Statistical Analysis Plan

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A Phase 2 Randomized, Double-Blind, Placebo-Controlled Study to Evaluate the Safety, Tolerability, and Pharmacodynamic Effects of GLY-200 in Type 2 Diabetic Patients

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TABLE OF CONTENTS

TITLE PAGE **1**
SIGNATURE OF STUDY STATISTICIAN **2**
SIGNATURE OF GLYSEND INC **3**
TABLE OF CONTENTS **4**

LIST OF ABBREVIATIONS **6**
1 INTRODUCTION **8**
2 STUDY OBJECTIVES **8**
2.1 Primary Objective 8
2.2 Secondary Objectives 8
2.3 Exploratory Objectives 8
3 STUDY OVERVIEW **8**
3.1 Study Design 8
3.2 Randomization and Blinding 9
4 STUDY ENDPOINTS **14**
4.1 Primary Endpoints 14
4.2 Secondary Endpoints 14
4.3 Exploratory Endpoints 14
5 ANALYSIS SETS **14**
5.1 Enrolled Analysis Set 14
5.2 Randomized Analysis Set 14
5.3 Safety Analysis Set 15
5.4 Full Analysis Set 15
5.5 Per-Protocol (PP) Analysis Set 15
5.6 Pharmacokinetic (PK) Analysis Set 15
6 STUDY SUBJECTS **15**
6.1 Analysis Sets 15
6.2 Subject Disposition 15
6.3 Demographic and Baseline Characteristics 16
6.4 Medical History 16
6.5 Inclusion/Exclusion Criteria and Subject Eligibility 16
6.6 Protocol Deviations 16
6.7 Study Drug Administration and Extent of Exposure 16
6.8 Concomitant Medications 16
7 STATISTICAL ANALYSIS METHODS **17**
7.1 General Considerations 17
7.1.1 Statistical Notation and Presentation 17
7.1.2 Baseline and Change from Baseline 18
7.1.3 Study Day 18
7.1.4 Handling of Missing or Partial Data 18
7.2 Pharmacodynamic and Pharmacokinetic Analyses 18
7.2.1 Data Handling 19
7.2.1.1 Plasma 19
7.2.1.2 Urine 19
7.2.1.3 Feces 19
7.2.1.4 Continuous Glucose Monitoring (CGM) 19
7.2.2 Pharmacodynamic (PD) Analyses 21
7.2.2.1 Analysis of Plasma MMTT PD Measurements 21
7.2.2.2 Analysis of Glucose Profile Derive from CGM Data 22
7.2.2.3 Analysis of Urine PD Measurements 22
7.2.3 Pharmacokinetic (PK) Analyses: Boron Elimination in Feces and Urine 23

7.2.3.1 Urine 22
7.2.3.2 Feces 23
7.3 Safety Analyses 23
7.3.1 Adverse Events 23
7.3.1.1 Adverse Events of Special Interest (AESI) 24
7.3.2 Clinical Laboratory Values 25
7.3.3 Vital Signs and Body Measurements 25
7.3.4 Physical Examinations 26
7.3.5 ECG (12-lead Electrocardiogram) 26
7.3.6 Fluid Balance 26
7.3.7 Food Intake and Appetite Assessments 26
8 CHANGE TO THE PLANNED ANALYSES FROM PROTOCOL 28
9 POWER AND SAMPLE SIZE 28
10 STATISTICAL SOFTWARE 28
11 REFERENCES 28

LIST OF TABLES

Table 1. Schedule of Activities for GLY-200-002 10
Table 2: Mixed-Meal Tolerance Test Schedule (Days -1, 1, 7 and 14) 12

LIST OF ABBREVIATIONS

Term	Definition
ADA	American Diabetes Association
AE(s)	adverse event(s)
AESI	adverse event of special interest
ANCOVA	analysis of covariance
ANOVA	analysis of variance
ATC	anatomical therapeutic chemical
AUC	area under the curve
BID	twice a day
BLOQ	below the limit of quantification
BMI	body mass index
BP	blood pressure
CGM	continuous glucose monitor
C_{max}	maximum observed blood drug concentration
ECG(s)	electrocardiogram(s)
EOS	end of study
ET	early termination
FSH	follicular stimulating hormone
HR	heart rate
LDL	low-density lipoprotein
LLOQ	lower limit of quantification
MedDRA	Medical Dictionary for Regulatory Activities
Min	minimum
Max	maximum
MMRM	mixed model with repeated measures
MMTT	mixed meal tolerance test
MWG	mean weighted glucose
PD	pharmacodynamic(s)
PI	primary investigator
PK	pharmacokinetic(s)
PR	PR interval of ECG
PT	preferred term
PYY	peptide YY
QRS	QRS interval of ECG
QT	QT interval of ECG
QTc	corrected QT interval of ECG
QTcB	corrected QT interval using Bazett's correction method
QTcF	corrected QT interval using Fridericia's correction method
RR	respiratory rate
SAE(s)	serious adverse event(s)
SAP	statistical analysis plan
SD	standard deviation
SE	standard error (of the mean)

Term	Definition
SOC	system organ class
T2DM	type 2 diabetes mellitus
TEAE(s)	treatment-emergent adverse event(s)
T_{max}	time to maximum blood concentration
VAS	visual analog scale
WHO-DD	World Health Organization Drug Dictionary

1 INTRODUCTION

This document details the planned statistical analyses for Glyscend Inc protocol GLY-200-02, a study titled "A Phase 2 Randomized, Double-Blind, Placebo-Controlled Study to Evaluate the Safety, Tolerability, and Pharmacodynamic Effects of GLY-200 in Type 2 Diabetic Patients".

This statistical analysis plan (SAP) was developed based on the Clinical Protocol GLY-200-02 (amendment 2 dated October 06, 2022).

2 STUDY OBJECTIVES

2.1 Primary Objective

- To assess the safety and tolerability of GLY-200 over the 14-day treatment period.

2.2 Secondary Objectives

- To assess the effect of GLY-200 on the postprandial plasma glucose and insulin profile following a standardized meal on Day 1, Day 7, and Day 14 compared to baseline.

2.3 Exploratory Objectives

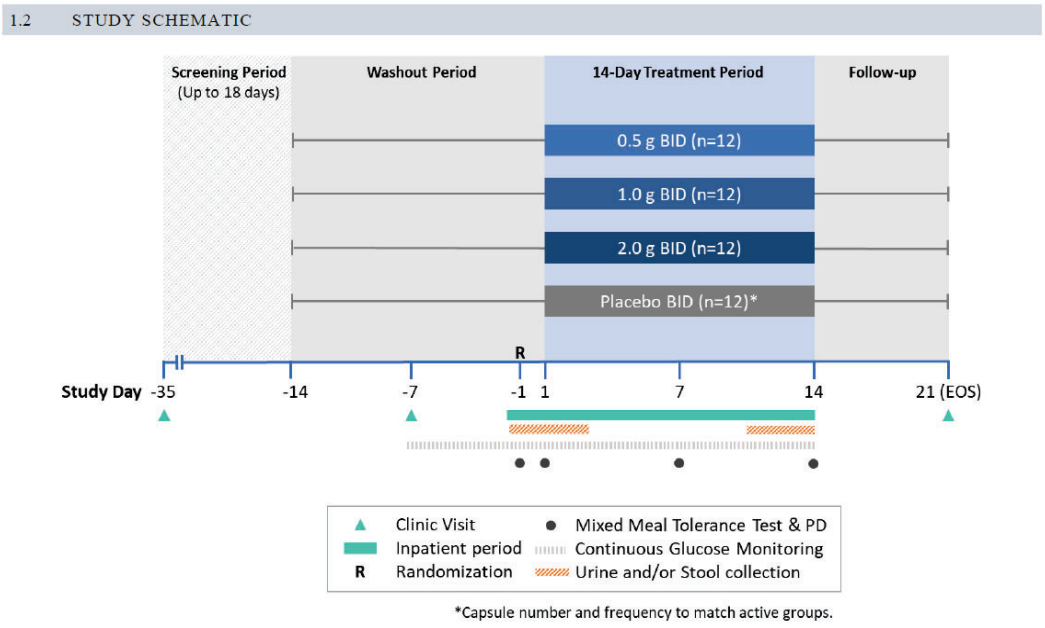
- To evaluate the pharmacokinetics (PK) of GLY-200 over 14 days of treatment as assessed by appearance of boron in the urine and feces.
- To assess the effect of GLY-200 on 24-hour glucose profiles as assessed by Continuous Glucose Monitoring (CGM).
- To assess the effect of GLY-200 on other pharmacodynamic measures including effects on GLP-1, GIP, PYY, ghrelin, glucagon, and bile acids (fractionated and total).
- To assess the effects of GLY-200 on oxalate, phosphate, and uric acid in the urine.

3 STUDY OVERVIEW

3.1 Study Design

This study is a Phase 2, randomized, double-blind, placebo-controlled, single-center study in adult patients with type 2 diabetes mellitus (T2DM). The study will evaluate the safety and tolerability of oral GLY-200. Following a 14-day washout of metformin, approximately 48 subjects will be randomized to 1 of 3 active treatment groups or 1 of 3 placebo groups. Subjects will participate in a ≤ 18-day screening period followed by a metformin washout period of 14 days, and an inpatient period of 16 days. Dosing will occur for 14 days. A Mixed Meal Tolerance Test will be performed on Day -1, post-dose on Days 1, 7 and 14. A follow-up clinic visit will occur on Day 21 [End of Study (EOS)] or Early Termination (ET). See Figure 1 for study schematic, Table 1 for Schedule of Activities and Table 2 for Mixed Meal Tolerance Test Schedule.

Figure 1



3.2 Randomization and Blinding

A total of 48 subjects with T2DM are planned for enrollment into the study. Subjects will be blinded to treatment assignment (active or placebo), but not dose level (group number).

The planned treatment groups are:

- Active Group 1: 0.5 g GLY-200 (1 x 0.5 g capsule)
- Active Group 2: 1.0 g GLY-200 (2 x 0.5 g capsules)
- Active Group 3: 2.0 g GLY-200 (4 x 0.5 g capsules)
- Placebo Group 1: 1 placebo capsule
- Placebo Group 2: 2 placebo capsules
- Placebo Group 3: 4 placebo capsules

Subjects will be randomly assigned to each treatment group based on a computer-generated randomization schedule, which will dictate the treatment assignment for the subject.

Subjects who prematurely discontinue from the study may be replaced at the discretion of the Sponsor and Investigator. Replacement subjects will have the same treatment assignment as the original subject.

Table 1. Schedule of Activities for GLY-200-002

STUDY PERIOD	**Screening**	**Washout**		**Confinement/Inpatient**							**Follow-Up/EOS**	**Early Term-ination (ET)**
STUDY DAY [1]	**Day -35 to Day -18 (Up to 2 visits)**	**Day -14 (+2 days) Phone Visit**[2]	**Day -7 (-3 days)**	**Day -1 (Check-in Day -2)**	**Day1**		**Days 2-6 (Each day)**	**Day 7**	**Days 8-13 (Each day)**	**Day 14 (Check-out)**	**Day 21 (±1 day)**	
					Pre-dose	**Post-dose**						
Informed Consent	X											
Eligibility Criteria [3]	X	X	X	X	X [3]							
Demographics	X											
Medical History	X											
Height [4]	X											
Body Weight	X			X			X	X	X	X	X	X
Prior/Concomitant Medication Assessment	X	X	X	X	X	X	X	X	X	X	X	X
Physical Examination (Full) [5]	X										X	X
Physical Examination (Abdominal Examination) [6]	X				X		X	X	X	X	X	X
Physical Examination (Symptom Directed) [7]					X	X	X	X	X	X		
Vital Signs (BP, HR, RR, Temp) [8]	X				X		X	X	X	X	X	X
12-Lead Safety ECG [9]	X				X			X		X	X	X
Clinical Laboratory Tests (Blood) [10]	X				X			X		X	X	X
Clinical Laboratory Tests (Urinalysis) [10]	X				X			X		X	X	X
Serology [11]	X											
Pregnancy Test (Serum; for females only)	X											
Pregnancy Test (Urine; for females only) [12]					X					X	X	X
Urine Drug Screen	X				X							
FSH Test (postmenopausal women)	X											
Adverse Events [13]	X	X	X	Ongoing							X	X
Dose Administration [14]					Inpatient Dosing							

STUDY PERIOD	**Screening**	**Washout**		**Confinement/Inpatient**							**Follow-Up/EOS**	**Early Term-ination (ET)**
STUDY DAY [1]	**Day -35 to Day -18 (Up to 2 visits)**	**Day -14 (+2 days) Phone Visit[2]**	**Day -7 (-3 days)**	**Day -1 (Check-in Day -2)**	**Day1 Pre-dose**	**Day1 Post-dose**	**Days 2-6 (Each day)**	**Day 7**	**Days 8-13 (Each day)**	**Day 14 (Check-out)**	**Day 21 (±1 day)**	
Fecal Sampling (Exploratory PK) [15]				X	X				Days 10-14		X [15]	X
Blood Sampling (PD: Glucose and Insulin) [16]				X	X	X				X	X	X
Blood Sampling (Exploratory PD: Hormones, Bile acids, Nutrients) [16]				X	X	X				X	X	X
Blood Sampling (Metabolites or exploratory PK/PD) [16]					X					X		
Urine Sampling (Exploratory PK and PD) [17]				X	X	X	Days 2-3		Days 10-14		X [17]	X
Fluid Balance [18]					X	X	Days 2-3		Days 10-14			
Documentation of food intake				Ongoing								
Food Intake and Appetite VAS Assessments [19]				X	X	X			Day 13 only			
Placement of CGM device [20]			X	X				X				
Mixed Meal Tolerance Test (MMTT) [21]				X		X		X		X		

Abbreviations: BP = blood pressure; CGM = continuous glucose monitor; ECG = electrocardiogram; EOS = End of Study; ET = Early Termination; FSH = follicular stimulating hormone; HR = heart rate; MMTT = mixed meal tolerance test; PD = pharmacodynamic(s); PK = pharmacokinetic(s); RR = respiratory rate; Temp = body temperature; VAS = visual analog scale.

Note: Prescreening laboratory values can be used to determine subject eligibility if they were collected within the screening window.

Footnotes:

1. Subjects are required to fast for at least 8 hours prior to every morning assessment. “Pre-dose” and “post-dose” refer to the first dose of the day.
2. At the Day -14 phone visit, site staff are to instruct subjects to begin the metformin washout.
3. Eligibility to be confirmed prior to dose administration on Day 1.
4. BMI to be calculated using the height value recorded at screening.
5. Full physical examination to include, at a minimum, assessment of the following: assessment of the following systems: HEENT, Mouth/Dental (if required), Neck (incl Thyroid & Nodes), Cardiovascular, Respiratory, Gastrointestinal, Renal, Neurological, Musculoskeletal, Skin, Other
6. Abdominal physical examination to include palpation, auscultation (noting presence and frequency of bowel sounds), percussion (looking for signs of peritonitis or localized tenderness). Abdominal examinations are to be conducted at indicated study visits prior to dosing, if applicable.
7. Symptom directed physical examination will include volunteer elicited examinations only.

8. Patients should be resting in a supine position for at least 5 minutes prior to and during vital signs measurements. Vital signs will be assessed at indicated study visits prior to dosing, if applicable.
9. At Screening and prior to first dose, triplicate 12-lead ECGs each separated by at least 1 minute will be taken to establish eligibility and baseline. The average value for the triplicate will be utilized for assessing QTcF exclusion criteria. Post-first dose ECGs will be single readings. In case of evident bad quality (e.g., muscle tremor) of the tracing, the ECG will be repeated. ECGs will be taken after at least 5 minutes resting quietly in a supine position. ECGs will be assessed at indicated study visits prior to dosing, if applicable.
10. Hematology, chemistry, coagulation tests, and urinalysis will be performed. Refer to protocol Section 8.2.7. Subjects will be required to fast for at least 8 hours prior to clinical laboratory blood sampling. Specific vitamin and mineral panels will only be assessed on Day 1 pre-dose and Day 14.
11. Human immunodeficiency virus, hepatitis B surface antigen, hepatitis C virus antibody testing. Subjects to be counselled if tests return positive result.
12. If the urine pregnancy test is positive, pregnancy will be confirmed by a serum pregnancy test.
13. Adverse events to be monitored at all times.
14. Subjects must refrain from food for a minimum of 2 hours prior to and a minimum of 1 hour following each administration of GLY-200 or placebo. At study visits where MMTT will be performed, dosing should occur 1 hour prior to the meal challenge (refer to MMTT Test Schedule in Table 2).
15. Subjects will be provided collection containers and will be instructed to collect all bowel movements, if possible, within 24 hours of their Day -1 and Day 21 (EOS) or ET visits. All bowel movements should also be collected at the site, if possible, anytime between check-in on Day -2 and Day 1 pre-dose. During the inpatient period starting on Day 10 until check-out on Day 14, all bowel movements should be collected. Additional samples are to be collected at the Day 21 (EOS) or ET visits if possible. Approximate weights of fecal collections should be recorded by site staff. Refer to the Laboratory Manual for additional details.
16. Refer to the MMTT Test Schedule in Table 2 for blood sample collection timepoints on MMTT days.
17. Urine is to be collected over 24 hours for exploratory PK/PD analysis (boron, etc.) from Day -1 to pre-dose on Day 1, post-dose on Day 1 to Day 2, Day 2 to Day 3, Day 3 to Day 4, and from Day 10 to 14. Urine samples for PD analysis are also to be collected by the subject from Day 14 to Day 17 and for the 24 hours prior to their Day 21 (EOS) or ET visit. All samples collected by the subject can be returned to the site at the Day 21 (EOS) or ET visit.
18. Recording of water and fluids in and output (urine volume out) over 24 hours daily.
19. The Appetite VAS Questionnaire will be completed by the subject pre-dose (or ~60 minutes before the meals on Day -1), pre-meal, immediately post-meal, and 30, 60, 90, 120, 150, 180, 210, and 240 minutes post-meal on the study days indicated. VAS assessments may be performed within a ± 10-minute window.
20. CGM automatic applicator will placed/replaced at indicated study visits for continuous measuring throughout the study; for the Day -1 visit, the CGM can be placed on Day -2 (check-in). The CGM is to be removed on Day 14 prior to check-out.
21. Refer to the MMTT Test Schedule in Table 2 for further details.

Table 2: Mixed-Meal Tolerance Test Schedule (Days -1, 1, 7 and 14)

Timepoint[1]	**Procedure**[2]
To be completed prior to MMTT timepoints (if applicable)	1. Vital Signs (BP, HR, RR, Temp) 2. 12-Lead Safety ECG 3. Collection of clinical laboratory samples (blood and urine)
-60 minutes (± 5 mins)	1. PD blood samples[3] 2. Metabolite/PK/PD blood samples (Day 1 only)[4] 3. GLY-200 Dosing (not applicable for Day -1 MMTT)
-30 minutes (± 5 mins)	PD blood samples[3]
0 minutes	1. PD blood samples[3] 2. Mixed-meal consumption (consume within 10 minutes)
15 minutes (± 5 mins)	PD blood samples[3]
30 minutes (± 5 mins)	1. PD blood samples[3] 2. Metabolite/PK/PD blood samples (Day 14 only)[4]
60 minutes (± 10 mins)	PD blood samples[3]
90 minutes (± 10 mins)	PD blood samples[3]
120 minutes (± 15 mins)	1. PD blood samples[3] 2. Metabolite/PK/PD blood samples (Day 14 only)[4]
180 minutes (± 15 mins)	PD blood samples[3]

1. All timepoints should be based on relative time from T=0 minutes (i.e., timepoints should not be adjusted based on the actual time of collection of the previous timepoint).
2. For timepoints at which more than 1 procedure is to be performed, procedures should be performed in the order presented.
3. Blood samples will be collected for PD analysis (including, but not limited to, glucose, insulin, GLP-1, GIP, PYY, ghrelin, glucagon, and fractionated and total bile acids); plasma samples should be collected for PD glucose assessments. Fractionated bile acids will be analyzed for all subjects at the -60 minutes (± 5 mins) and 60 minutes (± 10 mins) Mixed-Meal Tolerance Test Schedule timepoints on Day -1 and Day 14. Refer to the Laboratory Manual for further details.
4. Plasma will be collected for future GLY-200 metabolite and/or exploratory PK/PD analysis. Refer to the Laboratory Manual for further details.

4 STUDY ENDPOINTS

4.1 Primary Endpoints

- Safety and tolerability as measured by adverse events (AEs), serious adverse events (SAEs), vital signs, clinical laboratory parameters, and electrocardiograms (ECGs) for the duration of the study

4.2 Secondary Endpoints

- Actual and change from baseline in 3-hour plasma glucose and insulin profiles at Day 7 and Day 14
- Actual and change from baseline in fasting plasma glucose and insulin at Day 7 and Day 14

4.3 Exploratory Endpoints

The preliminary exploratory endpoints include:

- Boron analysis on urine and feces
- CGM glucose profile and parameters
- Blood: hormones and bile acids (fractionated and total)
- Urinalysis (oxalate, phosphate, uric acid)
- Macronutrients, Calories, hunger, and satisfaction as measured by the Appetite VAS Questionnaire and documentation of food intake
- Changes from baseline in nutritional parameters as measured by documentation of food intake

Additional exploratory markers may be analyzed from feces, plasma, and urine samples.

5 ANALYSIS SETS

5.1 Enrolled Analysis Set

The Enrolled Analysis Set will include all enrolled subjects (non-screen failure). This analysis set will be used for all listings, except Subject Violating Eligibility Criteria.

5.2 Randomized Analysis Set

The Randomized Analysis Set will include all randomized subjects, regardless of the administration of study drug. This analysis set will be used for subject disposition, demographics and baseline characteristics, medical history, protocol deviations, and concomitant medications. Subjects will be analyzed based on their randomized treatment assignment.

5.3 Safety Analysis Set

The Safety Analysis Set will include all subjects who were randomized and took at least 1 dose of the study drug. This analysis set will be used for adverse events, clinical laboratory tests, vital signs and body measurements, and ECG. Subjects will be analyzed based on the actual treatment they received.

5.4 Full Analysis Set

The Full Analysis Set will include all subjects who were randomized and took at least 1 dose of the study drug. This analysis set is identical to the Safety Analysis Set. This analysis set will be used for PD, PK, food intake and measurements, and CGM analyses. Subjects will be analyzed based on the actual treatment they received.

5.5 Per-Protocol (PP) Analysis Set

The Per-Protocol Analysis Set will include subjects in the Full Analysis Set who completed 14-days of treatment and all planned Mixed Meal Tolerance Tests inpatient study procedures and did not have any major protocol deviations that may affect pharmacodynamic endpoints. Protocol deviations will be reviewed by the sponsor to identify major protocol deviations that may affect pharmacodynamic endpoints and that list will be finalized prior to database lock. The Per-Protocol Analysis Set may be used for additional analysis on secondary and/or exploratory endpoints. Subjects will be analyzed based on the actual treatment they received.

5.6 Pharmacokinetic (PK) Analysis Set

The PK Analysis Set will include subjects in the Full Analysis Set who completed 14-days of treatment. Subjects will be analyzed based on the actual treatment they received.

6 STUDY SUBJECTS

Study subjects will be summarized by treatment group, GLY-200 overall, and study overall, unless specified otherwise.

6.1 Analysis Sets

The number and percentage of subjects in each analysis set will be summarized for the Enrolled Analysis Set. A listing of analysis sets will also be presented.

6.2 Subject Disposition

Subject disposition will be summarized for the Randomized Analysis Set. The number and percentage of subjects who are randomized, treated, completed, or discontinued from the study, as well as the reason for discontinuation will be tabulated. A listing of subject disposition will also be presented.

6.3 Demographic and Baseline Characteristics

Demographic and baseline characteristics will be summarized for the Randomized Analysis Set, including age, sex, ethnicity, race, height, weight, body mass index (BMI) and child bearing potential. A listing of demographics and baseline characteristics will also be presented.

6.4 Medical History

Medical history will be coded using version 24.1 or later of the Medical Dictionary for Regulatory Activities (MedDRA).

Medical history will be summarized by System Organ Class (SOC) and Preferred Term (PT) for the Randomized Analysis Set. Multiple histories in a summary level will be counted only once per subject. A listing of medical history will also be presented.

6.5 Inclusion/Exclusion Criteria and Subject Eligibility

Subject eligibility, e.g., inclusion and exclusion criteria failures will be listed for all screened subjects.

6.6 Protocol Deviations

A major protocol deviation is defined as a deviation classed as “Important Deviation”. A minor protocol deviation is defined as a deviation classed as “Not Important Deviation”.

To evaluate the incidence of major protocol deviations, the number and percent of subjects with a major protocol deviation will be summarized by treatment group, for the Randomized Analysis Set. Subjects will only be counted once for each deviation category. In addition, the total number of deviation events within a deviation category will be presented.

All protocol deviations (major and minor) will be presented in a listing, including a flag if the deviation resulted in exclusion from the PP Analysis Set (i.e., a major protocol deviation that may affect pharmacodynamic endpoints).

6.7 Study Drug Administration and Extent of Exposure

Duration of study drug exposure (days) will be summarized by treatment group only for the Safety Analysis Set. Duration of exposure to study drug is calculated as (date of last dose – date of first dose + 1). A listing of study drug administration and dosing information will also be presented.

6.8 Concomitant Medications

The WHO Drug Global - Mar 2022 will be used to categorize verbatim descriptions of non-study medications into the Anatomical Therapeutic Chemical (ATC) classification system. Each verbatim name will be classified by anatomical main group (ATC level 1), therapeutic subgroup

(ATC level 2), pharmacological subgroup (ATC level 3), chemical subgroup (ATC level 4), and trade name.

Prior medication is defined as any medication with a stop date prior to the date/time of the first dose of study drug. Prior medication will not be summarized but will be provided in a listing. Concomitant medications refer to non-study medications used after the date/time of the first dose of study drug. Among concomitant medications, those with a start date after the date/time of the first dose of study drug will be defined as new concomitant medication and will be flagged in the listing.

Concomitant medications, and new concomitant medications, will be summarized by ATC classification (ATC level 3 and preferred term) for the Randomized Analysis Set. All prior and concomitant medications will be presented in a listing.

7 STATISTICAL ANALYSIS METHODS

7.1 General Considerations

7.1.1 Statistical Notation and Presentation

For continuous variables the following descriptive statistics will be provided: number of subjects, mean, standard error of the mean (SE), standard deviation (SD), minimum (min), maximum (max), and median. For categorical variables, descriptive statistics will include the number and percentage of subjects in each category, using either the number of subjects in the treatment group or the number of subjects with non-missing values as the denominator for the percentages.

Min and max values will be rounded to the precision of the original value. Means and medians will be rounded to one decimal place greater than the precision of the original value. SDs and SEs will be rounded to two decimal places greater than the precision of the original value. Percentages for summarizing categorical data will be rounded to one decimal place. P-values will be presented with four decimal places and values less than 0.0001 will be presented as <0.0001. All inferential statistical testing will be two-sided.

In the case where a safety laboratory variable is recorded as "> x", "≥ x", "< x" or "≤ x", a value of x will be taken for summary. In listings, these data will be presented as recorded with the sign.

Data for subjects taking 1, 2, or 4 capsules of placebo will be pooled together as the placebo treatment group for analysis unless otherwise specified. By subject listings, including data at scheduled and unscheduled visits, will be sorted by treatment group, subject number, visit, and timepoint.

All values, scheduled or unscheduled, will be presented in data listings. Only the scheduled visits will be included in data summary and analysis.

7.1.2 Baseline and Change from Baseline

Baseline is defined as the last non-missing observation obtained prior to the administration of the study drug. Change from baseline is defined as post-baseline result minus baseline result.

7.1.3 Study Day

Study day will be calculated as the number of days from first dose of study drug.

For events/assessments on or after first dose of study drug:

- Study day = date of event (or date of assessment) – date of first dose of study drug + 1.

For events/assessments before first dose of study drug:

- Study day = date of event (or date of assessment) – date of first dose of study drug.

7.1.4 Handling of Missing or Partial Data

No missing data will be imputed for the safety analysis, except for missing or partial start dates of adverse events (AEs) with the imputation rule that missing component(s) will be assumed as the most conservative value(s) as possible. For example, AEs with missing start dates will be assumed as treatment-emergent adverse events (TEAEs), and will be conservatively captured as follow:

- If "day" is the only missing field, impute the "day" as the first randomized dose date if their "month" and "year" are the same; otherwise, the first day of the non-missing month.
- If "day" and "month" are the only missing fields, impute the "day" and "month" as the first randomized dose date if their "year" are the same; otherwise, January 1 of the non-missing year.

Missing or partial dates for non-study medications will be imputed similarly. If the start date is unknown and end date is "ongoing" then the non-study medication will be considered a concomitant medication, but not new concomitant medication.

Date imputation will only be used for computational purposes e.g., defining the treatment-emergent status. Actual data values as they appear in the original CRFs will be shown in data listings.

7.2 Pharmacodynamic and Pharmacokinetic Analyses

Listings, descriptive summaries, figures, and statistical analyses will be generated using SAS® (Version 9.4 or higher, SAS Institute Inc.).

Concentration values that are below the LLOQ will be imputed as LLOQ/2 for deriving amount and summary statistics, and displayed as reported in the data listings. Similarly, concentration values of "<X" will be imputed as X/2 for deriving amount and summary statistics. Concentration values that are missing will not be imputed.

7.2.1 Data Handling

7.2.1.1 Plasma

Plasma for PD analysis will be collected relative to the mixed-meal consumption (0 minutes) on Day -1 (Baseline), Day 1, Day 7 and Day 14, at the following timepoints: -60, -30, 0, 15, 30, 60, 90, 120, 180 minutes. See Mixed-Meal Tolerance Test Schedule (Table 2). Glucose, insulin, glucagon, GLP-1, GIP, PYY, ghrelin and total bile acids will be measured at each timepoint of the Mixed-Meal Tolerance Test. Fractionated bile acids, including ursodeoxycholic acids, cholic acids, chenodeoxycholic acids, and deoxycholic acids, will be measured at -60 minutes and 60 minutes on Day -1 (Baseline) and Day 14. A total fractionated bile acid result will also be reported for the fractionated samples.

A 5-minute window around each nominal timepoint up to 30 minutes, inclusive, is permitted. A 10-minute window is permitted for sampling at 60 and 90 minutes. A 15-minute window is permitted for the remaining collection times.

Samples collected outside of these windows will be listed, but excluded from analysis.

7.2.1.2 Urine

Urine is to be collected over 24 hours for exploratory PK/PD analysis (boron, etc.) from Day -1 to pre-dose on Day 1, post-dose on Day 1 to Day 2, Day 2 to Day 3, Day 3 to Day 4, and from Day 10 to 14. Urine samples for PD analysis are also to be collected by the subject from Day 14 to Day 17 and for the 24 hours prior to their Day 21 (EOS) or ET visit.

7.2.1.3 Feces

All bowel movements within 24 hours of Day -1 and Day 21 (EOS) or ET visits should be collected by the subject. At the site, all bowel movements between check-in on Day -2 and Day 1 pre-dose, and Day 10 until check-out on Day 14 should be collected.

7.2.1.4 Continuous Glucose Monitoring (CGM)

A CGM automatic applicator will be used for continuous blood glucose measuring throughout the study. Per protocol, the CGM device is to be placed on Day -7, and changed (i.e., replaced or removed) on Day -2, Day 7, and Day 14. A complete CGM profile for a 24-hour study day from 00:00 to 23:59 includes 288 data points per day assuming that CGM readings are recorded every 5 minutes. Values less than 30 will be considered invalid and imputed as missing.

A 24-hour CGM profile will be derived for three analysis visits: Week -1, Week 1, and Week 2. In addition, a 4-hour post-meal CGM profile will be derived for each meal (breakfast, lunch, and dinner), for Day -1, Week 1 and Week 2. Derived analysis values for Week -1, Week 1, and Week 2 will be the average of values from 3 days of sufficient data collection prior to protocol scheduled device changes on Day -2, Day 7, and Day 14, respectively.

CGM analysis values will be derived as follows:

1. Determine the closest 3 days prior to a protocol scheduled device change for which sufficient CGM data is available (see below for requirements for sufficient CGM data).
    a. Example: Week 1 analysis will first consider the 3 days prior to the protocol scheduled device change on Day 7: Day 6, Day 5, and Day 4. If Day 5 does not have sufficient CGM data, then Day 3 would next be considered. If Day 3 has sufficient CGM data, Week 1 analysis will use Day 6, Day 4, and Day 3. If Day 3 also does not have sufficient CGM data, then Day 2 would next be considered. This continues until 3 days with sufficient data have been found.
    b. Note: If there are not 3 days with sufficient CGM data in a given analysis period, then the analysis will use the available days with sufficient data.
2. Standardize the time of the daily 288 glucose readings on each of the days chosen in Step 1, into 5 minute windows from 00:00 – 23:55. Actual time of glucose reading will be rounded down to the start of the window.
    a. Example: A glucose reading at 09:37 falls within the 5 minute window between 09:35 and 09:39, thus, the analysis timepoint for the reading will be 09:35.
3. Standardize the timing of the 48 glucose readings in the 4 hours after the start of each meal (breakfast, lunch and dinner) on each of the days chosen in Step 1, into 00:00 (start time of meal) – 03:55 (3 hours, 55 minutes after start time of meal).
    a. Example: A subject starts lunch at 12:26 on Day 4, which falls in the window for analysis timepoint 12:25 (from Step 2). The records associated with analysis timepoints 12:25 – 16:20 (from Step 2) will be assigned to lunch meal times 00:00 – 03:55. On Day 5, the subject has lunch at 11:42, which falls in the window for analysis timepoint 11:40. The records associated with analysis timepoints 11:40 – 15:35 will be assigned to lunch meal times 00:00 – 03:55.
    b. Note: The 4 hour time block is independent of the start and/or end time of adjacent meals, as well as if a subject ate a snack.
4. Average the three glucose values from the days chosen in Step 1 for each of the analysis visits, and meals.

    Note: In the examples below, Day 4, Day 5 and Day 6 have sufficient CGM data.
    a. Example 1: The glucose value at timepoint 09:35 for Week 1 will be the average of the following glucose values: Day 4 at timepoint 09:35, Day 5 at timepoint 09:35, and Day 6 at timepoint 09:35.
    b. Example 2: The glucose value for dinner meal time 02:05 (2 hours, 5 minutes after meal start time) for analysis visit Week 1 will be the average of the following glucose

values: Day 4 dinner meal time 02:05, Day 5 dinner meal timepoint 02:05, and Day 6 dinner meal timepoint 02:05.

Requirements for a 24-hour day to have sufficient CGM data:

1. There is not a protocol scheduled device placement or change (i.e., Day -7, Day -2, Day 7, and Day 14) or a non-protocol scheduled device change due to issues with a sensor.
2. At least 85% of records (245 data points) are available.
3. There is no gap (missing data) lasting for >2 hours.

   Note: CGM data collected during the warmup period (e.g., 2 hours) will be excluded from statistical analysis. If a subject prematurely withdraws from the treatment period without meeting the criteria for useable CGM data as described above, the CGM data will be missing for the individual subject.

7.2.2 Pharmacodynamic (PD) Analyses

7.2.2.1 Analysis of Plasma MMTT PD Measurements

The C_{max}, T_{max}, and AUC will be derived for all MMTT PD measurements, except fractionated bile acids. AUC will be calculated using the trapezoidal rule. Analysis will be performed on both absolute and incremental (change from -60 minute timepoint) concentration and parameter values.

Baseline, post-baseline, and change from baseline values will be summarized by treatment group, visit, and timepoint (for concentrations) for the Full Analysis Set.

Listings of MMTT PD measurements concentrations and parameters will also be presented for the Enrolled Analysis Set. Listings will be reviewed and outliers may be removed from summary analysis.

An analysis of covariance (ANCOVA) model, with baseline (Day -1) value as a covariate, will be used to compare the GLY-200 treatment groups and the pooled placebo treatment group. The pooled placebo treatment group will be designated as the reference group for the pair-wise comparisons. The least squares mean (LSM) will be presented for each treatment group. Additionally, the difference between each GLY-200 treatment group and pooled placebo will be calculated, along with the two-sided 95% CIs, and p-value. Fractionated bile acid concentrations will be accessed at the -60 minute and 60 minute timepoints, on Day 14. For all other MMTT measurements, concentration values at each of the nine timepoints, and the AUC will be assessed for each study visit.

Mean and individual plasma concentration-time profiles will be presented graphically. Mean data will be plotted using nominal sample times and individual data will be plotted using actual times. For individual subject concentration-time data, spaghetti plots (four visits in one plot per subject) will be created. Mean plots will be presented ± standard error (SE).

7.2.2.2 Analysis of Glucose Profile Derive from CGM Data

Refer to Section 7.2.1.4 for the algorithms to derive analysis values for each of the analysis visits.

The mean weighted glucose (MWG) will be calculated for the 24-hour CGM profile, and the 4-hour post-meal CGM profile, for Day -1 (single day) and the derived analysis visits, Week -1, Week 1, and Week 2. The MWG is defined as the calculated AUC, divided by the actual time span the AUC was calculated for. Each non-missing reading will be counted as 5 minutes of actual time.

Week -1, Baseline (Day -1), post-baseline (Week 1 and Week 2), and change from baseline values will be summarized descriptively by treatment group, and visit for the 24-hour MWG. Baseline (Day -1), post-baseline (Week 1 and Week 2), and change from baseline values will be summarized descriptively by treatment group, visit, and meal, for the 4 hour post-meal MWG.

An analysis of covariance (ANCOVA) model will be used to evaluate the difference between the GLY-200 and pooled placebo treatment groups. The pooled placebo treatment group will be designated as the reference group for the pair-wise comparisons. Analyses will be performed on two models: with baseline as a covariate and without baseline as a covariate. The least squares mean (LSM) will be presented for each treatment group. Additionally, the difference between each GLY-200 treatment group and pooled placebo will be calculated, along with the two-sided 95% CIs, and p-value.

24-hr CGM profiles will be graphically displayed for each subject (three analysis visits in one plot per subject). The treatment group averaged 24-hour CGM profile will be graphically displayed by analysis visit (four treatment groups in one plot per analysis visit), and by treatment group (three analysis visits in one plot per treatment group).

In addition, time in range analysis will be performed on the 24-hour CGM profiles of Week -1, Day -1, Week 1, and Week 2. The following ranges will be used for analysis:

- Very low (<54 mg/dL)
- Low (54 - <70 mg/dL)
- In target range (70 - 180 mg/dL)
- High (>180 - 250 mg/dL)
- Very high (>250 mg/dL)

7.2.2.3 Analysis of Urine PD Measurements

Baseline, post-baseline, and change from baseline values may be summarized by treatment group and collection interval for concentrations of creatinine, oxalate, oxalate:creatinine ratio, phosphate, phosphate:creatinine ratio, uric acid, and uric acid:creatinine ratio. Listings of urine PD concentrations and parameters will also be presented.

7.2.3 Pharmacokinetic (PK) Analyses: Boron Elimination in Feces and Urine

7.2.3.1 Urine

The amount of boron in urine will be summarized by treatment group and study day. The amount of boron eliminated in the urine per day will be calculated by multiplying the reported concentration by the urinary volume collected. A listing of daily boron in urine will also be presented including boron concentration, volume of urine collected, and total daily boron amount. Values below the lower limit of quantification (BLOQ) will be set to LLOQ/2 for analysis.

7.2.3.2 Feces

The daily total amount of boron eliminated in the feces each day will be calculated by multiplying the reported daily concentration by the total weight of all fecal samples collected on that day.

Average daily boron amount will be summarized by treatment group for the following three analysis periods: prior to the date/time of first dose of study drug (Predose), between Day 10 – Day 14, and after the inpatient period has ended (Follow-up). Average daily boron amount will be derived as follows:

$$\frac{sum\ of\ daily\ total\ boron\ values\ during\ analysis\ period}{number\ of\ days\ that\ samples\ were\ collected\ during\ analysis\ period}$$

A listing of fecal sample analysis data will also be presented including individual fecal sample weights, daily total weight of all fecal samples, reported daily concentration, daily total boron amount, and average daily boron amount for each analysis period

7.3 Safety Analyses

Safety and tolerability will be assessed throughout the study by examination of adverse events (AEs), concomitant medications, clinical laboratory evaluations, vital signs, and physical examinations. Safety related tables and listings will be based on the Safety Analysis Set.

7.3.1 Adverse Events

An AE is any untoward medical occurrence in a subject, temporally associated with the use of study product, whether or not considered related to study drug. All AEs will be coded using MedDRA version 24.1 or later.

TEAE is defined as an AE with onset on or after the first dose of study drug, or any event already present that worsens in intensity following exposure to the study drug.

A study drug related AE is defined as an AE classed as "Possibly Related" or "Probably Related" to the study drug. If an AE has missing causality, it is assumed to be related to the study drug for analysis purposes.

Multiple events will be counted once per subject in each summary level.

Any AE that has worsened in the severity will be recorded as a separate AE.

To evaluate the incidence of TEAEs, the number and percent of subjects who experienced a TEAE within each SOC or PT summary level will be summarized by treatment group. Subjects will only be counted once within each summary level. In addition, the total number of TEAEs within a summary level will be presented. A subject is counted at most once per SOC and PT at the maximum severity for TEAE summary by maximum severity. AEs with missing severity will be classified as "Unknown."

An overall summary table of TEAEs will include:

- Any TEAEs (overall and by severity)
- Serious TEAEs (overall and by severity)
- TEAEs related to study drug (overall and by severity)
- TEAEs leading to discontinuation of study drug
- TEAEs leading to death

TEAEs will be summarized by treatment group, GLY-200 overall, and study overall for:

- TEAEs by PT (sorted by overall study incidence)
- TEAEs by SOC and PT
- Serious TEAEs by SOC and PT
- Related TEAEs by SOC and PT
- TEAEs by SOC, PT, and maximum severity
- TEAE leading to discontinuation of study drug by SOC and PT
- Gastrointestinal TEAEs (TEAEs in the gastrointestinal SOC) by study day (sorted by overall daily incidence)

AEs listings will be presented by treatment group, subject, start date, SOC and PT for:

- All AEs
- Serious AEs
- AEs leading to discontinuation of study drug
- Gastrointestinal AEs

Swimmer plots from Day 1 to Day 14 will be presented for GI TEAEs for each treatment group. Additional plot points will include meal start times and dosing times.

7.3.1.1 Adverse Events of Special Interest (AESI)

The following AESIs will be summarized by treatment group and overall:

- Hyperglycemia with evidence of diabetic ketoacidosis (DKA) or impending DKA: A blood glucose value > 300 mg/dL with symptoms of DKA (e.g., nausea, dizziness, abdominal pain, lethargy) and capillary ketones > 1.5 mmol/L or urine ketone positivity
- Clinically significant hypoglycemia: A blood glucose measurement of <54 mg/dL (<3.0 mmol/L) that may or may not have immediate symptoms depending on the level of hypoglycemia unawareness.

An AESI listing will also be presented.

7.3.2 Clinical Laboratory Values

Clinical laboratory data will be presented using the original unit of measurement as reported by the laboratory used. Each measurement will be classed as below, within, or above normal range, based on ranges supplied by the laboratory used.

For clinical laboratory (hematology, chemistry and urinalysis) parameters with continuous results, baseline, post-baseline, and change from baseline values will be summarized by treatment group and visit.

For clinical laboratory parameters with categorical results, the number and percentage of subjects with results in each category will be summarized by treatment group and visit. The denominator is the number of subjects with non-missing values for the given parameter and visit.

The number and percentage of subjects with abnormal laboratory results (i.e. those that are outside of normal ranges) will also be summarized be treatment group and visit.

In addition, normal and abnormal status changes from baseline will be presented in a shift table by treatment group. For the shift summary, the denominator is the number of subjects with non-missing value at baseline and the visit for the given parameter.

All laboratory (hematology, chemistry, urinalysis, and other) listings will be presented by treatment group, subject and visit (both scheduled and unscheduled). Abnormal laboratory results and/or with clinical significance will be flagged.

7.3.3 Vital Signs and Body Measurements

Vital signs will include body temperature, resting respiration rate, and sitting blood pressure (BP, systolic and diastolic) and heart rate (HR), and body measurements will include body weight, and BMI (weight (kg) / [height (m)]2).

Vital signs and body measurements will have baseline, post-baseline, and change from baseline values summarized by treatment group and visit. In addition, body weight will have percent change from baseline summarized. A vital signs and body measurements listing will also be presented.

Body weight will be analyzed using an ANCOVA model, with baseline weight as a covariate, to compare the GLY-200 treatment groups to the pooled placebo treatment group at each visit.

Absolute and percent change in body weight over time will be presented graphically by treatment group. Spaghetti plots (all subjects in one plot per treatment) will be created. Mean plots will be presented ± standard deviation (SD).

7.3.4 Physical Examinations

All physical examination data, including details of clinically significant findings will be listed. A separate listing of abdominal examination data will also be presented.

7.3.5 ECG (12-lead Electrocardiogram)

12-lead Electrocardiogram tracings and results (ventricular rate, PR, QRS, QT, QTc, QTcF and QTcB intervals) will be collected as triplicate recordings at screening and prior to first dose. The average of triplicate results at each visit will be used in data summary. All other post-first dose ECGs will be single readings. However, if any of the ECG measurements is out of normal range, it will be repeated in triplicate.

For overall interpretation at baseline, the most conservative (best case) of the respective readings will be reported. For overall interpretation post baseline, the most severe (worst case) of the respective readings will be reported.

ECG parameters will have baseline, post-baseline, and change from baseline values summarized by treatment group and visit using descriptive statistics.

In addition, change from baseline to post-baseline visits of the PI's interpretation of ECG results (normal/abnormal, not clinically significant/abnormal, clinically significant) will be presented in a shift table by treatment group and visit point using descriptive statistics. For the by-visit shift summary, the denominator is the number of subjects with non-missing cardiologist's interpretation at baseline and the given visit.

An ECG listing will also be presented.

7.3.6 Fluid Balance

Fluid balance data, including fluids in and output (urine volume out), will be listed.

7.3.7 Food Intake and Appetite Assessments

Food intake and appetite assessments will be summarized by treatment group, GLY-200 overall, and study overall.

For all meals during the inpatient period, percent meal consumed will be categorized as follows: 0% - 25%, 26% - 50%, 51% - 75%, and 76% - 100%. The number and percentage of subjects with results in each category will be summarized by study day, and meal. The denominator is the number of subjects with non-missing values for the given meal and study day. In addition, changes from Day -1 will be presented in a shift table by study day, and meal. For the shift summary, the denominator is the number of subjects with non-missing value at Day -1 and the study day for the given meal.

On Days -1, 1, and 13, subjects will also complete an appetite assessment at breakfast and dinner meals, and food intake measurements will be taken. Food intake measurements include total carbohydrates (g), total protein (g), total fat (g), and total calories consumed. Baseline (Day -1), post-baseline (Day 1 and Day 13), and change from baseline values for food intake measurements will be summarized by visit and meal (breakfast and dinner). A listing of food intake measurements will also be presented.

The Appetite VAS Questionnaire will be completed by the subject at breakfast and dinner on Day -1 (Baseline), Day 1, and Day 13, at the following timepoints: pre-dose (on Day -1 this will be '-60 minutes'), pre-meal, immediately post-meal, and 30, 60, 90, 120, 150, 180, 210, and 240 minutes post-meal. Assessments will include the following: overall appetite suppression, how much a subject can eat, the feelings of hunger, satisfaction and fullness, and the desire to eat sweet, salty, savory and fatty. Overall appetite suppression is derived as follows:

- Overall appetite suppression = (satisfaction + fullness + [100 – hunger] + [100 – how much can subject eat])/4.

Baseline, post-baseline, and change from baseline values will be summarized by treatment group, visit, meal (breakfast and dinner), timepoint, and assessment. A listing of Appetite VAS assessments will also be presented.

In addition, the AUC will be calculated at each visit and meal, for overall appetite suppression, how much can subject eat, and feelings of satisfaction, fullness and hunger. Baseline, post-baseline, and change from baseline AUC values will be summarized by treatment group, visit, and meal. A listing of Appetite VAS assessment AUCs will also be presented.

The treatment difference at each meal will be evaluated for the above specified assessments, with the pooled placebo treatment group used as reference. The AUCs from Day 1 and Day 13 will be analyzed using a mixed model for repeated measures (MMRM). The model will include terms for baseline AUC, treatment, visit, and the interaction of treatment and visit, with visit as a repeated measure. The least squares mean (LSM) will be presented for each meal, treatment group, and visit. Additionally, the difference between each GLY-200 treatment group and pooled

placebo will be calculated, along with the two-sided 95% CIs, and p-value, for each meal and visit. The following is reference code for this analysis:

```
proc mixed data=vas;
   by meal;
   class treatment subject visit;
   model auc = treatment visit treatment*visit baseauc / ddfm=kr;
   repeated visit / sub=subject type=cs;
   lsmeans treatment*visit / pdiff cl;
run;
```

Mean ±SD spaghetti plots for how much can subjects eat, overall appetite suppression, and feelings of satisfaction, fullness and hunger, will be presented for each visit and meal, comparing treatment groups. In addition, the results of the MMRM analysis will be presented in a forest plot of the estimated treatment difference, with the two-sided 95% CI, for each visit and meal.

8 CHANGE TO THE PLANNED ANALYSES FROM PROTOCOL

No changes were made to the planned analyses from the protocol (amendment 2 dated October 6, 2022).

9 POWER AND SAMPLE SIZE

The sample size chosen for this study was selected without statistical considerations.

10 STATISTICAL SOFTWARE

All statistical analyses will be performed using SAS® version 9.4 (or later).

11 REFERENCES