyed. Study drug may be destroyed on site according to good clinical practice (GCP) and site practice. Alternatively, the Sponsor may arrange for destruction with a third-party vendor operating in accordance with GCP and/or good manufacturing practice (GMP), as applicable. See the Study Procedures Manual for complete instructions on how to dispose of study drugs.

Detailed records will be maintained to allow for accurate accountability of bitopertin as per applicable Sponsor and clinical site procedures. All material containing bitopertin will be treated and disposed of as hazardous waste in accordance with governing regulations and clinical site procedures.

9.5. Study Drug Accountability, Control, and Return

The Investigator is responsible for bitopertin accountability, reconciliation, and record maintenance at the investigational site. In accordance with all applicable regulatory requirements, the Investigator or designated site staff must maintain accountability records throughout the course of the study. The amount of bitopertin received from the Sponsor, and the amount supplied and/or administered to participants will be documented.

The accountability logs should include dates, quantities, batch numbers, expiration dates (if applicable), and any unique pack numbers assigned to the study drug and/or participant. The accountability logs will also include general details related to the study including the protocol/amendment number, Sponsor, indication, and the Investigator.

The Investigator must maintain accurate records demonstrating dates and amount of study drug received and dispensed, and accounts of any study drug accidentally or deliberately destroyed. It will be the responsibility of the Sponsor to ensure that adequate samples of all study drug doses are retained in accordance with the relevant regulatory guidelines.

The study monitor will review bitopertin accountability logs and check returns (unused and used) prior to authorizing the destruction of used study drug by the study site.

Further details for study drug dispensing and accountability are included in the Pharmacy Manual.

9.6. Control, Return, and Retention of Non-study-drug Supplies

No non-study-drug supplies will be provided by the Sponsor.

10. STUDY ASSESSMENTS AND SCHEDULES

Refer to the Schedule of Assessments in Appendix 2 for the timing of all study assessments. All assessments on Day 1 and subsequent study visits will precede dosing. CCI visits are recommended to be performed at the study site. CCI visits, as noted on the Schedule of Assessments, may be conducted remotely with laboratory assessments obtained by a home-health study team member.

In the event of multiple procedures scheduled at the same time, the order, where possible, will be: PROs, vital signs, PK sampling, porphyrins, and then clinical laboratory sampling. Urinalysis may occur whenever is most convenient for the participant.

On Day 1, participants will receive instructions for study drug storage and administration and completion of paper and/or electronic PROs and other assessments/diaries. Repeat instruction will be provided as necessary throughout study.

No study assessments or procedures will occur until after the ICF is signed.

10.1. Demographics

Date of birth, sex, ethnicity, and race will be collected during Screening.

10.2. Medical and Medication History, Concomitant Medications

Complete medical history collected at Screening will include evaluation for past or present cardiovascular, respiratory, GI, renal, hepatic, neurological, endocrine, metabolic, lymphatic, hematologic, immunologic, dermatologic, psychiatric, and genitourinary disorders, medication and surgical history, and review of any other diseases or disorders.

A history of EPP will be recorded during Screening, including therapies and treatments previously received. If the participant's EPP genotype is not documented in the medical history, genotyping will occur during Screening, but medical history of EPP is sufficient for eligibility.

A review of prior and concomitant medications will be completed. Prior medications are those received from CCI days before informed consent up to first dose of study drug. Concomitant medications are those being taken at the time of informed consent and continuing through first dose of study drug and beyond, and those begun during the study (beginning Day 1 or later).

10.3. Physical Examination

At Screening, a full physical examination will be performed and will include assessment of the following: general appearance, head, ears, eyes, nose, throat, neck (including thyroid), skin, cardiovascular system, respiratory system, GI system, musculoskeletal system, lymph nodes, and nervous system. Any findings made during the physical examination must be noted even if they are part of the participant's medical history.

A complete physical examination will be repeated at the Day CCI (EOS) visit.

10.4. Targeted Neurological Examination

At CCI, a targeted neurologic exam that includes mental status, optical and eye movement cranial nerves (CNII, CNIII, CNIV, CNVI), motor function, gait, stance, and coordination will be performed. Any findings made during the physical examination must be noted even if they are part of the participant's medical history.

10.5. Body Weight and Height

Body weight (wearing light clothes, no shoes) and height will be measured at Screening. Body weight only will be assessed at EOS.

10.6. Vital Signs

Vital signs measurements will include resting systolic and diastolic blood pressure, body temperature (oral), respiratory rate, and pulse. Blood pressure must be taken before study drug administration and after the participant has rested comfortably in a supine position for at least 5 minutes. Vital signs will be recorded using consistent methods between participants. Vital signs will be collected at Screening, and at each visit. Abnormal vital signs may be repeated twice at Screening and once at subsequent visits.

10.7. Clinical Laboratory Tests

Participants will fast overnight (minimum 6 hours) prior to blood draws. Water is allowed. Blood draws should occur pre-dose and at the same time of day at each scheduled assessment (except for post-dose PK sampling). Scheduled assessment times for blood chemistry and hematology will be at all study visits except for Day 2. For urinalysis, collection will occur at Baseline (Day 1) and on Days CCI (EOS), **prior** to dosing.

The blood samples will be assessed for:

- Blood Chemistry: blood glucose, sodium, potassium, blood urea nitrogen (BUN), creatinine, AST, ALT, alkaline phosphatase, total bilirubin, direct bilirubin, gamma-glutamyl transferase (GGT), albumin, calcium, phosphorus, carbon dioxide (CO_2), and chloride
- Hematology: hemoglobin, hematocrit, RBC count, red cell distribution width, WBC count with differential, platelet count, MCV, MCH, and MCHC
- Special Hematology: reticulocyte (absolute), reticulocyte %, and CHr
- Urinalysis: color, pH, specific gravity, protein, blood, glucose, nitrites, and ketones. Urine sediment microscopy will be conducted in the instance of abnormal findings for blood.

A list of all laboratory parameters is provided in Appendix 3.

In the event of an unexplained, clinically significant abnormal laboratory test result, the test should be repeated once and followed until it has returned to the normal range and/or an adequate explanation of the abnormality is found.

During the study, all out of range (abnormal) laboratory values must be evaluated and commented on by the Investigator for clinical significance.

Clinical laboratory samples will be analyzed at a central laboratory. Please refer to the Laboratory Manual for sample collection and processing procedures.

10.8. Pregnancy Testing

Urine pregnancy test will be given to participants of females of childbearing potential at Screening and Baseline. If a urine test is positive, serum pregnancy test is required.

10.9. Serology/Virology

Testing for hepatitis B surface antigen (HBV), hepatitis C virus (HBC), and HIV will occur at Screening. Results must be available before the first dose. A positive result, indicating active disease status, should be discussed between the Investigator and Sponsor prior to enrollment.

10.10. Biomarker Assessments

10.10.1. Porphyrins

Porphyrins (metal-free whole blood PPIX, metal-free erythrocyte PPIX, whole blood total PPIX, plasma total PPIX) will be measured at Screening and all visits except Day 2.

10.10.2. Iron Studies

Blood for iron studies will be collected in the **fasted state (≥ 6 hours)**, between 6:00 AM and noon. Collection will occur at Baseline (Day 1) and on CCI (EOS).

The iron parameters to be assessed are: TIBC, serum iron, TSAT, and ferritin.

10.11. Pharmacokinetic Assessments

Blood sampling for PK analyses will occur on Day CCI . The Day CCI dose will be administered by the study site 24 hours (+/-1 hour) after the previous day's dose, and the subject's study drug supply may be used.

10.12. CCI

CCI

10.13. Sun Exposure Challenge Question (Time to Prodrome)

Participants will be required to expose their skin to sunlight once a week and measure the time it takes to experience a prodrome. The time (minutes) to first prodromal symptom (e.g., burning, tingling, itching, or stinging) associated with sunlight exposure will be recorded in a diary (Appendix 4, Appendix 5).

10.14. Patient Reported Outcomes

10.14.1. CCI

CCI

10.14.2. CCI

CCI

10.14.3. CCI

CCI

10.14.4. CCI

CCI

10.14.5. CCI

CCI

10.14.6. Exit Interview

Subjects who complete the double-blind treatment period will be invited to participate in an optional exit interview. The exit interview will be conducted after the last dose of the double-blind treatment period and within three days of the last dose. Information gathered from these interviews will support the interpretation of results of the patient reported outcome measures and diary.

10.15. Unscheduled Visits

If a study assessment (e.g., laboratory tests or vital signs) must be repeated, the results of the repeat assessment should be entered as an additional unscheduled visit in the eCRF. Complete instructions are found in the eCRF Completion Guidelines.

10.16. Open-Label Visits

Participants who continue on CCI will have study visits scheduled every C C weeks.

Any adjustment to the C CI mg dose must be pre-determined in consultation with the Sponsor.

10.17. Pregnancy Testing and Reporting and Contraception Recommendations

10.17.1. Pregnancy Testing Guidelines

For each female participant, the Investigator will document non-childbearing status or potential childbearing status during the screening period and record it in the eCRF.

Female participants of childbearing potential will have a urine pregnancy test at CCI If a urine pregnancy test is positive, it will be confirmed by a serum pregnancy test.

10.17.2. Contraception Recommendations for Female Participants of Childbearing Potential

Female participants of childbearing potential (prior menarche, no hysterectomy, no bilateral oophorectomy, not postmenopausal [at least 12 months natural, spontaneous amenorrhea]) who are sexually active with males must agree to use highly effective contraception (listed below) on Day 1 (or earlier) through at least 30 days after the last dose of study drug:

- Abstinence
- Stable hormonal contraceptive in conjunction with a barrier method (e.g., condom [male or female] or diaphragm)
- Intrauterine device, in place for at least 3 months

10.17.3. Contraceptive Recommendations for Male Participants

Male participants who are sexually active with females of childbearing potential (prior menarche, no hysterectomy, no bilateral oophorectomy, not postmenopausal [at least 12 months

natural, spontaneous amenorrhea]) must agree from Day 1 through at least 30 days after the last dose of study drug to have the female partner(s) use one of these contraceptive measures:

- Abstinence
- Stable (at least 3 months) hormonal contraceptive
- Barrier method (e.g., condom [male or female] or diaphragm)
- Intrauterine device, in place for at least 3 months
- Surgically sterile by hysterectomy, bilateral oophorectomy, or bilateral tubal ligation

10.17.4. Pregnancy Reporting

Pregnancy in a participant or the female partner of a male participant occurring up to 60 days after the participant's last dose of study drug must be reported to the Sponsor contract research organization pharmacovigilance group within 24 hours of the Investigator's knowledge of the pregnancy using a Pregnancy Report Form.

Pregnancy per se is not considered an AE unless there is a cause to believe that the study interventions may have interfered with the effectiveness of a contraceptive medication or if the outcome of the pregnancy meets SAE criteria (miscarriage/fetal death or congenital anomaly/birth defect), in which case it should be reported in the same manner and timelines as an SAE (Table 2). In addition, any infant death or congenital anomaly occurring 30 days or more after birth that the Investigator suspects is related to the in-utero exposure to the study interventions should also be reported as an SAE. Hospitalization for normal delivery of a healthy newborn is not an SAE.

If a female partner of a male participant becomes pregnant, the Investigator should request consent from the partner to collect relevant safety information. If a pregnancy occurs in a female participant, study interventions must be discontinued immediately. The pregnant woman should be referred to an obstetrician/gynecologist experienced in reproductive toxicity for further evaluation and counseling. The Investigator must notify the Sponsor contract research organization pharmacovigilance group of the outcome within 24 hours of the Investigator's knowledge of the pregnancy outcome using a Pregnancy Report Form.

At Screening, all participants (male and female) must agree to use a highly effective method of contraception (Section 10.17.2 and Section 10.17.3) during the duration of the study and for 30 days after the last dose of study drug.

10.18. Adverse Events

10.18.1. Definition of Adverse Events

An AE is defined as any untoward medical occurrence in a clinical study participant administered a medicinal product, which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including a clinically significant abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not it is related to the medicinal (investigational) product.

Untoward events that are part of the EPP/XLP pathophysiology and captured as study endpoints are not to be recorded as adverse events:

- Phototoxic reactions
- Pain and skin changes associated with phototoxic reactions
- PPIX concentrations

An AE includes but is not limited to any clinically significant worsening of a participant's preexisting condition. An abnormal laboratory finding that requires an action or intervention by the Investigator, should be reported as an AE.

AEs may be treatment emergent (i.e., occurring after initial receipt of study drug) or nontreatment emergent. A nontreatment-emergent AE is any new sign or symptom, disease, or other untoward medical event that begins after written informed consent has been obtained but before the participant has received study drug.

The term AE is used to include both serious and non-serious AEs.

It is the responsibility of the Investigator to document all AEs that occur during the study. AEs should be reported on the appropriate page of the eCRF.

10.18.2. Definition of an Adverse Event of Special Interest

AESIs based on clinical AEs observed during development include:

- CCI

10.18.3. Definition of an Unexpected Adverse Event

An unexpected AE is any event for which the nature or severity is not consistent with the information in the current Investigator's Brochure.

10.18.4. Definition of Serious Adverse Events

An SAE is any untoward medical occurrence at any dose (including those occurring after the ICF is signed and before dosing) that:

- Results in death
- Is life-threatening (subject is at immediate risk of death from the event as it occurred)
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability or incapacity
- Is a congenital anomaly/birth defect

Important medical events that may not result in death, are not immediately life-threatening, or do not require hospitalization may be considered SAEs when, based on appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

For SAE reporting purposes, hospitalization is defined as inpatient hospital stay. Hospitalizations for elective surgery or other medical procedures that are not related to a TEAE are not considered SAEs.

Death should not be reported as an SAE. The primary reason for a subject's death should be reported as the SAE, with death reported as the outcome.

10.18.5. Reporting of Adverse Events, Adverse Events of Special Interest, and Serious Adverse Events

In the event of a Grade 3 eye/visual AE, participants will be referred immediately to an ophthalmologist for a complete evaluation which includes visual acuity, color vision test, Humphrey visual field test, slit lamp biomicroscopy of the anterior and posterior segment to include evaluation of the macula, optic nerve , retinal vessels and peripheral retina, and intraocular pressure. For Grade 1 and Grade 2 eye/visual AEs that have symptoms persist for 1 week, regardless of dose reduction or discontinuation, the participant will be referred to an ophthalmologist for an evaluation that includes a slit lamp examination. All ophthalmologic evaluation results will be recorded in AE follow up and made available to the investigator. The evaluation by an ophthalmologist for eye/visual AEs also applies to all phases of the study. Additionally, all participants with treatment-related visual AEs must be followed until resolution.

The AE reporting period begins from the time the subject signs an ICF through End of Study visit. During the pre-screening period, only AEs resulting from study procedures will be reported. All participants with treatment-related AEs/AESIs/SAEs should be observed until resolution or stabilization of the event. Any SAE/AESI occurring after the reporting period must be promptly reported if a causal relationship to the study intervention(s) is suspected.

Elective or previously scheduled hospitalizations for pre-existing conditions that have not worsened after initiation of treatment should not be classified as SAEs. For example, an admission for a previously scheduled ventral hernia repair would not be classified as an SAE.

All AEs should be recorded individually unless, in the opinion of the Investigator or designated physician, the AEs constitute components of a recognized condition, disease, or syndrome. In the latter case, the condition, disease, or syndrome should be reported rather than each individual sign or symptom. If a constellation of signs and/or symptoms cannot be medically characterized as a single diagnosis or syndrome at the time of reporting, each individual event should be recorded as an AE, AESI, or SAE, as appropriate, on the relevant form(s) (SAE/AESI Report Form and/or AE eCRF). If a diagnosis is subsequently established, it should be reported as follow-up information becomes available. If a diagnosis is determined after the reporting of the constellation of symptoms, the signs/symptoms should be updated to reflect the diagnosis.

Each AE will be evaluated for duration, severity, seriousness, and causal relationship to each study intervention. The action taken with each study intervention and the outcome must also be recorded.

All SAEs/AESIs, regardless of relationship to each study intervention, must be reported immediately (within 24 hours of awareness of event by Investigator) to the Sponsor contract research organization pharmacovigilance group. Initial SAE/AESI notification should be made according to the information provided in Table 2.

Table 2: SAE Reporting Information

SAEs should be reported in the eCRF	Instructions found in the CRF Completion Guidelines document
If the eCRF is not accessible	A manual report is to be completed and sent to NorthAmerica_Medical@parexel.com or faxed to 1-781-434-5957
If the eCRF, email, and fax are not available	Contact the Parexel North America Safety Line via telephone at 1-781-434-5010

An initial SAE/AESI Report may be sent without the Investigator's signature but must be followed by a report signed by the Investigator within 48 hours of becoming aware of the event.

Follow-up SAE/AESI reports must be submitted by the Investigator as new information becomes available within the same reporting timelines as initial reports.

The Medical Monitors for this study may be contacted for advice or assistance. Contact details will be provided separately in a study contact list.

10.18.6. Severity of Adverse Events

The severity of the AE will be graded by the Investigator according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE), Version 5.0 (see web page https://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_50 for

details). Only AEs not listed in the CTCAE should be graded with the 'Equivalent To' text in Table 3.

Table 3: CTCAE (Version 5.0) AE Grading

CTCAE Grade	Equivalent To:	Definition
Grade 1	Mild	Discomfort noticed but no disruption of normal daily activity
Grade 2	Moderate	Discomfort sufficient to reduce or affect daily activity; no treatment or medical intervention is indicated, although this could improve the overall well-being or symptoms of the participant.
Grade 3	Severe	Inability to work or perform normal daily activity; treatment or medical intervention is indicated in order to improve the overall well-being or symptoms; delaying the onset of treatment is not putting the survival of the participant at direct risk
Grade 4	Life-threatening/ disabling	An immediate threat to life or leading to a permanent mental or physical condition that prevents work or performing normal daily activities; treatment or medical intervention is required in order to maintain survival
Grade 5	Death	AE resulting in death

Abbreviations: AE: adverse event, CTCAE: Common Terminology Criteria for Adverse Events.

10.18.7. Relationship of Adverse Events to Study Drug

The Investigator will make a judgment regarding whether the AE was related to study drug, as outlined below:

Definitely related: This category applies when, after careful medical consideration, there is almost no consideration of other causation.

Probably related: There is a clinically plausible time sequence between onset of the AE and study drug administration. The AE is unlikely to be caused by a concurrent or underlying illness, other drugs, or procedures. If applicable, the AE follows a clinically consistent resolution pattern upon withdrawal of study drug.

Possibly related: There is a clinically plausible time sequence between onset of the AE and study drug administration, but the AE could also have been caused by the concurrent or underlying illness, other drugs, or procedures. Information regarding study drug withdrawal may be lacking or unclear. "Possible" should be used when study drug administration is one of several biologically plausible causes of the AE.

Unlikely related: The AE is most likely due to a cause not related to study intervention administration. However, association with the study intervention cannot be completely ruled out.

Unrelated: Another cause of the AE is most plausible, and a clinically plausible temporal sequence is inconsistent with the onset of the AE and study intervention administration and/or a causal relationship is considered biologically implausible.

For the purpose of regulatory reporting requirements, causal relationships of definite, probable, and possible will be considered treatment-related, while unlikely and unrelated will be considered not treatment-related.

10.18.8. Clinical Laboratory Adverse Events

A clinical laboratory AE is any laboratory value that is deemed clinically significant by the Investigator and is accompanied by one of the following:

- requires a medical intervention
- requires a modification or interruption of study intervention
- is accompanied by clinical symptoms

Laboratory abnormalities that do not require medical intervention should not be recorded as AEs and will be captured and reported in the laboratory section of the clinical study report (CSR). If a medical intervention occurs, it should be recorded as a treatment with the abnormal laboratory finding as the AE (e.g., anemia with treatment required and blood transfusion recorded as a procedure; hyperglycemia with treatment required and change in insulin dose recorded on the concomitant medications eCRF).

The Investigator should decide, based upon the AE criteria and the clinical condition of the participant, whether a change in a laboratory parameter is clinically significant and therefore represents an AE.

If, at the end of the treatment phase with the study intervention, there are pathological laboratory values that were not present at Baseline, further clinical or laboratory investigations should be performed until the values return to within reference range or until a plausible explanation (i.e., concomitant disease) is found for the pathologic laboratory values.

10.19. Regulatory Aspects of Adverse Event Reporting

The Investigator must promptly report to his or her Institutional Review Board (IRB) all unanticipated problems involving risks to participants.

The Sponsor or designee will promptly notify FDA and all participating Investigators in a safety report of potential serious risks deriving from this clinical study or any other sources in accordance with 21 Code of Federal Regulations (CFR) 312.32.

11. STATISTICAL METHODS

11.1. General Considerations

All statistical analyses will be performed using Version 9.4 or later of Statistical Analysis Software (SAS®).

Data summaries will use descriptive statistics (n, mean, standard deviation [SD], median, minimum, and maximum) for continuous variables, and frequency and percentage for categorical and ordinal variables. Where there are missing values, the number missing will be presented without a percentage. All data collected will be included in by-participant data listings.

11.2. Determination of Sample Size

Approximately 75 participants will be randomized and allocated in a 1:1:1 ratio to receive bitopertin 20 mg QD, bitopertin 60 mg QD, or placebo.

The primary efficacy endpoint is percent change from baseline in whole blood metal-free PPIX level at Day CCI Means of percent change from baseline values will be compared for each bitopertin dose group and placebo. Assuming the difference in mean percent change in PPIX between bitopertin and placebo is 30% with a corresponding pooled SD of 35%, a sample size of 23 participants per group provides 80% power to detect a difference between each bitopertin dose group and placebo using a two-sample t-test with a 2-sided alpha=0.05. Assuming an 8% dropout rate, a total of 75 participants will be randomized.

The key secondary efficacy endpoint is total hours of sunlight exposure to skin on days with no pain from 1000 to 1800 hours (10:00 AM to 6:00 PM) over CCI days. Means of total hours of sunlight exposure on days with no pain will be compared for each bitopertin dose group and placebo. Assuming the difference in means between bitopertin and placebo is 55 hours with a corresponding pooled SD of 67 hours, a sample size of 25 participants per group provides 80% power to detect a difference between each bitopertin dose group and placebo using a two-sample t-test with a 2-sided alpha=0.05.

Given the nature of this phase 2 study, the sample sizes were calculated without a multiplicity adjustment for two dose comparisons versus placebo; however, a sequential testing strategy will be employed to control for testing of the primary endpoint followed by the key secondary endpoint as described in Section 11.9.2.

11.3. Analysis Sets

11.3.1. Intent-to-Treat Analysis Set

To be included in the Intent-to-Treat (ITT) analysis set, participants must be ≥18 years of age at baseline and be randomized to study drug.

All analyses of the ITT analysis set will be based on each participant's randomized treatment. If a participant is randomized according to the incorrect stratification, the participant will be analyzed under the randomized treatment for the stratum recorded in the randomization system. All efficacy analyses will be based on the ITT analysis set.

11.3.2. Full Analysis Set

To be included in the Full Analysis Set (FAS), participants must be ≥18 years of age at baseline, be randomized and take at least one dose of double-blind study drug, and have a baseline measurement and at least one post baseline measurement of the primary efficacy variable or key secondary efficacy variable.

All analyses of the FAS will be based on each participant's randomized treatment. If a participant is randomized according to the incorrect stratification, the participant will be analyzed under the randomized treatment for the stratum recorded in the randomization system. The FAS will be used for a sensitivity analysis of the primary and key secondary efficacy endpoints.

11.3.3. Per Protocol Analysis Set

The Per Protocol (PP) Analysis Set will include all participants in the FAS who meet the study eligibility requirements and have no major protocol deviations that affect the validity of the efficacy measurements. The PP Analysis Set will be used for a sensitivity analysis of the primary and key secondary efficacy endpoints. The criteria for inclusion in the PP Analysis Set will be finalized prior to study unblinding and detailed in the Statistical Analysis Plan (SAP).

11.3.4. Safety Analysis Set

All participants who are randomized and take at least one dose of double-blind study drug will be included in the Safety Analysis Set. Safety analyses will be based on the study drug that was dispensed to each participant.

11.4. Demographics and Baseline Characteristics

Demographic data and baseline characteristics for the Safety Analysis Set as well as ITT, FAS and PP Analysis Sets will be summarized overall and by treatment group using descriptive statistics. Demographic data will include but are not limited to sex, age (years), race/ethnicity, weight (kg), height (cm), and calculated BMI. Baseline characteristics will include all baseline PPIX measurements.

11.5. Participant Disposition

Frequencies and percentages will be displayed for the number of participants who: screened, failed screening, enrolled, were randomized, were treated, completed treatment, completed the study, discontinued treatment early, and withdrew from the study. The number and percentage of participants in each analysis set (ITT, FAS, PP, and Safety Analysis Sets) will be summarized.

11.6. Study Treatment Compliance

Compliance rates during the treatment period will be derived using the following formula:

100*((Total number of tablets dispensed – Total number of tablets returned)/
(Expected number of tablets to be taken based on whether the participant is on full or half dose)).

If a participant is lost to follow-up and does not return all unused tablets, it will be assumed that the participant did not take any tablets since the last visit date.

Compliance rates will be presented for the Safety Analysis Set using summary statistics and percentage for the frequency distributions (0-<20%, 20-<40%, 40-<60%, 60-<80%, 80-<100%, 100-<120%, and ≥120%) by treatment group and overall. Duration of treatment (in weeks) will be defined as:

(date of last dose of study drug - date of first dose of study drug + 1)/7

Duration of treatment will also be summarized by treatment group for the Safety Analysis Set.

11.7. Efficacy Analysis

All efficacy analyses will be performed based on the ITT. In addition, the primary and key secondary endpoint analyses will be conducted using the FAS and PP Analysis Sets.

11.7.1. Primary Efficacy Analysis

11.7.1.1. Primary Efficacy Endpoint: Percent Change from Baseline Metal-Free PPIX

For the primary endpoint of percent change from baseline in whole blood metal-free PPIX level, a mixed model repeated measures (MMRM) analysis of covariance (ANCOVA) model will be fitted using all data as observed. The dependent variable is the percent change from baseline whole blood metal-free PPIX level for all post-baseline assessments for each participant. The model will include fixed effects for treatment, sunlight exposure time to prodromal symptom randomization stratification factor , baseline metal-free PPIX level, visit (Days CCI and CCI and visit-by-treatment interaction and a random effect for the participant. The mixed model will utilize restricted maximum likelihood estimation with the Kenward-Roger method used to compute the denominator degrees of freedom for tests of fixed effects. The model will assume an unstructured covariance matrix. If the model does not converge under the unstructured covariance matrix, alternative covariance structures will be used until convergence is reached based on an ordered list of pre-specified covariance structures which will be documented in the SAP. If a structured covariance is used, then a robust sandwich estimator will be utilized for estimating the variance of the treatment effect estimate.

The primary efficacy analysis will be based on comparisons of bitopertin and placebo at the Day CCI visit. From the MMRM model, the Day CCI pairwise differences between least square (LS) means of bitopertin treatment groups and placebo will be presented along with the

corresponding 95% CIs and 2-sided p-values. This analysis will be repeated for the FAS and PP Analysis Set.

The primary estimand for this study is the difference in LS means of bitopertin and placebo with respect to percent change from baseline in whole blood metal-free PPIX level at Day CCI based on the primary endpoint MMRM model fitted to the ITT. There will be no adjustments made for the use of prohibited therapies or other intercurrent events. The p-values associated with the primary estimand will be used to test the formal hypotheses of this study, that mean percent change from baseline to Day CCI whole blood metal-free PPIX level for each dose of bitopertin is different from that of placebo. All other p-values to be displayed will be considered descriptive and will not be used for formal hypothesis testing.

Since this endpoint may be skewed, a Kruskal-Wallis test will be used to test the treatment effect as a sensitivity analysis. The 2-sided p-values will be presented. The Hodges–Lehmann estimates of the median differences between the bitopertin treatment groups and placebo, and their 95% CIs, will also be presented.

11.7.2. Key Secondary Efficacy Analysis

The key secondary endpoint for this study is total hours of sunlight exposure on days with no pain from 1000 to 1800 hours (10:00 AM to 6:00 PM) summed over the entire treatment period from randomization to Day CCI An ANOVA model will be used for the ITT with effects for randomized treatment group and the sunlight exposure time to prodromal symptom randomization stratification factor. Pairwise differences between LS means of bitopertin treatment groups and placebo will be presented along with the corresponding 95% CIs and 2--sided p-values. This analysis will be repeated for the FAS and PP analysis set.

Since a highly skewed distribution for this endpoint is expected, a Kruskal-Wallis test will be used to test the treatment effect as a sensitivity analysis. The 2-sided p-values will be presented. The Hodges–Lehmann estimates of the median differences between the bitopertin treatment groups and placebo, and their 95% CIs will also be presented.

11.7.3. Secondary Efficacy Analyses

Two-week averages of daily sunlight exposure time (minutes) prior to first prodromal symptom (e.g., burning, tingling, itching, or stinging) associated with sunlight exposure between 1 hour post-sunrise and 1 hour pre-sunset will be calculated for the 2 weeks immediately prior to randomization (baseline) and for the treatment period from randomization to Day CCI visit for each participant (see Appendix 4, Appendix 5). An MMRM ANCOVA model for the change from baseline will be fitted for this endpoint with fixed effects for randomized treatment, timepoint (2-week intervals), timepoint-by-treatment interaction, sunlight exposure time to prodromal symptom randomization stratification factor and the corresponding baseline as a covariate. An unstructured within -participant covariance structure will be specified as the first choice. If the model converges, the Kenward-Roger approximation will be used to estimate the denominator degrees of freedom. If the model does not converge under the unstructured covariance matrix, alternative covariance structures will be used until convergence is reached

based on an ordered list of pre-specified covariance structures which will be documented in the SAP. If a structured covariance is used, then a robust sandwich estimator will be utilized for estimating the variance of the treatment effect estimate. From the model, the differences between LS means of bitopertin groups and placebo will be presented for the 2-week interval ending in Day CCI along with the corresponding 95% CIs and 2-sided p-values.

Percent Change from baseline in total PPIX concentration and change from baseline in plasma PPIX concentration will be analyzed in the same manner as the primary endpoint.

Pain intensity of phototoxic reactions is captured daily according to a Likert scale (0-10) on the Sun Exposure Diary (Appendix 4). Pain intensity will be analyzed as a total severity score whereby all as-observed pain scores will be summed over the entire treatment period from randomization to Day CCI Additionally, the maximum pain intensity post day C CI through end of study will be summarized. An ANOVA model will be used for the ITT population with effects for randomized treatment group and the sunlight exposure time to prodromal symptom randomization stratification factor. Pairwise differences between LS means of bitopertin treatment groups and placebo will be presented along with the corresponding 95% CIs and 2-sided p-values. This analysis will be repeated for the FAS and PP analysis set.

11.8. Safety Analyses

Safety data will include AEs, physical examination results, vital signs, CCI , and clinical laboratory measurements. Observed data will be listed by participant and summarized using descriptive statistics by treatment group.

11.8.1. Clinical Laboratory Tests

Clinical laboratory parameters will be measured at baseline and post-baseline visits. Each continuous laboratory variable will be summarized as changes from baseline by treatment group.

Laboratory data will also be classified as low, normal, or high relative to the parameter's reference range. Laboratory abnormalities for each treatment group will be summarized using shift tables.

11.8.2. Adverse Events

AEs will be mapped to preferred term and body system using the Medical Dictionary for Regulatory Activities (MedDRA). AEs that begin after the first administration of study drug, or existing AEs that worsen after the first dose of study drug will be considered TEAEs. The number and percentage of participants reporting TEAEs will be summarized for each treatment group by MedDRA system organ class and preferred term, by severity, and by relationship to study drug (drug-related TEAEs versus TEAEs not related to study drug). Drug-related TEAEs will be considered those to be at least possibly related to study drug based on the Investigator's assessment. The number and percentage of participants reporting SAEs, and the number and percentage of participants reporting AEs leading to study drug discontinuation will also be summarized for each treatment group by MedDRA system organ class and preferred term.

11.8.3. Physical Examination and Vital Signs

The number and percentage of participants with physical examination abnormalities at each visit will be summarized and presented for each body system by treatment group. A listing of abnormalities will also be provided.

Vital signs data will be summarized as changes from baseline and will be classified as low, normal, or high based on reference ranges pre-specified in the SAP. Vital sign abnormalities for each treatment will be summarized using shift tables.

11.8.4. Prior Medications, Concomitant Medications and Procedures

Concomitant medications will be coded using the World Health Organization Drug Dictionary (WHO-DD), and concomitant medical procedures will be coded using MedDRA. The incidence of prior medications, concomitant medications, and concomitant medical procedures will be summarized using frequencies and percentages.

11.8.5. Pharmacokinetics

Trough plasma study drug levels as well as PK parameters will be listed and summarized by dose group using descriptive statistics.

11.9. Other Statistical Issues

11.9.1. Significance Levels

All tests will be 2-tailed, using an overall 0.05 level of significance. All CIs will be 2-sided, 95% CIs.

11.9.2. Multiple Comparisons/Multiplicity

Given the nature of this phase 2 study, no adjustment for multiple comparisons of the primary endpoint will be made to account for multiple dose comparisons (i.e., there are two bitopertin groups being tested vs. placebo). Each bitopertin group comparison versus placebo will be tested using an $\alpha = 0.05$.

If and only if the comparison of the primary endpoint for a bitopertin group and placebo meets statistical significance at $\alpha = 0.05$, will the key secondary endpoint be tested for that same bitopertin group and placebo. In this way, the testing from the primary analysis to the secondary follows a hierarchical sequential design.

All p-values to be presented for the rest of the secondary endpoints will be considered descriptive.

11.9.3. Missing Data

Participants who discontinue the study prematurely will not be replaced but will be encouraged to remain in the study and follow the schedule of assessments even if they meet study drug discontinuation criteria. To the extent possible, attempts will be made to minimize the amount of missing data through measures planned in the study conduct. However, if missing data are present, they will be handled as follows, for the primary efficacy endpoint:

For the primary analysis of whole blood metal-free PPIX levels, inference will be performed in three steps. In step 1, missing data will be imputed with multiple imputation (MI) using SAS PROC MI. The MCMC option in PROC MI will be used for imputing the percent change from baseline in whole blood metal-free PPIX levels over time, including baseline as a covariate in the imputation model. Since classification variables such as treatment group cannot be included in the MCMC statement directly, a BY statement will be included in the PROC MI code to provide separate imputation models for each treatment group. If convergence is not attained, the regression option in PROC MI will be utilized for imputation. The output from PROC MI will be a data set containing multiple repetitions of the original data set, along with the newly imputed values. A minimum of 30 repetitions will be performed.

In step 2 of the primary analysis, each MI repetition will be analyzed separately to test the equality of the overall percent change from baseline means of the treatment groups and the placebo group. In step 3, the results from each separate analysis in step 2 will be pooled using SAS PROC MIANALYZE. The pooled results will contain the estimate for the mean difference between bitopertin treatment groups and placebo for the percent change from baseline in whole blood metal-free PPIX levels at Day 121, as well as the corresponding standard error, 95% CI, and p-value.

If the primary analysis renders a significant treatment difference, a tipping point analysis will be performed in order to examine the sensitivity of inferences to departures from the missing at random (MAR) assumption. Details will be provided in the SAP and a summary is provided below:

As missing values following early discontinuations may not be consistent with a MAR assumption, a sensitivity analysis using multiple imputation under a missing not at random (MNAR) assumption will be performed searching for a tipping point that reverses the primary analysis conclusion. The imputed values will be made worse by adding a delta defined as k times the treatment difference between the bitopertin and placebo values obtained from the MI MAR analysis, where k is a shift parameter that is incremented in order to identify the point at which the primary analysis result becomes non-significant (the tipping point). Consideration will then be given to how plausible the imputed values are at the tipping point. If not plausible, then the conclusion for the primary analysis under the MAR assumption is supported.

Given the key secondary endpoint is total hours of sunlight exposure on days with no pain from 1000 to 1800 hours (10:00 AM to 6:00 PM) summed over the entire treatment period from randomization to Day 121, participants with missing data for any given day and for any reason (including premature discontinuation) will be assumed to have no sunlight exposure (i.e., 0 hours). This methodology ensures missing data is treated conservatively.

12. SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

12.1. Regulatory and Ethical Considerations

This study will be conducted in accordance with the protocol and with the following:

The ethical principles that have their origin in the Declaration of Helsinki, and that are consistent with International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), all applicable local Good Clinical Practice (GCP) guidelines, and the applicable regulatory requirement(s).

Applicable laws and regulations.

- The protocol, protocol amendments, ICF, Investigator Brochure, and other relevant documents (e.g., advertisements) must be submitted to an IRB by the Investigator and reviewed and approved by the IRB before the study is initiated.
- Any amendments to the protocol will require IRB approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study participants.

The Investigator will be responsible for the following:

- Providing written summaries of the status of the study to the IRB/ IEC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/IEC.
- Notifying the IRB/IEC of SAEs or other significant safety findings as required by IRB/IEC procedures.
- Providing oversight of the conduct of the study at the site and adherence to requirements of 21 CFR, ICH guidelines, the IRB, and all other applicable local regulations.
- Conduct the study in compliance with the protocol. Any deviation from the protocol must be documented.

12.2. Financial Disclosure

Investigators and sub-investigators will provide the Sponsor with sufficient, accurate financial information as requested to allow the Sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities. Investigators are responsible for providing information on financial interests during the course of the study and for 1 year after completion of the study.

12.3. Informed Consent Process

The Investigator or his/her representative will explain the nature of the study to the participant or his/her healthcare proxy and answer all questions regarding the study.

Participants must be informed that their participation is voluntary. Participants or their healthcare proxy will be required to sign a statement of informed consent that meets the requirements of 21 CFR 50, local regulations, ICH guidelines, Health Insurance Portability and Accountability Act (HIPAA) requirements, where applicable, and the IRB/IEC or study center.

The medical record must include a statement that written informed consent was obtained before the participant was enrolled in the study and the date the written consent was obtained. The authorized person obtaining the informed consent must also sign the ICF.

Participants must be re-consented to the most current version of the ICF(s) during their participation in the study. A participant who is rescreened is not required to sign another ICF if the rescreening occurs within 30 days from the previous ICF signature date.

A copy of the ICF(s) must be provided to the participant or the participant's healthcare proxy.

12.4. Participant Data Protection

Participants will be assigned a unique identifier by the Sponsor. Any participant records or datasets that are transferred to the Sponsor will contain the identifier only; participant names or any information which would make the participant identifiable will not be transferred.

The participant must be informed that his/her personal study-related data will be used by the Sponsor in accordance with local data protection law. The level of disclosure must also be explained to the participant.

The participant must be informed that his/her medical records may be examined by Clinical Quality Assurance auditors or other authorized personnel appointed by the Sponsor, by appropriate IRB members, and by inspectors from regulatory authorities.

The Investigator and Investigator's staff shall maintain the confidentiality of all participant records.

12.5. Dissemination of Clinical Study Data

The Sponsor will comply with current regulatory requirements for disclosure and submission of study results. The Sponsor's policy on publication of study results is described in Section 13.

12.6. Data Quality Assurance

All participant data relating to the study will be recorded on printed or eCRF unless transmitted to the Sponsor or designee electronically (e.g., laboratory data). The Investigator is responsible

for verifying that data entries are accurate and correct by physically or electronically signing the eCRF.

The Investigator must maintain accurate documentation (source data) that supports the information entered in the eCRF.

The Investigator must permit study-related monitoring, audits, IRB/IEC review, and regulatory agency inspections and provide direct access to source data documents.

Monitoring details describing strategy (e.g., risk-based initiatives in operations and quality such as Risk Management and Mitigation Strategies and Analytical Risk-Based Monitoring), methods, responsibilities, and requirements, including handling of noncompliance issues and monitoring techniques (central, remote, or on-site monitoring) are provided in the Monitoring Plan.

The Sponsor or designee is responsible for the data management of this study including quality checking of the data.

The Sponsor assumes accountability for trial-related duties and functions delegated to other individuals (e.g., contract research organizations).

Study monitors will perform ongoing source data verification to confirm that data entered into the eCRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of participants are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.

Records and documents, including signed ICFs, pertaining to the conduct of this study must be retained by the Investigator for at least 2 years after the last marketing application approval or 2 years after formal discontinuation of the clinical development of the investigational product or according to applicable regulatory requirement(s). If the Investigator withdraws from the responsibility of keeping the study records, custody must be transferred to a person willing to accept the responsibility. The Sponsor must be notified immediately by telephone or e-mail and the notification confirmed in writing if a custodial change occurs.

12.7. Source Documents

Source documents provide evidence for the existence of the participant and substantiate the integrity of the data collected. Source documents are filed at the Investigator's site.

Data entered in the eCRF that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The Investigator may need to request previous medical records or transfer records, depending on the study. Also, current medical records must be available.

Any electronic study data are to be entered into a secure, validated data processing system and a backup maintained. Any changes to electronic study data will be documented.

Definition of what constitutes source data can be found in ICH guidance for industry E6 Good Clinical Practice: Consolidated Guidance.

12.8. Study and Site Opening and Closure

The study start date is the date on which the clinical study will be open for recruitment of participants.

The first act of recruitment is the first site open and will be the study start date.

The Sponsor designee reserves the right to close a study site or terminate the study at any time for any reason (e.g., as necessary for participant safety) at the sole discretion of the Sponsor. Study sites will be closed upon study completion. A study site is considered closed when all required documents and study supplies have been collected and a study-site closure visit has been performed.

Reasons for the early closure of a study site by the Sponsor may include but are not limited to:

- Failure of the Investigator to comply with the protocol, the requirements of the IRB/IEC or local health authorities, the Sponsor's procedures, or GCP guidelines.
- Inadequate recruitment of participants by the Investigator.
- Discontinuation of further study drug development.

If the study is prematurely terminated or suspended, the Sponsor shall promptly inform the Investigators, the IRBs/IECs, the regulatory authorities, and any contract research organization(s) used in the study of the reason for termination or suspension, as specified by the applicable regulatory requirements. The Investigator shall promptly inform the participant and should assure appropriate participant therapy and/or follow-up.

12.9. COVID-19 Mitigation Plan

The protocol includes the following adaptations to minimize risk while prioritizing the overall well-being and best interests of all involved in the trial. This protocol was written considering the impact of COVID-19 on trial participants, site staff and Sponsor staff. With these priorities in mind, these protocol design modifications have been made, but will still permit assessment of safety and efficacy of bitopertin:

- the number of study visits has been minimized to align with the trial endpoints
- some visits may be remote, with conduct of the study procedures at the subject's location

In addition to the above items included in the protocol, ongoing risk assessments and monitoring of the COVID-19 situation will be conducted by the Sponsor with input from the local investigator at a site and country (if applicable) level. These ongoing assessments include changes to any of the following:

- Potential impact on trial participants

- Potential impact on trial site staff
- Potential impact on Sponsor/CRO staff conducting site monitoring and central review of data

Other mitigation plans, as appropriate, may be instituted.

12.10. Future Use of Stored Specimens and Data

Blood and urine samples may be stored and used for future research to develop methods, assays, prognostics, and/or companion diagnostics related to EPP and related conditions, and/or to further understand the mechanism of action or safety of bitopertin.

Samples may be stored for a maximum of 10 years following study closure. The collection, labelling, storage, and shipment of samples will be detailed in the Laboratory Manual.

13. PUBLICATION POLICY

All information regarding bitopertin supplied by the Sponsor to the Investigator or generated as a result of any clinical studies is privileged and confidential information belonging to the Sponsor. The Investigator agrees to use Sponsor's confidential information solely to accomplish the study and will not use such information for any other purposes without the prior written consent of Disc Medicine. It is understood that there is an obligation to provide the Sponsor with complete and accurate data obtained during the study. The information obtained from the clinical study will be used towards the development of bitopertin and may be disclosed by Disc Medicine to regulatory authority(ies), other investigators, corporate partners, or consultants as required.

14. APPENDICES

APPENDIX 1. Strong CYP3A4 Inhibitors and Inducers

Strong Inhibitors	**Strong Inducers**
Atazanavir	Apalutamide
Ceritinib	Carbamazepine
Clarithromycin	Enzalutamide
Cobicistat and cobicistat-containing coformulations	Fosphenytoin
Darunavir	Lumacaftor
Idelalisib	Lumacaftor-ivacaftor
Indinavir	Mitotane
Itraconazole	Phenobarbital
Ketoconazole	Phenytoin
Levoketoconazole	Primidone
Lonafarnib	Rifampin (rifampicin)
Lopinavir	
Mifepristone	
Nefazodone	
Nelfinavir	
Ombitasvir-paritaprevir-ritonavir	
Ombitasvir-paritaprevir-ritonavir plus dasabuvir	
Posaconazole	
Ritonavir and ritonavir-containing coformulations	
Saquinavir	
Telithromycin	
Tucatinib	
Voriconazole	

APPENDIX 2. Schedule of Assessments

Procedure	Screening	Baseline	Study Visits[a]						
	CCI							CCI	OL visits q 8 wks
Visit Window	C								(±3)
Informed consent	X								
Eligibility criteria	X								
Demographics	X								
Medical history	X								
Complete physical examination	X							X	
Targeted neurological examination[b]	X				X			X	
Vital signs	X	X	X	X	X	X	X	X	X
Body weight and height[c]	X							X	
Serum or urine pregnancy test[d]	X	X							
Genotyping (unless documented in medical history)	Anytime during Screening or Treatment Period								
Randomization[e]	X[e]	X[e]							
Pharmacokinetic sampling[f]		C	C		C				
Blood chemistry[g]	X	X		X	X	X	X	X	X
Hematology[h]	X	X		X	X	X	X	X	X
Porphyrins[i]	X	X		X	X	X	X	X	X
Serology/Virology[j]	X								
Serum iron, TIBC, TSAT, ferritin		X			X		X	X	X

Procedure	Screening	Baseline	Study Visits[a]						OL visits q 8 wks
	CCI							CCI (EOS)	
Visit Window	C								**(±3)**
Urinalysis		X			X		X	X	
Light dosimeter[k]	X	Daily							
Daily sun exposure diary[k]	X	Daily							
'Sun Exposure Challenge Question' – (time to prodrome)[l]	X	Weekly							
PRO questionnaires[m]		X				X	X	X	X
CCI	X	X		X	X	X	X	X	X
Concomitant medications	X	X		X	X	X	X	X	X
Adverse events[n]	X	X		X	X	X	X	X	X
Study drug		Daily dosing of study drug						X[o]	X
Study drug diary[p]		X	X	X	X	X	X	X	X
Exit interview[q]								X	

HBV = Hepatitis B virus; HCV = Hepatitis C virus; HIV = Human immunodeficiency virus; OL=open-label; PRO = Patient reported outcomes.

Footnotes:

a. After Day 1, all study visits may occur remotely with the exception of the Day C and EOS visits.
b. A targeted neurologic exam that includes mental status, optical and eye movement cranial nerves (CNII, CNIII, CNIV, CNVI), motor function, gait, stance, and coordination will be performed.
c. Height is only measured at Screening.
d. For females of child-bearing potential, a pregnancy test must be performed. The preferred method is a urine screen, but a serum test may be performed if urine is not feasible. If the urine test is positive, a serum test must be performed to confirm.
e. Participants randomized to treatment group upon confirmation of eligibility Day -1 or Day 1.
f. Day C: Pre-dose and 2±0.25, 4±0.25, 6±0.25 hours post-dose. Day C: 24±1 hours post Day 1 dose, but prior to Day C dosing. Day C Pre-dose and 4±0.5 hours post-dose.
g. Chemistry includes sodium, potassium, carbon dioxide (CO2), chloride, blood urea nitrogen (BUN), creatinine, glucose, calcium, alkaline phosphatase, total bilirubin, direct bilirubin, AST, ALT, GGT, and albumin.

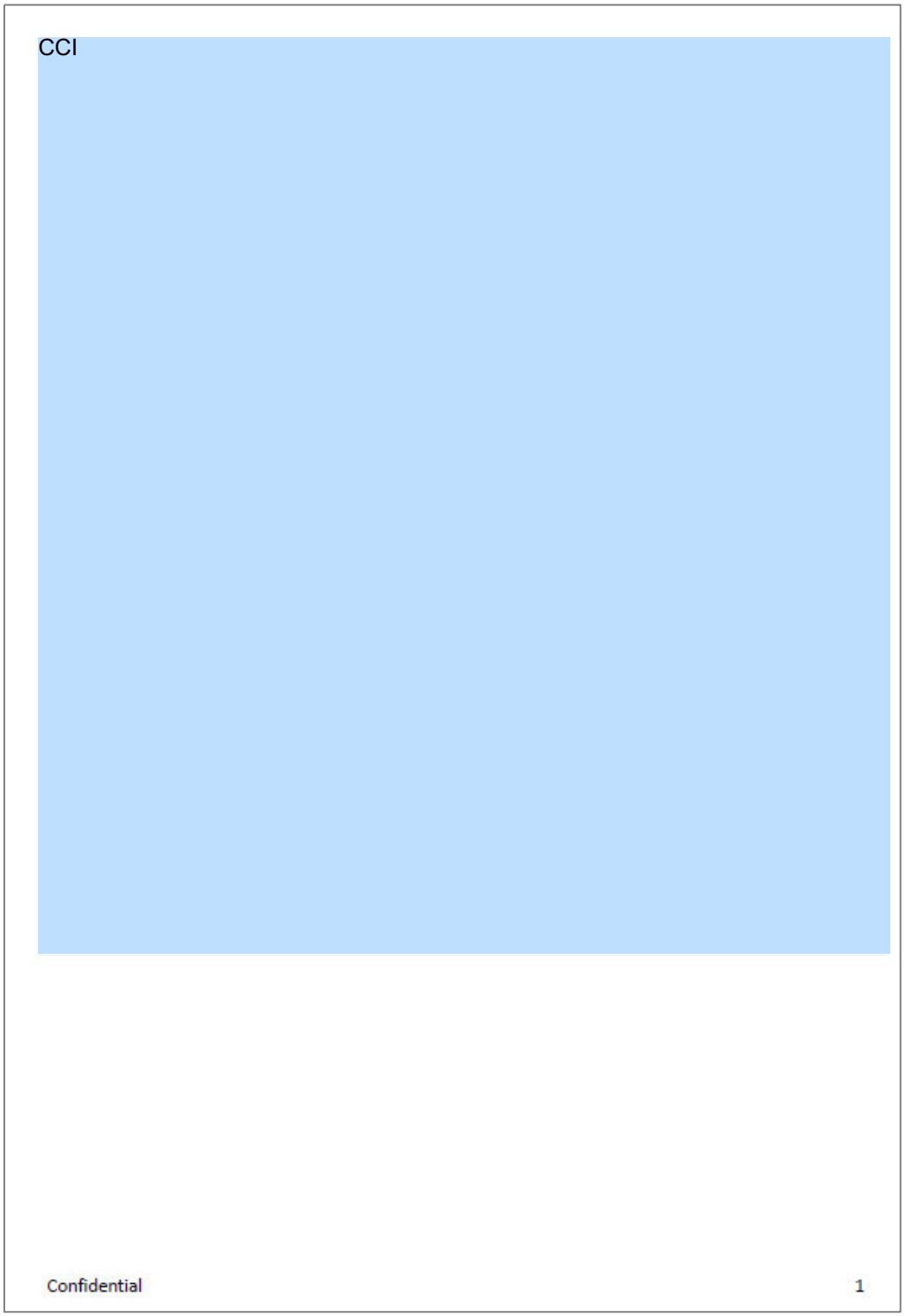
h. Hematology parameters include a complete white blood cell count (WBC) with differential, red blood cell count (RBC), hemoglobin, hematocrit, platelet count, mean corpuscular hemoglobin (MCV), red cell distribution width, mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular hemoglobin (MCH). Special hematology parameters include reticulocyte (absolute) and reticulocyte %, and CHr.
i. Porphyrins, including metal-free whole blood PPIX, metal-free erythrocyte PPIX, whole blood total PPIX, and plasma total PPIX.
j. Includes hepatitis B surface antigen (HBV), hepatitis C virus (HBC), and human immunodeficiency virus (HIV). Results must be available before the first dose.
k. Light dosimeter and daily sun exposure diary are to be completed for 2 weeks during Screening and daily during the double-blind treatment phase. Light dosimeter is optional during open-label phase.
l. 'Sun Exposure Challenge Question' (time to prodrome) is to be completed twice during Screening, if possible, and weekly thereafter (Appendix 5).
m. Assessment tools: CCI
Appendix 6).
n. Only SAEs and AEs related to study procedures are to be collected prior to first dose of study drug. AEs may be assessed by phone.
o. Dosing may continue if participant continues on open-label study drug.
p. A study drug diary will be dispensed to the subject at baseline. Subjects will bring their diary with them to each study visit. Diary information will be reviewed and/or collected by the study team during study visits.
q. Subjects who complete the double-blind treatment period will be invited to participate in an optional exit interview after the last double-blind period dose and before the first open label period dose.

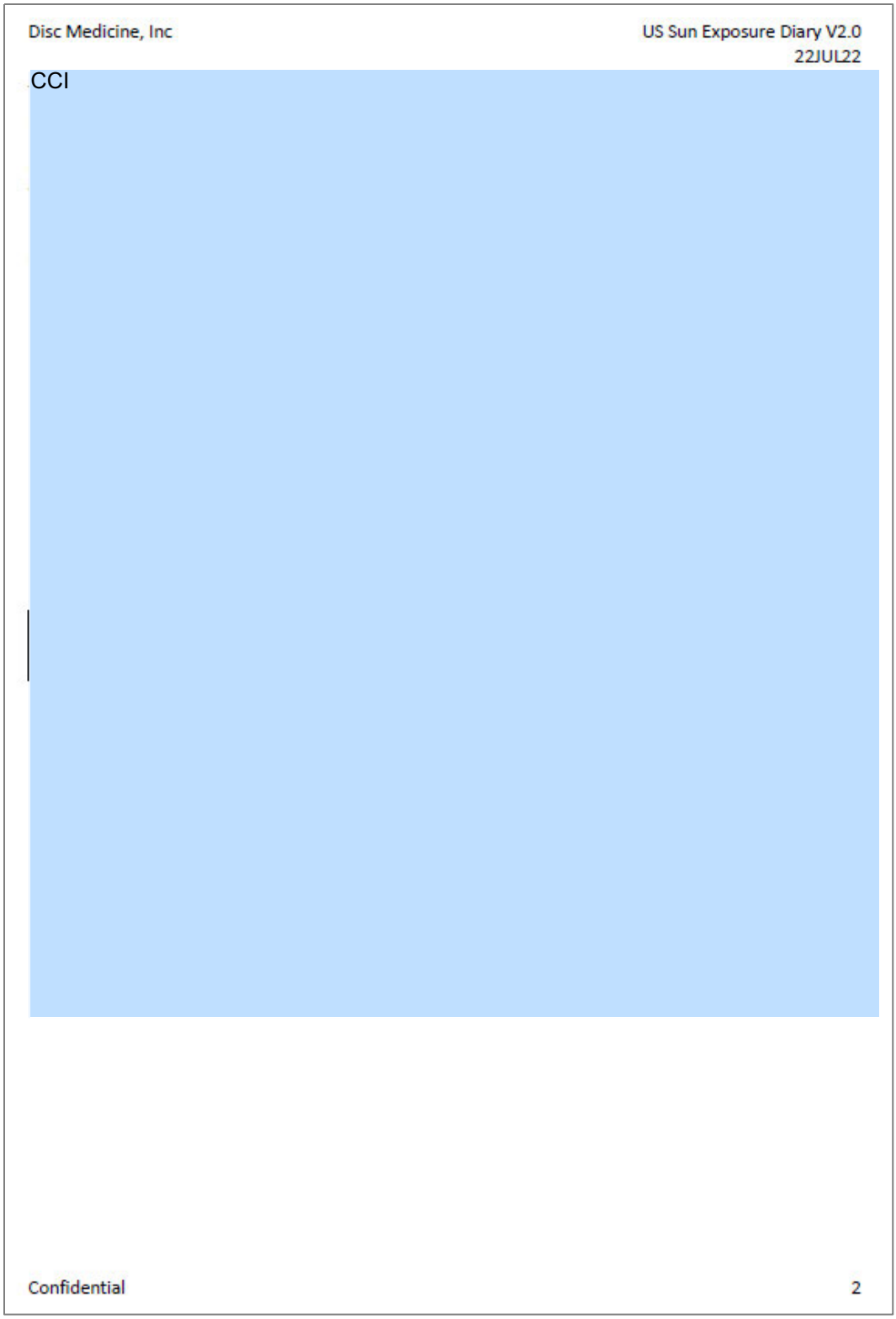
APPENDIX 3. Clinical Laboratory Parameters

Refer to Schedule of Assessments (Appendix 2) for sampling times.

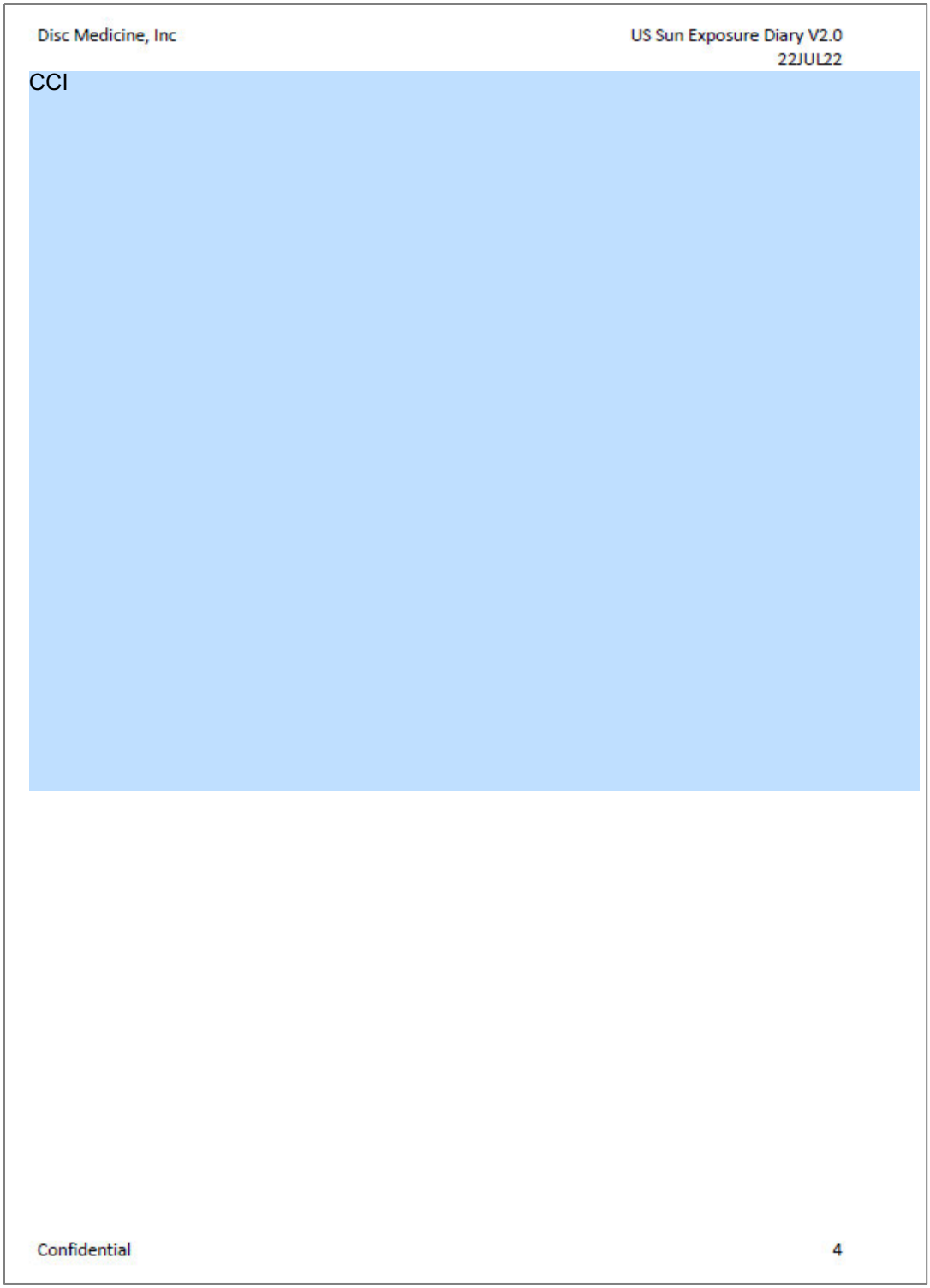
CCI		
	CCI	

APPENDIX 4. Sun Exposure Diary

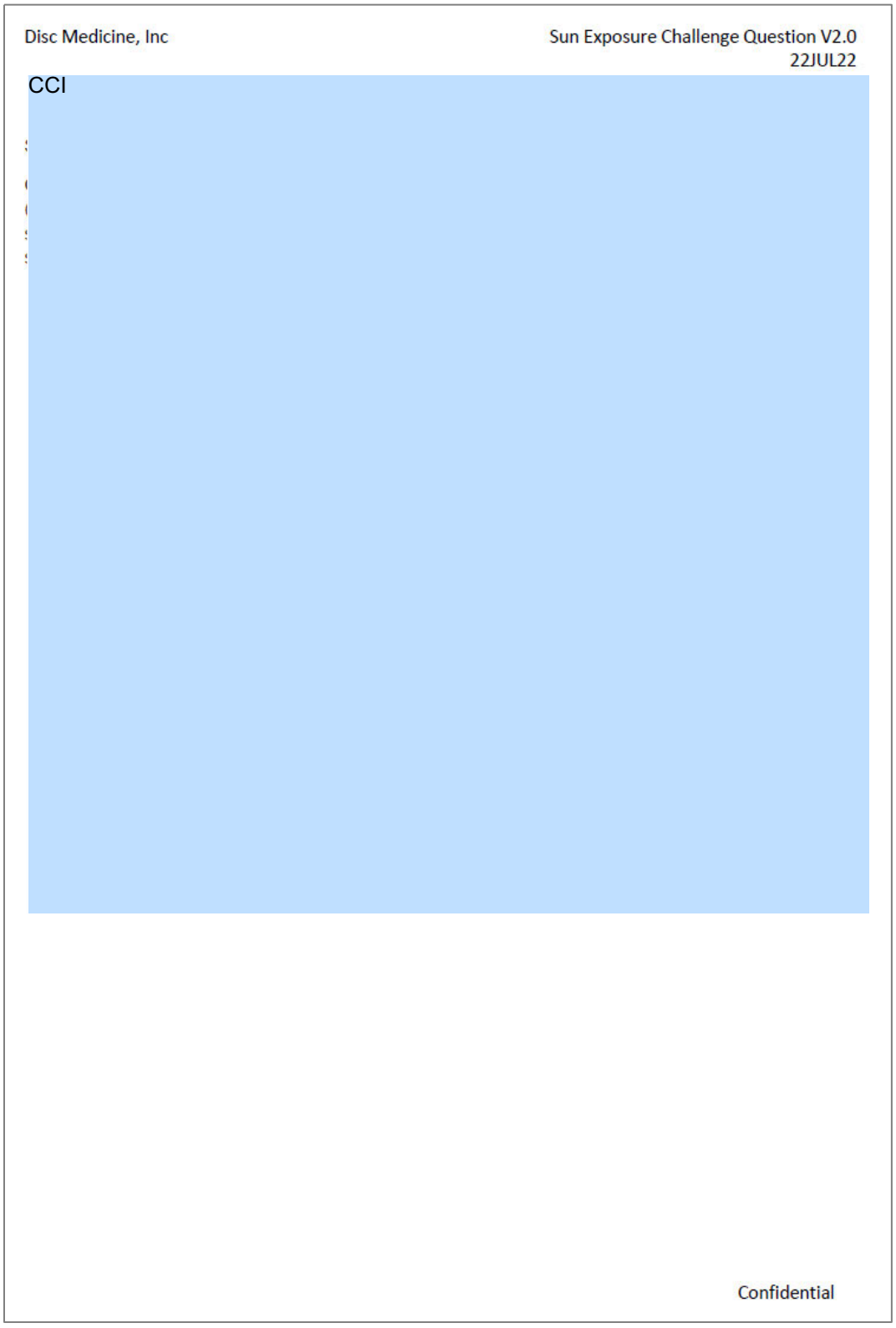



Disc Medicine, Inc
US Sun Exposure Diary V2.0
22JUL22
CCI
Confidential
2


CCI
Confidential
3


Disc Medicine, Inc
US Sun Exposure Diary V2.0
22JUL22
CCI
Confidential
4

APPENDIX 5. Sun Exposure Challenge Question



APPENDIX 6. CCI

CCI

15. REFERENCES

1. Wang P, Sachar M, Lu J, Shehu AI, Zhu J, Chen J, Liu K, Anderson KE, Xie W, Gonzalez FJ, Klaassen CD, Ma X. The essential role of the transporter ABCG2 in the pathophysiology of erythropoietic protoporphyria. Sci Adv. 2019 Sep;5(9):eaaw6127.
2. Balwani M, Naik H, Anderson KE, Bissell DM, Bloomer J, Bonkovsky HL, Phillips JD, Overbey JR, Wang B, Singal AK, Liu LU, Desnick RJ. Clinical, Biochemical, and Genetic Characterization of North American Patients with Erythropoietic Protoporphyria and X-linked Protoporphyria. JAMA Dermatol. 2017 Aug 01;153(8):789-796.
3. Snast I, Kaftory R, Sherman S, Edel Y, Hodak E, Levi A, Lapidoth M. Acquired erythropoietic protoporphyria: A systematic review of the literature. Photodermatol Photoimmunol Photomed. 2020 Jan;36(1):29-33.
4. Sachar M et al. Protoporphyrin IX: the Good, the Bad, and the Ugly. J Pharmacol Exp Ther. 2016;356(2):267-75.
5. Phillips JD et al. Heme biosynthesis and the porphyrias. Mol Genet Metab. 2019;128(3):164–177.
6. Wulf HC, Nissen CV, Philipsen PA. Inactivation of protoporphyrin IX in erythrocytes in patients with erythropoietic protoporphyria: A new treatment modality. Photodiagnosis Photodyn Ther. 2020 Mar;29:101582
7. Heerfordt IM et al. Patients with Erythropoietic Protoporphyria Have Reduced Protoporphyrin IX from Early in Pregnancy. Br J Dermatol. 2017;177:38-40.
8. Cui, L., Hung, H.M., and Wang, S.J. “Modification of sample size in group sequential clinical trials.” Biometrics 55, pp. 853-857 (1999).
9. Lawrence, J. and Hung, HMJ. Estimation and Confidence Intervals after Adjusting the Maximum Information. Biometrical Journal, 2003; 45 (2): 143-152.