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Rhythm Pharmaceuticals, Inc.

RM-493-032

A Randomized, Double-Blind, 3-arm, Parallel Group, Placebo- and Positive-controlled Study to Investigate the Effects of Setmelanotide on QTc Interval in Healthy Subjects

Statistical Analysis Plan

Version: 3.0

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SPONSOR SIGNATURE PAGE

Approved by: [redacted]

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Rhythm Pharmaceuticals, Inc.

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Date

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Signatures below confirm that the Statistical Analysis Plan was developed in accordance with [redacted] and that it is approved for release.

This document has been approved and signed electronically on the final page by the following:

Signatory	
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REVISION HISTORY

[redacted]

LIST OF ABBREVIATIONS

Abbreviation/Acronym	Definition/Expansion
Δ	Change from baseline
ΔΔ	Placebo-corrected change from baseline
AE	Adverse event
ATC	Anatomical Therapeutic Chemical
AUC_{tau}	Area under the plasma concentration-time curve during the dosing interval
AUC_{inf}	AUC from time zero extrapolated to infinity
AUC_{last}	AUC from time zero to the last quantifiable concentration
BLQ	Below the lower limit of quantification
BMI	Body mass index
BP	Blood pressure
bpm	Beats per minute
CI	Confidence interval
C-SSRS	Columbia Suicide Severity Rating Scale
Cl/F	Apparent clearance
C_{max}	Maximum observed concentration
CPMS	Clinical Pharmacology, Modelling and Simulation
CRU	Clinical Research Unit
CS	Clinically significant
CS	Clinically significant
CSR	Clinical Study Report
CTCAE	Common Terminology Criteria for Adverse Events
CV	Coefficient of variation
ECG	Electrocardiogram
EOS	End of study
BLQ	Below the limit of quantification
ET	Early termination
eCFR	Electronic Case Report Form
H	High

Abbreviation/Acronym	Definition/Expansion
HR	Heart rate
ICF	Informed consent form
IMP	Investigational medicinal product
L	Low
LEPR	Leptin receptor
LLOQ	Lower limit of quantification
MC4R	Melanocortin-4 receptor
MedDRA	Medical Dictionary for Regulatory Activities
N	Normal
NCA	Noncompartmental analysis
NCS	Not clinically significant
NR	Not reportable
NS	No sample
PCS	Potentially clinically significant
PK	Pharmacokinetics
POMC	Pro-opiomelanocortin
PCSK1	Proprotein convertase subtilisin/kexin type 1
PT	Preferred Term
QD	Once daily dosing
QTc	corrected QT interval
QTcF	QT corrected using Fridericia's formula
RGDO	Rare genetic disorders of obesity
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SC	Subcutaneous
SD	Standard deviation
SI	Standard international
SoA	Schedule of Assessments
SOC	System Organ Class
TEAE	Treatment-emergent adverse event
TEMA	Treatment-emergent markedly abnormal

Abbreviation/Acronym	Definition/Expansion
T_{max}	Time of the maximum observed plasma concentration
V_d/F	Apparent volume of distribution
WHO-DD	World Health Organization - Drug Dictionary

1 INTRODUCTION

Human genetics studies have identified several diseases that are the result of genetic defects affecting the melanocortin-4 receptor (MC4R) pathway, including, but not limited to, pro-opiomelanocortin (POMC) deficiency obesity due to mutations in the *POMC* gene; heterozygous proprotein convertase subtilisin/kexin type 1 (PCSK1) deficiency due to mutations in the *PCSK1* gene, leading to a hormone processing defect that also causes POMC deficiency obesity; and leptin receptor (LEPR) deficiency obesity due to mutations in the *LEPR* gene. These MC4R pathway mutations cause rare genetic disorders of obesity (RGDO) that start early in childhood, progress over time, and can become life-threatening in severity.

Setmelanotide (also known as RM-493) is a synthetic, cyclic octapeptide (8-amino acid containing peptide) that functions as a potent MC4R agonist. Setmelanotide is an 8-amino acid, cyclic peptide that binds with high affinity (inhibitory constant [Ki] = 2.1 nM) to the human MC4R and is efficient in activating MC4R (50% effective concentration [EC50] = 0.27 nM). Setmelanotide is indicated for chronic weight management in adult and pediatric patients 6 years of age and older with obesity due to POMC, PCSK1, or LEPR deficiency confirmed by genetic testing demonstrating variants in POMC, PCSK1, or LEPR genes that are interpreted as pathogenic, likely pathogenic, or of uncertain significance.

This study is being conducted to evaluate the effects of therapeutic and supratherapeutic setmelanotide concentrations on QTc corrected by the Fridericia method (QTcF) interval in healthy subjects.

This Statistical Analysis Plan (SAP) describes the rules and conventions to be used in the presentation and analysis of PK and Safety data for Clinical Study Report (CSR) of Study RM-493-032. It describes the data to be analysed, including details of the statistical analyses to be performed. Should circumstances arise during the study rendering these analyses inappropriate, or if improved methods of analysis arise, updates to the analyses may be made. Any deviations from the final SAP before database lock and all alternative or additional statistical analyses that may be performed may be described in an updated SAP version and/or will be discussed in the CSR.

The content of this SAP is based on following study documents:

- Study protocol, Version 2.0 (Nov 24, 2021).

2 STUDY OBJECTIVES

2.1 Primary Objective

The primary objective of this study is:

- To evaluate the effects of therapeutic and supratherapeutic setmelanotide concentrations on QTc corrected by the Fridericia method (QTcF) interval in healthy subjects.

2.2 Secondary Objectives

The secondary objectives of this study are:

- To assess the effects of therapeutic and supratherapeutic setmelanotide concentrations on Heart rate (HR), PR interval, QRS interval, T-wave morphology, and cardiac safety in healthy subjects.
- To evaluate assay sensitivity and the effect of a positive control, a single 400 mg oral dose of moxifloxacin, on the QT/QTcF interval in healthy subjects.

- To assess the pharmacokinetics (PK) of setmelanotide following administration of therapeutic and supratherapeutic doses of subcutaneous (SC) setmelanotide in healthy subjects.
- To evaluate the safety and tolerability of therapeutic and supratherapeutic doses of SC setmelanotide in healthy subjects.

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3 INVESTIGATIONAL PLAN

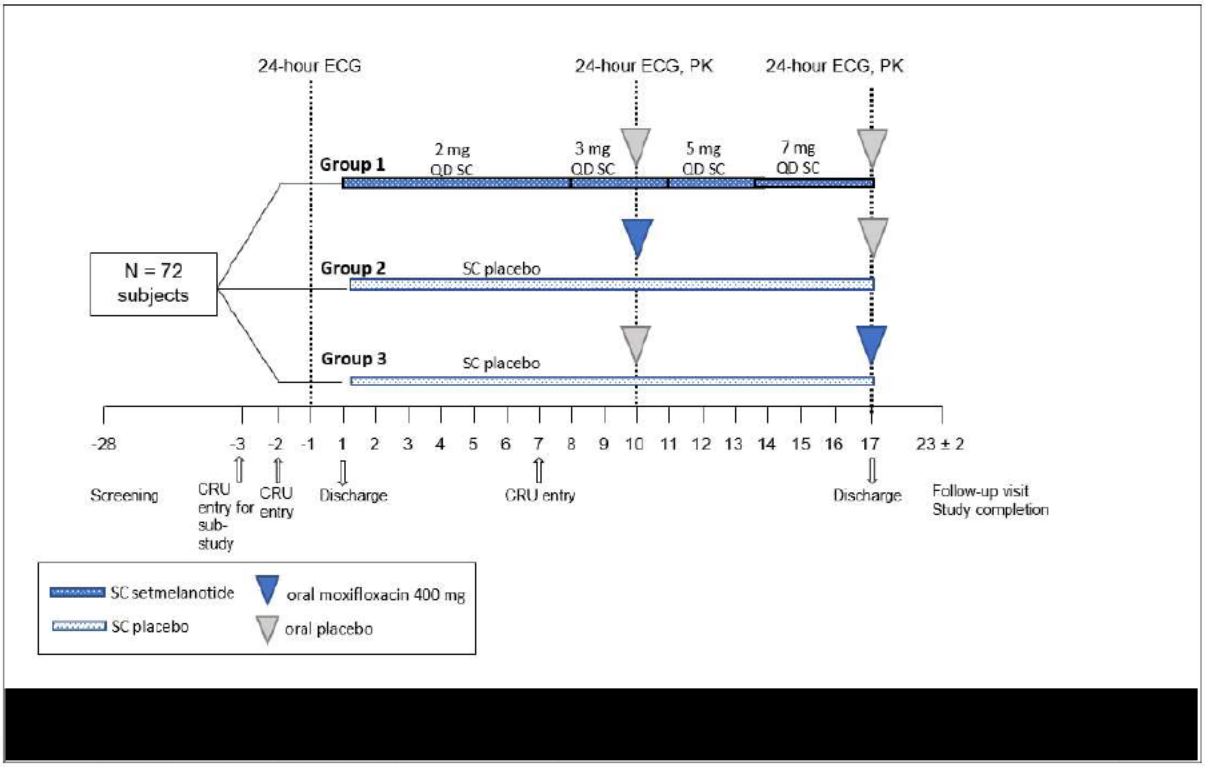
3.1 Overall Study Design and Plan

This is a double-blind, randomized, placebo- and positive-controlled, parallel group, 3-arm study to assess the potential for therapeutic and supratherapeutic concentrations of SC setmelanotide administered as IMCIVREE to affect the QTcF interval compared with placebo. Approximately 72 healthy subjects aged 18 to 50 years of age, inclusive, are planned to be enrolled in the United States. Subjects will be enrolled into 1 of 3 groups as follows:

- Group 1: SC setmelanotide; oral placebo on Days 10 and 16
- Group 2: SC placebo; oral moxifloxacin on Day 10 and oral placebo on Day 16
- Group 3: SC placebo; oral placebo on Day 10 and oral moxifloxacin on Day 16.

The study design is presented in Figure 3-1.

Figure 3-1 Study Design Schematic



Upon providing informed consent, subjects will enter a 28-day Screening period, during which they will be assessed for eligibility and complete all screening procedures as described in the Schedule of Assessments (SoA) (Table 6-1).

Following the Screening period, eligible subjects will check-in to the Clinical Research Unit (CRU) on the morning of Day -2. Subjects will be randomized 1:1:1 into Group 1, 2, or 3 and will be fasted overnight (for at least 10 hours). On the morning of Day -1 following a standard breakfast, subjects will undergo 24-hour continuous ECG Holter monitoring beginning 30 minutes after the meal (at the time of anticipated dosing on Days 10 and 16). Baseline assessments (Table 6-1) will occur on Day -1.

On the morning of Day 1, subjects will receive SC setmelanotide 2 mg (Group 1) or SC placebo

(Groups 2 and 3) in a double-blinded fashion.

From Days 2 to 16 following a standardized breakfast, subjects will continue receiving SC setmelanotide (3 mg QD from Days 8 to 10; 5 mg QD from Days 11 to 13; 7 mg QD from Days 14 to 16) or SC placebo according to their assigned group. On Days 10 and 16, oral placebo or oral moxifloxacin 400 mg will be administered with the SC dose according to the assigned dose group. Continuous Holter ECGs and PK sampling will be conducted on Days 10 and 16 pre-dose and for up to 24 hours post-dose according to the SoA (Table 6-1). All treatments administered in the CRU will be double-blinded and will be given at the same time every day (approximately 30 minutes after the start of breakfast).

Subjects will remain in the CRU through the morning of Day 17 and will be discharged after completion of all study assessments if safety parameters are acceptable to the Investigator. Subjects will return to the CRU for a Follow-up visit 7 ± 2 days after receiving the last SC dose (Day 23 ± 2).

Adverse events (AEs), 12-lead safety ECGs, Columbia Suicide Severity Rating Scale (C-SSRS) score, clinical laboratory tests, vital signs, and concomitant medication monitoring will be collected throughout the study for safety assessments as described in the SoA (Table 6-1).

3.2 Endpoints and Associated Variables

Triplicate values for HR (bpm), RR (msec), PR (msec), QRS (msec) and QT (msec) will be extracted by the ECG core lab at Day -1 and on Days 10 and 16 at the following timepoints: pre-dose and 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 9, 10, 12, 16, and 24 hours post-dose. The Day -1 extractions will begin at 7:30 AM (the time of anticipated dosing on Days 10 and 16).

Heart rate corrected QT intervals will be derived from the QT and RR intervals for each replicate at each time point:

- Fridericia's cube-root corrected QT([1]): QTcF (msec) = QT (msec) / $(RR(msec)/1000)^{1/3}$
- Bazett's square-root corrected QT([2]): QTcB (msec) = QT (msec) / $(RR(msec)/1000)^{1/2}$

At each time point, the triplicate values will be averaged in order to obtain 1 single value per subject and time point. The averaged values will be rounded to the nearest integer. Changes from baseline and percent changes from baseline will be computed as:

- $\Delta Y_{i,k,j} = Y_{i,k,j} - Y_{i,\,D\text{-}1,\,j}$
- $\Delta_{rel} Y_{i,k,j}(\%) = 100 \times (Y_{i,k,j} - Y_{i,\,D\text{-}1,\,j}) / Y_{i,\,D\text{-}1,\,j}$
- With i=Subject, j=time, k=Day

3.2.1 Primary Cardiac Endpoint

The primary cardiac endpoint of the study is:

- Setmelanotide concentration-related change from baseline in QTc (Δ) and placebo-adjusted change from baseline in QTc (ΔΔ) corrected for HR using QTcF (ΔQTcF and ΔΔQTcF)

3.2.2 Secondary Cardiac Endpoints

The secondary cardiac endpoints of the study are:

- Change from baseline (Δ) and placebo-corrected change from baseline (ΔΔ) in HR, RR, QTcF, QTcB, PR, and QRS intervals after administration of SC setmelanotide or oral moxifloxacin
- Categorical outliers for QTcF, QTcB, HR, PR, QRS, ΔQTcF, ΔQTcB, Δ_{rel}HR, Δ_{rel}PR and Δ_{rel}QRS intervals after administration of SC setmelanotide
- Frequency of treatment-emergent changes of T-wave morphology and U-wave presence after administration of SC setmelanotide
- Relationship between moxifloxacin concentrations and ΔQTcF

3.2.3 Pharmacokinetic Endpoints

Pharmacokinetic concentration data will be obtained at time point(s) described in the protocol as follows:

Plasma PK concentrations will be determined at the following nominal times: pre-dose, 0.5 (±2min), 1 (±5min), 1.5 (±5min), 2 (±5min), 2.5 (±5min), 3 (±10min), 4 (±10min), 5 (±10min), 6 (±10min), 7 (±10min), 8 (±10min), 9 (±10min), 10 (±10min), 12 (±20min), 16 (±20min) and 24 (±20min) hours post-dose

Unless otherwise stated, derivation of PK parameters will be the responsibility of Clinical Pharmacology, Modeling and Simulation (CPMS) group, [redacted]

If calculable, the following PK parameters listed in Table 3-1 will be determined for setmelanotide in plasma following repeated SC administration.

Table 3-1 Plasma Pharmacokinetic Parameters after Multiple Dose Administration of Setmelanotide

Parameter	WNL Name	CDISC Name	Definition
Cmax	Cmax	CMAX	Maximum observed concentration in a dosing interval after last dose administration
Tmax	Tmax	TMAX	Time corresponding to occurrence of Cmax after last dose administration
AUCtau	AUC_TAU	AUCTAU	AUC over the dosing interval after last dose administration
AUClast	AUClast	AUCLST	AUC from time zero to the last quantifiable concentration

Parameter	WNL Name	CDISC Name	Definition
Cl/F	CLss_F	CLFTAU	Apparent clearance following oral administration at after last dose administration
Vd/F	Vz_F	VZFTAU	Apparent volume of distribution during terminal phase after last dose administration

If calculable, the following PK parameters listed in Table 3-2 will be determined for moxifloxacin in plasma following single oral administration.

Table 3-2 Plasma Pharmacokinetic Parameters After Single Dose Administration of Moxifloxacin Administration

Parameter	WNL Name	CDISC Name	Definition
Cmax	Cmax	CMAX	Maximum observed concentration
Tmax	Tmax	TMAX	Time corresponding to occurrence of C_{max}
AUClast	AUClast	AUCLST	AUC from time zero to the last quantifiable concentration
AUCinf	AUCINF_obs	AUCIFO	AUC from time zero extrapolated to infinity

3.2.4 Safety Endpoints

The safety endpoints are:

- Frequency and severity of AEs (assessed separately for relation to setmelanotide and/or moxifloxacin)
- Changes from baseline in clinical laboratory evaluations, vital signs, 12-lead safety ECGs, C-SSRS score, and physical examinations

[redacted]

- [redacted]

4 STATISTICAL METHODS

4.1 Data Quality Assurance

All tables, figures, and data listings to be included in the report will be independently checked for consistency, integrity, and in accordance with standard [redacted] procedures.

4.2 General Presentation Considerations

This section is not applicable to PK data.

4.2.1 Treatment

The following treatments will be administered as follows:

- SC setmelanotide: SC injection: 2 mg × 7 days followed by 3, 5, and 7 mg × 3 days per dose level
- SC placebo (matched to active setmelanotide): SC placebo injection × 16 days
- Oral moxifloxacin: single oral dose of 400 mg moxifloxacin
- Oral placebo (matched to oral moxifloxacin): single oral placebo dose

4.2.2 Study Day

Study days will be numbered relative to the first day of study drug administration.

- If the date of event is before the study drug administration, then:

  Study day = (Date of measurement – Date of study drug administration in each group)

- If the date of event is on or after the study drug administration, then:

  Study day = (Date of measurement – Date of study drug administration in each group) + 1

4.2.3 End of Study

The end of the study is defined as the date of the last visit of the last subject under the auspices of the current study.

4.2.4 Baseline

Baseline is defined as the last non-missing measurement recorded before first dose of the study drug administration for res.

Time-matched Measurements:

For Continuous 12-lead ECG using Holter's readings for Cardiac endpoints, baseline is defined as the time-matched measurements on Day -1.

No imputation will be done for missing baseline value for derivation of change from baseline or summary tables and shift tables.

4.2.5 Summary and Representation of Data

Continuous data will be summarized in terms of mean, standard deviation (SD), median, minimum, maximum and number of observations, unless otherwise stated.

Categorical data will be summarized in terms of the number of participants providing data at the relevant time point (n), frequency counts, and percentages.

The minimum and maximum will be reported to the same number of decimal places as the raw data recorded in the database. The mean and median will be reported to one more decimal place than the raw data recorded in the database. The SD will be reported to two more decimal places than the raw data recorded in the database. In general, the maximum number of decimal places reported shall be four for any summary statistics.

Percentages will be presented to one decimal place. Percentages will not be presented for zero counts. Percentages will be calculated using N as the denominator. If sample sizes are small, the data displays will show the percentages, but any textual report will describe frequencies only. Percentage will be presented as whole number if percentage is 100.

If for any summary table, n is less than three then only n, minimum, and maximum should be presented, and other summary statistics will be left blank.

All ECG data recorded during the course of the study or derived during the programming phase will be presented in the individual data listings. All listings will be presented sorted by treatment group, subject number, visit number, date and time point.

Abnormality on ECG parameters will be flagged using the flag system defined in Table 4-2.

Unless specified otherwise, quantitative ECG parameters will be described per treatment group, day and time point using the following statistics: N (number of observations), mean, median, standard deviation (SD), minimum (Min), and maximum (Max) for raw and change from baseline values. 95% confidence interval (CI) of the mean will be added on changes from baseline.

4.3 Software

All report outputs will be produced using SAS® version 9.4 or later in a secure and validated environment.

The PK analyses will be conducted using Phoenix® WinNonlin (WNL) version 8.2 or later in a secure and validated environment.

All report outputs will be provided to the Sponsor in Microsoft RTF format.

4.4 Study Participants

4.4.1 Disposition of Participants

A clear accounting of the disposition of all subjects who enter the study will be provided, from randomization to study completion.

Subject disposition will be summarized by treatment group for the randomized analysis population. This display will show the number and percentage of subjects who have completed the study or have discontinued study treatment and a summary of the primary reasons for discontinuation of study treatment.

Primary reason for discontinuation of subjects are:

- Adverse event

- Withdrew consent
- Protocol deviation
- Loss to follow up
- Death
- Pregnancy
- Study terminated by sponsor
- Investigator decision
- Other

Disposition data will be presented for the Randomized Analysis Population.

Subject discontinuations will be listed including the date of study exit, details of IMP and reason for discontinuation in the randomized analysis population.

Listing of screening failure data will be provided including reason for screen failure in screened subjects. A disposition table during screening phase will also be provided in all screened subjects.

4.4.2 Protocol Deviations

All protocol deviations are predefined in the separate document, Protocol Deviation Specification.

4.4.2.1 Protocol Deviations with Non-PK Implications

The defined protocol deviations will be collected during the study period by site monitor/clinical team and programming team. All deviations related to study inclusion or exclusion criteria, conduct of the study, participant management or participant assessment, and handling of the participant's rights will be described.

4.4.2.2 Protocol Deviations with PK Implications

Protocol deviations that may potentially impact PK parameter derivations include, but are not limited to:

- Emetic episode in case of orally administered study drug
- IV administration deviations – interruption of administration, etc.
- Missed PK samples that impact estimation of PK parameter(s)
- Concomitant medications not authorized by protocol
- PK samples obtained out of allowance window that may impact the estimation of PK parameter(s)

[redacted]

Protocol deviations (mentioned in Sections 4.4.2.1 and 4.4.2.2) and analysis population will be reviewed in the data review meeting to decide inclusion or exclusion of participant(s) from analyses Population. Decisions regarding the exclusion of participants and/or participant data from analyses will be made after database lock and receipt of PK data, and will be documented and approved.

A by-participant listing of major and minor protocol deviations will be provided including participant identifier; exclusion from specific analysis population; and protocol deviation classification, and protocol deviation description and exclusion from specific analysis Population.

4.5 Analysis Populations

The following populations are defined for this study:

- **Randomized Population:** All participants who signed Informed Consent Form (ICF) and are assigned a randomization number are included in the randomized population.
- **Safety Population:** all subjects who received at least one dose of study drug (SC setmelanotide, SC placebo, oral moxifloxacin, or oral placebo).
- **Pharmacokinetic Population:** all subjects who received at least 1 dose of setmelanotide or moxifloxacin and have at least one quantifiable concentration of setmelanotide or moxifloxacin. A subject may be excluded from the PK summary statistics and statistical analysis if the subject has an AE of vomiting that occurs at or before 2 times the median T_{max} during moxifloxacin dosing.
- **ECG Population:** all subjects in the Safety Population with measurements at baseline as well as on-treatment with at least one post-dose timepoint with a valid value. The ECG population will be used for the by-timepoint and categorical analyses of the cardiodynamic ECG parameters.
- **PK/QTc Population:** all subjects who are in both the ECG and PK populations with at least 1 pair of post-dose PK and QTcF data from the same timepoint as well as subjects in the ECG population who received placebo. The PK/QTc population will be used for the C-QTc analysis. The PK/QTc population will be defined for setmelanotide and for moxifloxacin. Placebo subjects will be included in the C-QTc analysis set by setting their concentrations to 0.

A summary table with the number of participants in each of the analysis population will be provided and this table will be displayed by treatment group and overall, for randomized population. A listing of subjects excluded from analysis population will also be provided including reason of exclusion for randomized analysis population.

4.6 Demographics and Baseline Characteristics

The demographic characteristics (age, race, ethnicity, sex, height, body weight, body mass index [BMI]) will be summarized by treatment group and listed by participant for the Safety Population.

Age, height, BMI and weight will be summarized using the mean, SD, minimum, median, and maximum. The count and percentage will be computed for sex, race, and ethnicity. The summary table will be displayed by treatment group and overall, for the Safety Population.

4.7 Medical History

Based on the Safety Population, all subjects who had prior medical history will be presented in a by-subject listing including but not limited to subject ID, group, description of disease/procedure, MedDRA System Organ Class (SOC), MedDRA Preferred Term (PT), start date and stop date (or ongoing if applicable) of disease/procedure. All medical history will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). The version used for coding will be identified in the Data Management Plan.

4.8 Concomitant Medications

Medications will be considered as concomitant if they are taken at least once in the treatment period

starting with the first study administration and ending after the last study drug administration during the study.

Medications will be coded using the latest version of World Health Organization-Drug Dictionary (WHO-DD) and will be classified by Anatomical Therapeutic Chemical (ATC) categories.

By-participant listings of prior and concomitant medications will be provided for the Safety Population .

4.9 Treatment Exposure and Compliance

4.9.1 Treatment Exposure

A by-participant listing of participant exposure to study drug will be generated. The listing will include dose, date and time, unit, formulation, route, and frequency.

4.9.2 Compliance

Not applicable.

4.10 Efficacy Evaluation

Not Applicable.

4.10.1 Analysis and Data Conventions

Not Applicable.

4.10.2 Primary Cardiac Variable

4.10.2.1.1 Cardiodynamic ECG Assessment (Concentration-QTc analysis)
Primary concentration-response relationship will be investigated between ΔQTcF measurements and setmelanotide concentration. The same methodology can be used to analyze other ECG parameters (HR, PR, QRS) if an effect was evidenced from the central tendency analysis.
The C-QTc analysis will be performed according to the methodology detailed in Garnett et al.( ) white paper.

4.10.2.1.1 Graphical exploratory analysis
As a preliminary step, graphical exploration supporting model assumptions will be performed as detailed in Table 4-1.

Table 4-1 Graphical exploration analysis

Model assumption tested	Plot	Investigation	Model Impact
No drug effect on HR	Time course of mean HR/ΔHR stratified by dose (Day 10 vs. 16) Time course of estimated ΔΔHR stratified by dose (Day 10 vs. 16)	Homogeneous pattern between doses. No dose-related trend	Relationship between QT and RR may differ between on- and off treatment. The QTc correction could be inadequate on-treatment
QTc is independent of HR: Adequacy of the QTc correction in the population	<u>Drug-free and/or placebo data:</u> Scatter plot of QTc (y-axis) versus HR (x-axis) for baseline and placebo data with a regression line	The regression line should tend toward a horizontal line and show the lack of relationship between QTc and HR	Correction factor is potentially poorly estimated due to narrow range of HR intervals within each subject which could bias the C-QTc model. An alternative correction obtained on wider range of HR should be investigated (see section 4.10.2.1.2)
No Impact of a treatment effect on HR on the correction method	<u>Change in QTc is independent of change in HR on-treatment:</u> Scatter plot of on-treatment ΔQTc (y-axis) versus ΔHR (x-axis) with a regression line	The regression line should tend toward a horizontal line and show the lack of relationship between ΔQTc and ΔHR	The QTc correction could be inadequate on-treatment and could bias the C-QTc model. An alternative correction taking better into account HR variations should be investigated (see section 4.10.2.1.2)
Direct effect assumption. Impact of metabolite PK/PD hysteresis	Time course of GM concentrations and ΔΔQTc intervals	Shape of time course of effect, matched time of peaks, return to baseline Magnitude of variability in PK and QTc	Direct concentration-response model is inadequate. Alternative model (inclusion of metabolite, effect compartment, turn-over models) should be considered.
Drug effect on QTc	Time course of mean QTc/ΔQTc stratified by dose (Day 10 vs. 16)	Heterogeneity pattern between doses. Dose-related trend	A priori none since high inter-subject variability in ΔQTc can mask signal in mean curves.

Model assumption tested	Plot	Investigation	Model Impact
	Time course of estimated ΔΔQTc stratified by dose (Day 10 vs. 16)		However, largest ΔΔQTc should be consistent with ΔΔQTc predicted from the C-QTc model if the model is correct
Evaluate linearity and heterogeneity assumptions between exposure and QTc across doses and studies	C-ΔQTc: Scatterplot of individual ΔQTc (y-axis) vs. time-matched concentrations (x-axis) including a nonparametric trend curve	Shape of C-QTc relationship. Magnitude of ΔQTc over observed concentration range. Concentration range versus known clinical exposure scenario (therapeutic dose, worst case exposure, etc.)	A non-linear pattern could bias the C-QTc model and predictions. An alternative model should be envisaged Heterogeneity between doses/trials that should be taken into account in the C-QTc model

4.10.2.1.2 Study specific QT correction

For all analyses performed on healthy volunteers, the preferred, *a priori*, heart-rate correction method is the Fridericia -corrected QT interval (QTcF).

In the case that the individual correction factor is poorly estimated (due to narrow range of RR/HR intervals within each subject) or that setmelanotide has a chronotopic effect (resulting in change of HR after drug administration and a relationship between QT and RR that may differ between on- and off-treatment) it might be necessary to determine an alternative correction method for the QT interval in order to limit the bias in the assessment of QT/QTc prolongation.

In such case, an individual QT correction, QTcI, will be obtained from data recorded at baseline (Day -1) and from placebo subjects by modelling the QT/RR relationship (general power model) using a mixed model. The formula obtained for the QT/RR relation will then be converted to individual heart rate correction formulae.

The individual prediction of model parameters will be then applied to the QT and RR parameters at each time point to form the QTcI intervals.

The general power model relating QT and RR intervals can be written as follows:

$$QT=\alpha(RR/1000)^{\beta}, \alpha \text{ and } \beta \text{ being the model parameters to be estimated.}$$

After log transformation the model can be expressed as:

$$Log(QT)= \alpha'+\beta log(RR/1000), \text{ where } \alpha'=log(\alpha).$$

In order to estimate the parameters, log(QT) and log(RR/1000) obtained at baseline and in placebo subjects will be submitted to a mixed regression model where the slope and the intercept will be considered as random terms.

The statistical expression of the mixed model is:

$$Log(QT_{ij})= a_i + b_i log(RR_{ij}/1000) + e_{ij}$$

Where:

$log(QT_{ij})$ and $log(RR_{ij})$ are respectively, the j^{th} log QT/RR measurements performed on subject i

a_i and b_i are the random intercept and slope, respectively

4.10.2.1.3 Model development

The model development will start with the pre-specified mixed linear model as detailed in Garnett *etal* considering ΔQTcF as dependent variable. In case of the graphical exploration suggests that Fridericia correction does not account properly for HR/RR variations, alternative corrections should be investigated if possible (refer to previous Section 4.10.2.1.2).

The fixed effect parameters of the pre-specified model will be intercept, slope for linear effect of setmelanotide concentrations, influence of baseline (centered on mean) covariate, treatment specific intercept (0=placebo, 1= setmelanotide) and time (as factor).

Subject-specific random effects (between subject variability) will be added on intercept and slope parameters with an unstructured covariance matrix. In case of the unstructured covariance matrix is not supported by the data, other simplified or reduced structures will be investigated (ex. variance components).

$$\Delta QTcF_{ijk} = (\theta_0 + \eta_{0,i}) + (\theta_1 + \eta_{1,i})C_{ijk} + \theta_2 TRT_j + \theta_3(QTcF_{ijk=0} - \overline{QTcF_0}) + \theta_{4,k} Time_k + \varepsilon_{ijk}$$ (Eq.1)

Where

- i is the subject, j the treatment and k the time;
- $\Delta QTcF_{ijk}$ is the change from baseline in QTcF for subject i in treatment j at time k;
- θ_0 is the population mean intercept in the absence of a treatment effect;
- $\eta_{0,i}$ is the subject-specific random effect associated with the intercept term θ_0;
- θ_1 is the population mean slope of the assumed linear association between concentration and $\Delta QTcF_{ijk}$;
- $\eta_{1,i}$ is the subject-specific random effect associated with the slope θ_1;
- C_{ijk} is the concentration for subject i in treatment j and time k;
- TRT_j is variable associated with treatment (j=0 for placebo subjects, j=1 for setmelanotide subjects);
- θ_2 is the fixed effect parameter associated with the treatment specific intercept TRT_j;
- θ_3 is the fixed effect parameter associated with baseline and $\overline{QTcF_0}$ is the mean of all baseline;
- θ_{4k} is a vector of *k-1* parameters associated to the time factor, $Time_k$, having *K* levels. Time is defined as the scheduled time after dose;
- The influence of Day and the interaction between time and Day will be investigated within the model.
- $QTcF_{ijk=0}$ is the baseline QTcF

It is assumed the random effects are normally distributed with mean [0,0] and an unstructured covariance matrix G, whereas the residuals are normally distributed with mean 0 and variance R.

No additional covariate investigation will be performed and in order to avoid unnecessary model-building steps, non-significant fixed parameters will not be removed from the model. Random effects not supported by the model or resulting in null estimates will be removed as they may result in non-convergence problems.

The selection of best model will be based on AIC criteria.

The estimated mean, ΔΔQTcF and their 2-sided 90% confidence intervals will be calculated at each setmelanotide dose C_{max} geometric means (C_d) as follows (Est refers to an estimate from the model):

- $\Delta\Delta QTcF(C_d)_{Est} = \theta_{2,Est} + \theta_{1,Est} C_d$
- 90% CI= $\Delta\Delta QTcF(C_d)_{Est} \pm t(0.95,DF) \times SE_{Est}$
- With $SE_{Est} = \sqrt{var(\theta_{2,Est}) + C_d^2 var(\theta_{1,Est}) + 2C_d(cov(\theta_{2,Est}, \theta_{1,Est})}$

4.10.2.1.4 Model evaluation

Goodness of fit plots will be provided, consisting on:

- Quantile-Quantile plots of residuals;
- Concentrations versus residuals;
- Time, baseline and treatment versus residuals;
- Model predicted ΔQTc versus observed ΔQTc plotted versus with a regression line.

Any marked bias in residual plots may suggest model misspecification. Moreover, a large and significant treatment specific intercept (TRT term in the model) may also indicate model misspecification (i.e. lack of linearity or hysteresis).

Evaluation of the final model will be performed using:

- Visual predictive checks (VPC) obtained by simulating ΔQTc according to the final model selected and generated by binning the concentrations into deciles. VPC will be performed to assess the adequacy of the structural model.
- Mean observed and predicted ΔQTc by deciles of concentrations (quantile plot) with model slope and 90% CI.

4.10.2.1.5 Alternative to direct linear model

➢ **Nonlinear model**

In case of the linear relationship between ΔQTc and setmelanotide concentrations cannot be accepted, an alternative model such as saturable model will be considered:

$$\Delta QTcF_{ijk} = \left(\theta_0 + \eta_{0,i}\right) + \frac{(\theta_1+\eta_{1,i})C_{ijk}^{\gamma}}{(\theta_2+\eta_{2,i})^{\gamma}+C_{ijk}^{\gamma}} + \theta_3 TRT_j + \theta_4\left(QTcF_{ijk=0} - \overline{QTcF_0}\right) + \theta_{5,k} Time_k + \varepsilon_{ijk}$$

(Eq.2)

In Eq.2, the parameter θ_1 is the maximum asymptotic effect due to treatment, and the parameter θ_2 is the concentration at which the effect is half θ_1.

In that case, the mean and 90% CI for ΔΔQTc can be computed by non-parametric bootstrap methods with subject identifier used as the unit for resampling. Resampling should be stratified by dose level within the treatment group and the placebo subjects. Model parameters and ΔΔQTc are determined from each of the replicate bootstrapped datasets. Two-sided 90% CIs are computed from the 5th and 95th percentile of the rank-ordered ΔΔQTc values from all replicates.

➢ **Indirect relationship**

If the effect cannot be directly related (time-matched) to exposure to setmelanotide, it will be investigated whether any metabolite can explain the delayed effect.

In that case, a multivariate model combining parent and metabolite, as a linear or non-linear contribution, will be investigated using the same methodology as described above.

With such model, predictions of ΔΔQTc (and 90% CI) will be performed independently at C_{max} GM of each compound, setting the components for the other compounds at the mean value obtained at time of occurrence of C_{max} (t_{max}) of the compound of interest.

If the inclusion of the metabolite is not sufficient to explain the indirect/delayed effect and a hysteresis phenomenon cannot be ruled out, alternative models such as effect compartment or turn over models combining population PK and PKPD models should be envisaged. Such kind of modelling is out of the scope of this analysis plan and if it must be implemented will be detailed in a specific modelling plan.

4.10.2.1.6 Display of results

Parameters estimated from the selected model will be tabulated with their standard errors and 95% CIs. ΔΔQTcF predicted for each dose level will be presented with their 2-sided 90% CIs and a

graphical display of predicted ΔΔQTcF over the concentration range observed during the study will be provided.

4.10.2.1.7 Decision rule

The impact of setmelanotide on QT/QTc prolongation will be considered as below the threshold of regulatory concern if the upper bound of the 90% confidence interval of ΔΔQTcF predicted at highest dose C_{max} is below 10 ms.

4.10.3 Secondary Cardiac Variables

4.10.3.1 Central tendency analysis

The holter extracted ECG parameters will be summarized separately using descriptive statistics, on raw values and changes from baseline (Δ). Descriptive statistics will be computed per treatment group, day, dose level and time points.

Mean changes with their 95% CI will be plotted over time per treatment.

For all parameters, the changes from baseline (Δ) will be analysed using an analysis of variance model (ANOVA) including treatment, time and their interaction as main factors and structuring the residual matrix in order to account for the repeated measurement pattern of the data. The baseline value (centered on population mean) will be considered as covariate. This analysis will be performed by day in order to evaluate the therapeutic setmelanotide dose at day 10 and the supra-therapeutic setmelanotide dose at day 16.

At each time point, estimates (LSmeans) of treatment effects and differences between setmelanotide dose levels and placebo (ΔΔ) will be obtained with their 2-sided 90% CIs.

ΔΔ Estimates with their 90% CI will be plotted over time per day/dose level.

For moxifloxacin *vs.* placebo evaluations, the changes from baseline will be analysed per time point, for each parameter separately, using analysis of variance model including terms for treatment and considering the subject as random effect. The analysis will be performed by day (day 10 vs. day 16).

At each time point, estimates (LSmeans) of treatment effects and differences between moxifloxacin and placebo (ΔΔ) will be obtained with their 2-sided 90% CIs.

ΔΔ Estimates with their 90% CI will be plotted over time per day.

4.10.3.2 Categorical analyses

For the analyses described in this section, a treatment-emergent abnormality/finding will be defined as any abnormality/finding not already reported on any of the ECGs collected before the administration.

The analysis will be performed for setmelanotide and moxifloxacin evaluation.

4.10.3.2.1 Incidence of abnormal values

Average of the replicates extracted at each time point will be considered for this categorical analysis consisting on the display of the number and percentage of subjects by day and treatment group presenting at least one treatment emergent abnormality according to pre-defined thresholds presented in Table 4-2.

Table 4-2 Thresholds for abnormalities and corresponding flags for listings

Criteria	Flag for listings
Actual values	
HR<40 beats/min	L
HR>120 beats/min	H
PR>220 msec	H
QRS>120 msec	H
450<QTc≤480 msec	B
480<QTc≤500 msec	H
QTc>500 msec	P
Changes from baseline	
HR relative change >25%	I
PR relative change >25%	I
QRS relative change >25%	I
30<QTc increase≤60 msec	I
QTc increase>60 msec	I+

Flagging system will be used in listings to identify values above thresholds, also not necessarily related to clinically significant events, the flags can be interpreted as follows:
L: Low value - B: Borderline value - H: High value - P: Prolonged value
I: Noticeable increase from baseline - I+: marked increase from baseline
QTc denotes all correction methods

4.10.3.2.2 Incidence of morphological abnormalities

A qualitative interpretation of the ECG will be performed by the cardiologist according to the charter referenced as Standard Banook-Cardiabase Code List. This qualitative interpretation will provide a codification of the abnormalities detected on the recordings according to a code list grouping the abnormalities as:

- Q or Qs pattern
- Axis and Voltage
- Hypertrophy
- ST depression and elevation
- T/U wave abnormalities
- AV conduction
- Intraventricular conduction defects
- Rhythm
- Technical issue
- Overall conclusion

Each single extracted ECG will be analyzed resulting on the description of one or several morphological findings. Each unique finding will be classified according to the charter as normal,

abnormal not clinically significant or abnormal clinically significant. Then a global conclusion will be drawn on the whole ECG as the worst case reported over all findings.

Number and percentage of subjects presenting at least one treatment emergent abnormality, grouped according to the categories defined above, will be computed. The notion of abnormality in this analysis will refer to each individual finding for which the interpretation according to the charter is abnormal (either clinically or not clinically significant) and not ECG global conclusion which can mix normal and abnormal finding for the same ECG.

4.10.3.3 Assay sensitivity

The moxifloxacin C-QTc model will follow the same methods as described for setmelanotide model from Section 4.10.2.1.1 to 4.10.2.1.4. The sensitivity analysis will be performed separately for day 10 and day 16, due to the different baseline for moxifloxacin in group 2 and 3.

The assay sensitivity will be accepted if the slope for the relationship is significant at the 5% level and if the lower bound of the 90% CI of ΔΔQTc predicted at moxifloxacin C_{max} is above 5 ms.

4.10.4 Pharmacokinetics

4.10.4.1 Pharmacokinetic Concentrations

Setmelanotide and moxifloxacin plasma concentration versus time data will be summarized by treatment group and nominal time using descriptive statistics (number of observations [n], mean, median, CV%, SD, minimum, and maximum). Individual and mean concentration versus time plots will be presented.

Concentration Listings:

Pharmacokinetic concentration data for setmelanotide and moxifloxacin, will be listed by treatment group, and participant for the Safety Population. Concentration listings will include nominal PK sampling time, actual sampling times relative to dose administration, deviation from nominal time, and percent deviation from nominal time, and concentrations. Setmelanotide and moxifloxacin plasma concentrations below the lower limit of quantification (LLOQ) will be presented as below the limit of quantification (BLQ) in the listings and the LLOQ value presented as a footnote. Missing PK samples will be reported as no sample (NS) and/or not reportable (NR) and considered excluded from PK analysis.

Concentration Summary Tables:

Source data as reported from the laboratory will be used for calculation of concentration summary statistics. Tabular summaries for concentration-time data will report N (number of subjects who received treatment), n (number of subjects with non-missing values), and n (BLQ) (the number of subjects with BLQ samples).

Concentration for setmelanotide and moxifloxacin will be summarized by treatment group and nominal timepoint for the Pharmacokinetic Population. The following descriptive statistics will be presented for plasma setmelanotide and moxifloxacin concentrations obtained at each nominal time point: N, n, n(BLQ), arithmetic mean, SD, coefficient of variation (CV%), geometric mean, geometric CV% (calculated as: gCV% =SQRT(e^{s^2}-1)*100; where s is the SD of the log-transformed values), median, minimum, and maximum values.

For summary tables, all BLQs will be considered zero, and the number of BLQs and non-BLQs at each scheduled time point will be reported. Summary Statistics will not be calculated if non-BLQ concentrations at a scheduled time point are <3 and will be reported as NC.

The rules followed for calculation and presentation of concentration data with regards to the number of decimal places/significant digits for the listings of participant level concentrations and summary tables of concentration are as follows:

Concentration Listings and Tables	Rounding
Individual concentrations	*n* s f. as supplied by bioanalytical laboratory
Minimum and Maximum	*n* s.f. capped at 4
Mean/SD/Median/Geomean	*n+1* s f. capped at 4
CV%/gCV%	*1* d.p.
N/n	Whole number

s.f = significant figures, d.p. = decimal place

Concentration Figures:

For arithmetic mean linear/linear graphs, all BLQ values will be substituted with zero for calculation of arithmetic mean and for log/linear graphs the log transformed arithmetic mean will be displayed (this should not include zero).

For individual linear/linear and log/linear graphs all BLQ values will be substituted as follows:

- BLQs at the beginning of a participant profile (ie, before the first incidence of a measurable concentration) will be assigned to zero (except for intravenous administration when these BLQs should not be displayed). When using log/linear scale, these timepoints will be considered missing.
- BLQs at the end of a participant profile (ie, after the last incidence of a measurable concentration) will be set to missing.
- Single BLQs which fall between two measurable concentrations will be set to missing.
- Consecutive BLQs which fall between measurable concentrations will be set to missing. Measurable concentrations after consecutive BLQs will be set to missing.

To visualize participant-level concentrations and the comparison between groups for each treatment, the descriptive PK graphs listed below will be generated.

- Figure x.x.x: Individual participant profiles for Setmelanotide Plasma Concentration Time Data – (Linear Scale and Semi-Logarithmic Scale) (Safety Population)
- Figure x.x.x : Overlaid individual participant profiles for Setmelanotide Plasma Concentration Time Data – (Linear Scale and Semi-Logarithmic Scale) (Safety Population)
- Figure x.x.x : Mean (± SD) Setmelanotide Plasma Concentration Time Data – (Linear Scale and Semi-Logarithmic Scale) (PK Population)
- Figure x.x.x: Individual participant profiles for Moxifloxacin Plasma Concentration Time Data – (Linear Scale and Semi-Logarithmic Scale) (Safety Population)
- Figure x.x.x : Overlaid individual participant profiles for Moxifloxacin Plasma Concentration Time Data – (Linear Scale and Semi-Logarithmic Scale) (Safety Population)

- Figure x.x.x : Mean (± SD) Moxifloxacin Plasma Concentration Time Data – (Linear Scale and Semi-Logarithmic Scale) (PK Population)

Figures will be generated in black and white or color using unique line style and marker for each plot in the graph. For all PK concentration-time plots, linear scale will be used for x-axis (eg, do not use an ordinal scale).

4.10.4.2 Pharmacokinetic Parameters

PK parameters for setmelanotide will be calculated by NCA methods from the concentration-time data using Phoenix® WinNonlin® Version 8.2 or higher following these guidelines:

- Actual time from dose/injection will be used in the calculation of all derived pharmacokinetic parameters, except when parameters are calculated for safety/dose escalation meetings when nominal times may be used to calculate PK parameters.
- There will be no imputation of missing data.
- Handling of BLQ samples for derivation of plasma PK parameters
  - BLQs at the beginning of a participant profile (ie, before the first incidence of a measurable concentration) will be assigned to zero.
  - BLQs at the end of a participant profile (ie, after the last incidence of a measurable concentration) will be set to missing.
  - Single BLQs which fall between two measurable concentrations will be set to missing.
  - Consecutive BLQs which fall between measurable concentrations will be set to missing. Measurable concentrations after consecutive BLQs will also be set to missing.

Pharmacokinetic parameters will be estimated according to the guidelines presented in Table 4-3.

Table 4-3 Pharmacokinetic Parameter and Estimation

Parameter	Guideline for Derivation
C_{max}, t_{max}	Obtained directly from the observed concentration-time data
AUC_{tau}	Area under the plasma concentration-time curve during the dosing interval. If T_{last} < tau and extrapolation is not possible, AUC_{tau} will be replaced by AUC_{last} under condition that T_{last} is at least 95% of tau.
AUC_{last}	The AUC from zero time (pre-dose) to the time of last quantifiable concentration will be calculated by a combination of linear and logarithmic trapezoidal methods. Unless specifically requested and justified, the linear up/log down trapezoidal method will be employed.
$AUC_{0\text{-}inf}$	The area from zero time extrapolated to infinite time will be calculated as follows: $$AUC_{0\text{-}inf} = AUC_{last} + \frac{C_{last}}{\lambda_z}$$ where C_{last} is the last observed quantifiable concentration.
λz	1. The apparent terminal phase rate-constant (λz) will be estimated by linear regression of concentration versus time data presented in a log-linear scale. 2. Data are primarily monotonically decreasing in magnitude and are representative of the actual decline in the log concentration-time curve. 3. Only those data points that are judged to describe the terminal log-linear decline will be used in the regression. 4. A minimum number of three data points in the terminal phase will be used in calculating λz with the line of regression starting at any post-C_{max} data point (C_{max} should not be part of the regression slope). Unless otherwise determined by PK Scientist's best knowledge and judgment, if the adjusted correlation coefficient (R^2 adjusted) is <0.8, it will be excluded from the summary tables and statistical analysis of PK parameters, and λz and all the λz dependent parameters (i.e. t½, $AUC_{0\text{-}inf}$, CL/F, MRT, and V_z/F) will also be flagged and excluded from summary tables and statistical analysis. The reason for exclusion will be listed/footnoted in parameter listings. In case $0.8 \leq R^2$ adjusted < 0.9, it will be flagged together with all dependent parameters.

Parameter	Guideline for Derivation
	5. Unless otherwise determined by PK Scientist's best knowledge and judgment, the interval used to determine λz should be equal or greater than 1.5-fold the estimated t½, and if less than 1.5-fold, λz will be flagged in listings and excluded from summary tables and statistical analysis of PK parameters. All the derived parameters (i.e. t½, $AUC_{0\text{-}inf}$, CL, MRT, and Vz) will also be flagged from listings and excluded from statistical analysis of PK parameters. The reason for exclusion will be listed/footnoted in parameter listings. 6. Data points may be dropped from the linear regression if the PK Scientist considers the reported values to be anomalous. Any data points so designated should remain in the listings with a footnote and be identified in the study report with a rationale for exclusion.
CL/F	Apparent clearance of parent drug will be calculated from: $CL/F = \frac{Dose}{AUC_{0\text{-}inf}}$
V_z/F	Apparent volume of distribution at terminal phase may be calculated from: $V_z/F = \frac{Dose}{\lambda_z \times AUC_{0\text{-}tau}} = (CL/F)/\lambda_z$

PK Parameters Listings:

PK parameters will be listed by participant for the Safety Population. PK parameters that will be excluded from summary tables and statistical analyses of PK parameters will be flagged and footnoted with the reason for exclusion.

PK Parameter Summary Tables:

PK parameters will be provided by CPMS group. Biostatistics group will consider this the PK parameters source data and will use this data without rounding for calculation of PK parameters summary statistics tables.

PK parameters will be summarized by treatment group and for the PK Population.

Tabular summaries for PK parameters will report N (number of subjects who received treatment) and n (number of subjects with non-missing values).

Descriptive statistics for calculated PK parameters will include N, n, arithmetic mean, SD, CV%, geometric mean, median, minimum, and maximum values. For t_{max}, only N, n, median, minimum and maximum values will be presented. No descriptive statistics will be determined when fewer than three individual PK parameters are available.

The rules followed for presentation of PK parameters data with regards to the number of decimal places/significant digits for the listings of participant level PK parameters and summary tables of PK parameters are as follows:

PK Parameter Listings and Tables	Rounding
Derived Individual parameters	4 s f.
Directly Derived Individual parameters (C_{max})	*n* s.f. as supplied by the analytical laboratory but not more than 4 s f.
Minimum and Maximum	4 s f.
Mean/SD/Median/Geomean	4 s f.
CV%	1 d.p.
N/n	Whole number
Exceptions for PK Tables	
t_{max} individuals and min/max	2 d.p
t_{max} median only	2 d.p

s.f = significant figures, d.p. = decimal place

4.11 Safety Evaluation

All safety summaries and analyses will be based upon the Safety Population as defined in Section 4.4 of this document.

All summaries will be provided by treatment group.

Placebo across treatment group will be pooled.

4.11.1 Adverse Events

A treatment-emergent adverse event (TEAE) is defined as an AE that begins or that worsens in severity after at least one dose of the study drug has been administered.

Any AEs with incomplete start and end dates/times will be treated as per

Table 6-2.

Adverse events will be coded using the MedDRA Version 23.1 or higher.

A table of the number (percentage) of subjects and number of adverse events with treatment emergent adverse events (TEAEs) will be presented and summarized by SOC, PT and treatment group. If more than one event with the same preferred term occurred for the same subject, then the subject will be counted only once for that preferred term.

A table of the number (percentage) of subjects and number of adverse events with TEAEs will be presented and summarized by SOC, PT, relationship, and treatment group. If more than one event with the same preferred term occurred for the same subject, then the subject will be counted only once with the highest relationship for that preferred term.

A table of the number (percentage) of subjects and number of adverse events with TEAEs, summarized by SOC, PT, severity, and treatment group will be presented. If more than one event occurred with the same preferred term for the same subject, then the subject will be counted only once with the maximum severity level of that preferred term.

Adverse event summaries will be ordered in terms of decreasing frequency for SOC, and PT within SOC, in the test treatment group, and then similarly by decreasing frequency in the reference treatment group, and then alphabetically for SOC, and PT within SOC.

For each subject and each adverse event, the worst severity recorded will be attributed and used in the by-severity summaries. Similarly, the worst causality (most related to treatment) will be attributed and used in the by-causality summaries. If severity or causality is missing, a conservative approach for AE assessment (taking into account the worst case) will be followed.

A by-subject listing of all adverse events (including non-treatment-emergent events) will be provided. This listing will be presented by treatment group and will include: subject identifier, adverse event (SOC, PT, and verbatim term), date of onset, date of resolution, duration, severity, seriousness, action taken, outcome and causality.

4.11.2 Deaths, Serious Adverse Events, and Other Significant Adverse Events

Listings of SAEs, deaths and other significant adverse events will be provided along with SAEs and AEs leading to withdrawal from treatment or study.

4.11.3 Clinical Laboratory Evaluation

Blood samples for clinical laboratory tests are to be drawn as per shown in Table 6-1.

Specific tests are described below:

- **Hematology:** complete blood count with platelet count and standard indices will be obtained.

[redacted]

- **Chemistry:** sodium, potassium, chloride, carbon dioxide (CO_2), albumin, total protein, glucose, blood urea nitrogen (BUN), creatinine, uric acid, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltranspeptidase (GGT), creatine phosphokinase (CPK), alkaline phosphatase, total bilirubin, direct bilirubin, lactate dehydrogenase (LDH), calcium and phosphorus.
- **Coagulation Profile:** prothrombin time (PT) or international normalized ratio (INR), and partial thromboplastin time (PTT), also referred to as activated partial thromboplastin time (aPTT).
- **Urinalysis:** pH, glucose, protein, ketones, bilirubin, blood, urobilinogen, specific gravity, nitrite, and leukocytes by dipstick analysis or machine urinalysis. Urine microscopic examination will be performed if positive findings on dipsticks warrant further examination.

Clinical laboratory test results of hematology, chemistry, coagulation profile and urinalysis will be provided by treatment group.

All TLFs will display only the standard international (SI) units after conversion by means of standard conversion factors.

Quantitative clinical laboratory variables, ie, hematology, chemistry, coagulation profile, and urinalysis will be summarized using descriptive statistics (n, mean, SD, minimum, maximum and median) by treatment group and time-point. Additionally, a within-participant change will be calculated as the post-baseline measurement minus the baseline measurement and summarized in the same way.

Baseline definition will be defined in section 4.2.4.

Any quantitative laboratory parameters that are given as '<xx' or '>xx' in the database will be imputed with the absolute value of the number without the sign (e.g., <2.2 will be imputed as 2.2) for the calculation of the changes from baseline and for the descriptive statistics. In the listings, no imputations will be performed, and all data will be displayed as recorded in the database.

Each laboratory result will be classified as low (L), normal (N), or high (H) at each time point according to the laboratory supplied reference ranges. For hematology and biochemistry, shift tables will be presented showing the number and percentage of subjects with shifts from baseline to each postdose time point. Tabulations will be presented by treatment group.

Measurements obtained at Screening and End of Study (EOS) will not be included in the shift tables.

Measurements obtained prior to dosing in each period will be included in the tabulations for the treatment received in that specific treatment period.

Frequency tabulations of qualitative clinical laboratory variables (urinalysis) will be presented by treatment group and time-point.

All laboratory data will be displayed in listings.

Laboratory abnormalities that are considered clinically significant (CS) are recorded in the database as AEs. Therefore, no tabulation of laboratory values meeting any CS criteria (except liver chemistry) will be presented as all relevant information will be presented in the AE summaries.

Results of pregnancy tests (females only), urine drugs screen tests will be listed only.

4.11.4 Vital Signs

Vital signs will be performed as shown in Table 6-1. The following vital signs measurements will be obtained:

- Supine Systolic blood pressure (SBP) [mmHg].
- Supine Diastolic blood pressure (DBP) [mmHg].
- Supine Heart rate (beats per minute [bpm])
- Pulse rate (bpm).
- Tympanic (ear) Body temperature [°C].

Vital signs data will be listed by subject including changes from baseline. The baseline for the vital signs measurements will be the measurement on Day -1.

Descriptive statistics (n, mean, SD, median, minimum, maximum) for absolute values and changes from baseline will be summarized descriptively in tabular format by time of collection and by treatment group.

4.11.5 12-Lead Electrocardiogram (ECG)

Standard safety 12-lead ECGs will be performed as shown in Table 6-1.

The following ECG parameters will be recorded:

PR interval (msec)

QRS interval (msec)

QT interval (msec)

QTc interval (msec)

QT interval corrected using the Fridericia correction formula (QTcF) (msec).

HR (beats per minute [bpm]).

The ECG will be evaluated by the Investigator as ‘Normal’, ‘Abnormal, Not clinically significant (NCS)’ or ‘Abnormal, CS’.

All ECG parameters will be listed by participant for each treatment group and time point including changes from baseline.

Baseline is defined in section 4.2.4.

Descriptive statistics for absolute values and changes from baseline will be presented by treatment group. A categorical QTc analysis will also be performed.

Measurements obtained at Screening and EOS will not be included in the shift tables.

Measurements obtained prior to dosing in each period will be included in the tabulations for the treatment received in that specific treatment period.

4.11.6 12-Lead Holter ECG

A 12-lead Holter ECG recording will be performed for 24 hours on Day -1, Day 10, and Day 16 of each treatment group. The Holter recording will be used to obtain a measurement of mean HR per hour on each day.

Mean HR per hour on Day -1, Day 10, and Day 16 will be summarized (mean, SD, minimum, maximum and median) by time point (each hour) and treatment.

Day -1 measurements will be considered as baseline.

Individual measurements of mean HR per hour will be displayed in a listing including the time-matched changes from baseline from Day -1 to Day 1 and the placebo-corrected time-matched changes and Holter's comment.

4.11.7 Physical Examination

Physical examinations will be performed as shown in Table 6-1.

The full physical examination includes an assessment of general appearance and a review of systems (dermatologic, head, eyes, ears, nose, mouth/throat/neck, thyroid, lymph nodes, respiratory, cardiovascular, gastrointestinal, extremities, musculoskeletal, neurologic systems).

Changes from baseline in any physical examination findings identified by the Investigator as clinically significant must be recorded as an AE on the appropriate eCRF.

Clinically significant physical examination, with respect to number frequencies and percentage, will also be tabulated by time of collection and treatment group Clinically significant physical examination findings will be listed.

Other safety evaluations of comprehensive skin examination and Fitzpatrick scale will be listed.

[redacted]

[redacted]

[redacted]

[redacted]

4.12.1 Columbia Suicide Severity Rating Scale (C-SSRS)

C-SSRS will be summarized by time of collection and treatment group.

C-SSRS score will be listed.

4.13 Determination of Sample Size

Using a 2-sample t-test with a 1-sided 0.05 significance level, a sample size of 60 evaluable subjects who complete the study (20 subjects in Group 1 receiving SC setmelanotide and 20 subjects each in Groups 2 and 3 receiving SC placebo) will provide at least 85% power to exclude that setmelanotide causes more than a 10 msec QTc effect at clinically relevant plasma levels, as shown by the upper bound of the 2-sided 90% confidence interval (CI) of the model-predicted QTc effect (ΔΔQTcF) at the observed geometric mean C_{max} of setmelanotide on Day 10 and Day 16 in the study individually.

To demonstrate assay sensitivity using moxifloxacin with a 1-sided 0.05 significance level, a sample size of 20 evaluable subjects receiving moxifloxacin and 20 evaluable subjects receiving placebo at each ECG assessment day (Days 10 and 16) will provide at least 81% power to demonstrate assay sensitivity with a 1-sided 0.05 significant level. The power to detect the effect of moxifloxacin on QTc over the entire crossover period is at least 86%.

As such, a total of approximately 72 subjects are planned for enrollment to achieve 60 completers, accounting for dropouts.

4.14 Changes in the Conduct of the Study or Planned Analysis

Not Applicable.

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vi. SAS® Version 9.4 of the SAS System for Personal Computers. Copyright©®2002-2003. SAS Institute Inc. SAS and all other SAS Institute Inc. product or service names are registered trademarks or trademarks of SAS Institute Inc., Cary, NC, USA.
vii. Phoenix®WinNonlin® Professional Software Version 8.2. https://www.certara.com

[redacted]

6 APPENDICES

6.1 Schedule of Assessments

Table 6-1 Schedule of Assessments

Study Period	Screening	[redacted]	Check-In	Baseline	Treatment										Follow-up	Treatment Discontinuation
Study Day Assessment	-28 to -2		-2	-1	1	2-6	7	8	9	10	11-14	15	16	17	23 ± 2	7 ± 4 days after last SC dose
Informed consent	X															
Inclusion/exclusion review	X		X													
Medical history review	X															
Physical exam [a]	X			X										X	X	X
Comprehensive skin exam [b]	X															
Fitzpatrick classification scale	X															
Height and weight [c]	X			X										X	X	X
Vital signs [d]	X			X										X	X	X
Pregnancy test [e]	X		X												X	X
FSH test	X		X													
Urine drug screen	X		X													
CRU confinement			X	X	X	X	X	X	X	X	X	X	X			
CRU discharge														X		
Fasting [f]			X						X			X				
Meal administration [g]				X				X	X	X	X	X	X			

Study Period	Screening	[redacted]	Check-In	Baseline	Treatment										Follow-up	Treatment Discontinuation
Study Day Assessment	-28 to -2		-2	-1	1	2-6	7	8	9	10	11-14	15	16	17	23 ± 2	7 ± 4 days after last SC dose
Setmelanotide administration [h]					X	X	X	X	X	X	X	X	X			
Moxifloxacin administration [i]										X			X			
PK sampling [j]										X			X			
Continuous 12-lead ECGs (Holter monitor) [k, l]				X						X			X			
Safety 12-lead ECGs [l, m]	X			X			X							X	X	X
Clinical labs [n]	X			X			X							X	X	X
Adverse events	X		←		Continuous							→				X
Concomitant medication review	X		←		Continuous							→				X
C-SSRS [o]	X			X										X		X

[redacted]

CRU = Clinical Research Unit; C-SSRS = Columbia Suicide Severity Rating Scale; ECG = electrocardiogram; FSH = follicle stimulating hormone; INR = international normalized ratio; PK = pharmacokinetic; PT = prothrombin time; PTT (or aPTT) = partial thromboplastin time

[a] A full physical exam will be conducted at Screening. A brief physical exam will be conducted at each check-in, study discharge, and follow-up.

[b] A comprehensive skin evaluation will be performed by the Investigator at Screening. If any concerning lesions are identified during the screening period, the subject should be referred to a dermatologist for assessment and potential biopsy and results should be confirmed as to whether the lesion is benign prior to first dose of setmelanotide. If the pre-treatment biopsy results are of concern, the subject will be excluded from the study.

[c] Height will only be collected at Screening.
[d] Supine blood pressure, supine heart rate, pulse rate, and tympanic (ear) body temperature. Heart rate and blood pressure will be measured using the same arm for each reading after the subject has been resting in the supine position for at least 5 minutes. For timepoints at which vital signs, PK blood samples, and/or ECGs are all to be collected, ECGs should be collected first followed by vital signs then PK samples, with the timing of the PK sample to occur at the nominal time.
[e] Serum pregnancy test will be performed at Screening. Urine pregnancy test will be performed on all other designated days.
[f] On the evening before continuous ECG sampling, subjects will undergo a 10-hour overnight fast.
[g] Breakfast is to be administered at approximately 7:00 AM every day in the CRU (30 minutes pre-dose). Lunch, dinner, and a snack will be administered at approximately 12:00 PM (after 4-hour assessments), 6:00 PM (after 10-hour assessments), and 9:00 PM, respectively.
[h] **Group 1** will receive SC setmelanotide QD (approximately 7:30 AM) at a dose of 2 mg from Days 1 to 7; 3 mg from Days 8 to 10; 5 mg from Days 11 to 13; and 7 mg from Days 14 to 16.
Groups 2 and 3 will receive SC placebo QD (approximately 7:30 AM) from Days 1 through 16.
All doses will be double-blinded.
[i] **Group 1** will receive oral placebo with their SC setmelanotide dose on Days 10 and 16.
Group 2 will receive oral moxifloxacin 400 mg on Day 10 and oral placebo on Day 16.
Group 3 will receive oral placebo on Day 10 and oral moxifloxacin 400 mg on Day 16.
All doses will be double-blinded. On Days 10 and 16, subjects should receive SC setmelanotide/placebo first. Oral moxifloxacin/placebo should then be administered within 2 minutes of the SC dose.
[j] Blood samples for quantification of setmelanotide and moxifloxacin concentrations will be collected at pre-dose and 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 9, 10, 12, 16, and 24 hours post-dose on Days 10 and 16. The PK samples collected at 0.5 hours post-dose will have a sampling window of ± 2 minutes, samples collected from 1 hour though 2.5 hours post-dose will have a sampling window of ± 5 minutes, samples collected from 3 hours through 10 hours post-dose will have a sampling window of ± 10 minutes, and samples collected from 12 through 24 hours postdose will have a sampling window of ± 20 minutes; however, collection of samples outside of the window will not be considered protocol deviations. The timing of all PK samples should be recorded to the nearest minute. For timepoints at which vital signs, PK blood samples, and/or ECGs are all to be collected, ECGs should be collected first followed by vital signs then PK samples, with the timing of the PK sample to occur at the nominal time.
[k] ECGs will be extracted at Day -1 and on Days 10 and 16 at the following timepoints: pre-dose and 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 9, 10, 12, 16, and 24 hours post-dose. The Day -1 extractions will begin at 7:30 AM (the time of anticipated dosing on Days 10 and 16).
[l] For ECG collection, subjects will rest in the supine position for at least 10 minutes before and 5 minutes after each timepoint. For timepoints at which vital, PK blood samples, and/or ECGs are all to be collected, ECGs should be collected first followed by vital signs then PK samples, with the timing of the PK sample
to occur at the nominal time.
[m] Safety ECGs will be collected at the Screening visit, CRU Baseline (Day -1), final CRU discharge, and the Follow-up visit.
[n] Safety clinical laboratory tests will include: hematology with platelet count and standard indices, chemistry panel (includes sodium, potassium, chloride, CO_2, albumin, total protein, glucose, BUN, creatinine, uric acid, AST, ALT, GGT, CPK, alkaline phosphatase, total bilirubin, direct bilirubin, LDH, calcium, phosphorus, magnesium), and urinalysis with microscopic analysis if positive findings on dipsticks warrant further examination. Safety laboratories will also include a coagulation profile (PT or INR, and PTT also referred to as aPTT).
[o] In order to be eligible for the study, at Screening, a subject cannot have a suicidal ideation of type 4 or 5, a history of a suicide attempt in the past 20 years, or any suicidal behavior in the last month. If at any time during the study a subject has a suicidal ideation of type 4 or 5, or any suicidal behavior, the subject should be referred to a mental health professional.

Table 6-2 Algorithm for Treatment-Emergent Adverse Events:

Start/Increase Severity Date	Stop Date	Action
Known	Known	Considered as a treatment-emergent adverse event (TEAE) if start date on or after the date of the first dose of investigational product (IP)
	Partial	Considered as a TEAE if start date on or after the date of the first dose of IP. The last day of the month and the last month (ie, December) will be used if the stop day/month is missing.
	Missing	Considered as a TEAE if start date on or after the date of the first dose of IP
Partial, but known components show that it cannot be on or after first IP taken date	Known	Not a TEAE. The first day of the month and January will be used if the start day/month is missing.
	Partial	Not a TEAE. The first day of the month and January will be used if the start day/month is missing. The last day of the month and the last month (ie, December) will be used if the stop day/month is missing.
	Missing	Not a TEAE. The first day of the month and January will be used if the start day/month is missing.
Partial, could be on or after first IP taken date	Known	Considered as TEAE, if stop date is after first IP taken date. The first IP taken date will be used if start date is in the same month/year with first IP taken date, or the first day of the month and January will be used if the start day/month is after first IP taken date Considered as not TEAE, if stop date is prior to first IP taken date. The first day of the month and January will be used if the start day/month is missing.
	Partial	Considered as TEAE. The first IP taken date will be used if start date is in the same month/year with first IP taken date, or the first day of the month and January will be used if the start day/month is after first IP taken date. The last day of the month and the last month (ie, December) will be used if the stop day/month is missing.
	Missing	Considered as TEAE. The first IP taken date will be used if start date is in the same month/year with first IP taken date, or the first day of the month and January will be used if the start day/month is after first IP taken date.
Missing	Known	Considered as TEAE if stop date is on or after the date of the first dose of IP.
	Partial	The last day of the month and the last month (ie, December) will be used if the stop day/month is missing. If the imputed stop date is on or after the first dose of IP considered as a TEAE; if the year is missing, considered as a TEAE
	Missing	Considered as a TEAE

Table 6-3 Algorithm for Prior/Concomitant Medications:

Start Date	Stop Date	Action
Known	Known	If stop date is prior to the date for the first dose of IP, considered as prior; if stop date is on or after the date for the first dose of IP, considered as concomitant.
	Partial	The last day of the month and the last month (ie, December) will be used if the day/month of stop date is missing. If the impuated stop date is prior to the date for the first dose of IP, considered as prior; if the imputed stop date is on or after the date for the first dose of IP, considered as concomitant.
	Missing	Considered as concomitant.
Partial	Known	If stop date is prior to the date for the first dose of IP, considered as prior; If stop date is on or after the date for the first dose of IP, considered as concomitant. The first day of the month and January will be used if the start day/month is missing.
	Partial	The last day of the month and the last month (ie, December) will be used if the day/month of stop date is missing. If the impuated stop date is prior to the date for the first dose of IP, considered as prior; if the imputed stop date is on or after the date for the first dose of IP, considered as concomitant. The first day of the month and January will be used if the start day/month is missing.
	Missing	Considered as concomitant. The first day of the month and January will be used if the start day/month is missing.
Missing	Known	If stop date is prior to the date for the first dose of IP, considered as prior; if stop date is on or after the date for the first dose of IP, considered as concomitant.
	Partial	The last day of the month and the last month (ie, December) will be used if the day/month of stop date is missing. If the impuated stop date is prior to the date for the first dose of IP, considered as prior; if he imputed stop date is on or after the date for the first dose of IP, considered as concomitant.
	Missing	Considered as concomitant.