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

Study Number: GLY-200-02

Sponsor: Glyscend, Inc.

Version Number: Amendment 02 Version 01

Date: 06 October 2022

Supersedes:

Amendment 01 Version 01 Dated: 19 July 2022

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SPONSOR SIGNATURE PAGE

Study Title: 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

Protocol Number: GLY-200-02

Version: Amendment 02 Version 01

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The above titled study protocol was subjected to critical review. The information presented is consistent with current knowledge of the risks and benefits of the investigational product, as well as with the moral, ethical, and scientific principles governing clinical research as set out in the current Declaration of Helsinki and the guidelines on Good Clinical Practice.

By my signature, I confirm that I have reviewed this protocol and find its content to be acceptable.

Name & Title	Signature and Date
[redacted]	[redacted]
[redacted]	[redacted]

INVESTIGATOR SIGNATURE PAGE

Study Title: 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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By signing this Protocol, the Investigator acknowledges and agrees:

- The Protocol contains all necessary details for conducting the study. The Investigator will conduct this study as detailed herein, in compliance with International Council for Harmonisation (ICH) Good Clinical Practice (GCP) and the applicable regulatory requirements and will make every reasonable effort to complete the study within the time designated.
- The Protocol and all relevant information on the drug relating to pre-clinical and prior clinical experience, which was furnished by the Sponsor, Glyscend, Inc. (Glyscend), will be made available to all physicians, nurses, and other personnel who participate in the conducting of this study. The Investigator will discuss this material with them to assure that they are fully informed regarding the drug and the conduct of the study.
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- The conduct and results of this study will be kept confidential. The results of this study may be published. Upon completion of the Study, it is the intention of the parties to prepare a joint publication regarding or describing the Study and all the results there from and both parties shall co-operate in this regard. Where it is the intention of Glyscend to file for a patent or other intellectual property right protection, publication may be deferred at the option of Glyscend for up to twelve months from the date of completion of the proposed joint publication to allow Glyscend to make all filings it deems appropriate.

Investigator's Printed Name	Signature and Date

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1. PROTOCOL SUMMARY

1.1 SYNOPSIS

Title:	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	
Phase:	2	
Population:	Subjects with Type 2 diabetes on a stable dose of metformin for at least the past 3 months who are willing to wash-off metformin treatment during the study period	
Description of Study Agent:	GLY-200 (non-absorbable polymer)	
Number of Subjects:	A total of 48 completed subjects. Subjects that terminate prior to completing the study may be replaced at the Sponsor's discretion.	
Study Duration:	The entire study is estimated to be completed in 12 months.	
Participant Duration:	14-day treatment and a 7-day follow-up	
Number of Sites Enrolling Subjects:	Single-Center	
Study Objectives and Endpoints:	*Primary Objectives*	*Primary Endpoints*
	• To assess the safety and tolerability of GLY-200 over the 14-day treatment period	• 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
	Secondary Objectives	*Secondary Endpoints*
	• 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	• 3-hour plasma glucose and insulin profiles • Fasting plasma glucose and insulin

	Exploratory Objectives	*Exploratory Endpoints*
	• 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	The preliminary PK and pharmacodynamics (PD) will include (but will not be limited to): • boron analysis on urine and feces • CGM glucose parameters • Blood: hormones and bile acids (fractionated and total) • Urinalysis (oxalate, phosphate, uric acid) • Macronutrients, Calories, hunger, and satiety • Changes from baseline in nutritional parameters Additional exploratory markers may be analyzed from stool, plasma, and urine samples.
Methodology:	This study is a Phase 2, randomized, double-blind, placebo-controlled, single-center study in adult patients with type 2 diabetes. The study will evaluate the safety and tolerability of oral GLY-200. Approximately 48 subjects will be randomized. Placebo groups will match each active dose regimen to preserve the blind. The dose levels planned are: • Active Group 1: 0.5 g GLY-200 (1 x 0.5 g capsule) BID (n=12) • Active Group 2: 1.0 g GLY-200 (2 x 0.5 g capsules) BID (n=12) • Active Group 3: 2.0 g GLY-200 (4 x 0.5 g capsules) BID (n=12) • Placebo Group 1: 1 placebo capsule BID (n=4) • Placebo Group 2: 2 placebo capsules BID (n=4) • Placebo Group 3: 4 placebo capsules BID (n=4) 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 follow-up clinic visit will occur on Day 21 [End of Study (EOS)] or Early Termination (ET).	

Inclusion/ Exclusion Criteria:	**Criteria for inclusion:** 1. Patients diagnosed with type 2 diabetes: a. treated with a stable dose of metformin ≥750 mg/day for at least 3 months prior to screening b. willing to come off current metformin treatment during washout and treatment period c. HbA1c ≥ 6.0 and ≤ 8.5% at screening d. completed at least 12 days of metformin washout without meeting rescue criteria prior to dosing 2. Male or female, ≥ 30 and ≤ 70 years old at the time of screening 3. BMI ≥ 18 and ≤ 40 at screening 4. Capable of consent, and written informed consent signed prior to entry into the study 5. Willing to consume standard meals provided by the study center 6. Able and willing to attend the necessary visits to the study center 7. Willing to stop antacid use at screening 8. Male subjects with female partners of childbearing potential must comply with the contraception requirements as described in Appendix 3 from the time of first dose of study medication until 90 days after the last dose of study medication (i.e., one sperm cycle) 9. Female subjects must comply with contraception requirements as described in Appendix 3 from the screening period through the end of study or be of non-childbearing potential, defined as meeting at least one of the following criteria: a. Achieved postmenopausal status, defined as: cessation of regular menses for at least 12 consecutive months with no alternative pathological or physiological cause; and have a serum follicular stimulating hormone (FSH) level within the laboratory's reference range for postmenopausal women b. Have undergone a documented hysterectomy and/or bilateral oophorectomy c. Have medically confirmed ovarian failure All other female subjects (including women with tubal ligations and women who do NOT have a documented hysterectomy, bilateral oophorectomy and/or ovarian failure) will be considered to be of childbearing potential. **PLEASE NOTE**: ORAL CONTRACEPTION IS **NOT ALLOWED** (and is exclusionary) IN THIS STUDY AS THE STUDY DRUG (GLY-200) MAY REDUCE THE EFFECTIVENESS OF ORAL CONTRACEPTION DUE TO POTENTIAL DISRUPTION ON GI ABSORPTION. 10. Fully vaccinated against COVID-19 based on the recommendations of the

region at least 14 days prior to dosing

Criteria for exclusion:

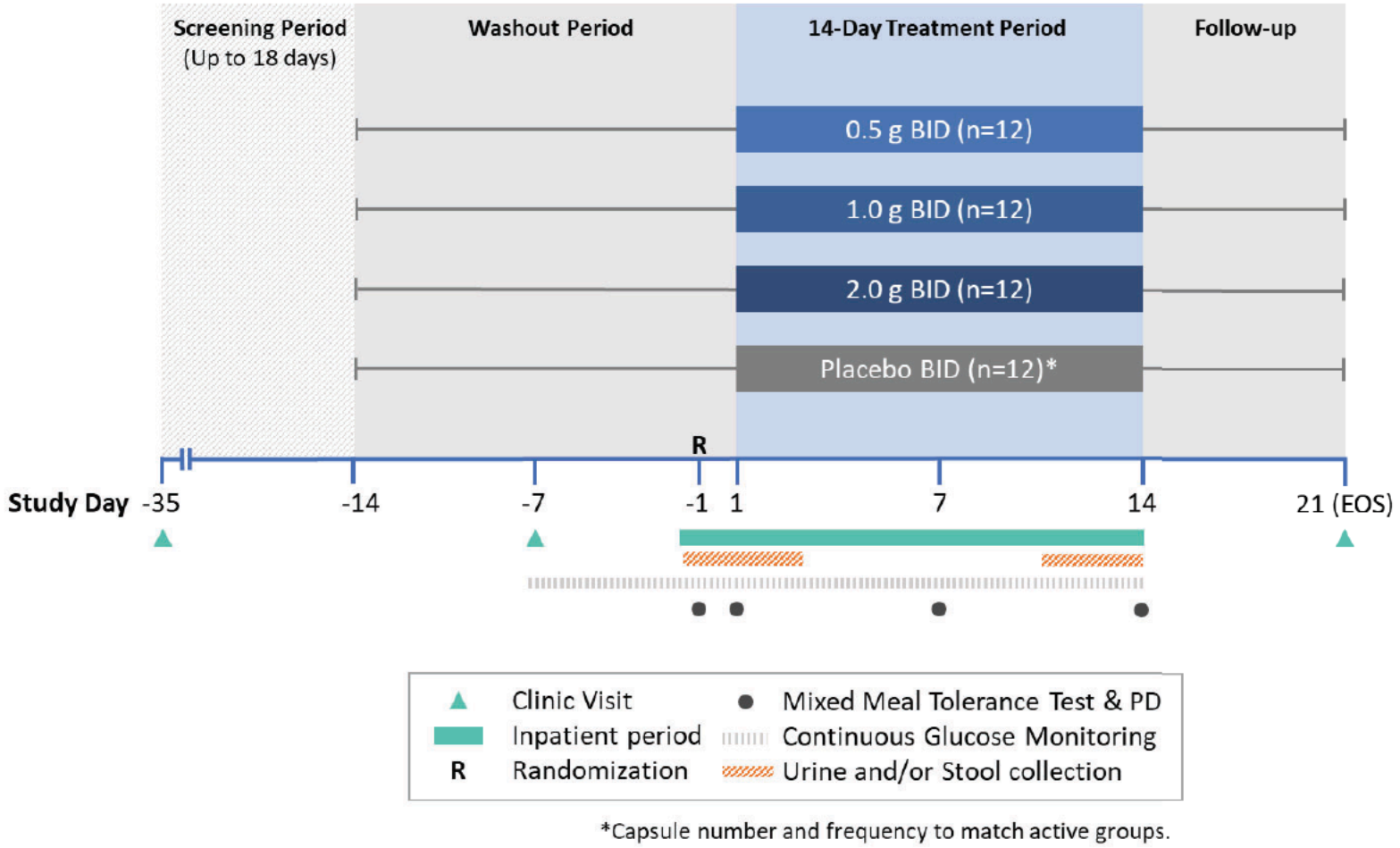
1. Women who are pregnant (as confirmed by a serum or urine human chorionic gonadotrophin [hCG] test) or lactating
2. Women of childbearing potential who are using oral contraception
3. Vegetarian or vegan
4. Treated with any prescription medication for the treatment of type 2 diabetes or weight loss other than metformin in the last 3 months prior to screening
5. Use of any drug treatment that affects gastric pH (prescription or over-the counter), such as H2-receptor antagonists and proton pump inhibitors for 3 months prior to screening (e.g., famotidine, ranitidine, esomeprazole, dexlansoprazole, omeprazole, pantoprazole)
6. Use of any drug treatment that affects gastrointestinal motility including but not limited to cholinergic agonists and antagonists, prokinetic agents, opioid antagonists, antidiarrheals, and antibiotics in the last 3 months prior to screening (e.g., cevimeline, pilocarpine, loperamide, erythromycin, metoclopramide, domperidone, cisapride, mosapride, sincalide)
7. Any chronic condition that requires oral drug treatment that would violate concomitant medication use restrictions described in Section 6.8 (i.e., all orally administered concomitant medications must be administered at least 2 hours before and 4 hours after GLY-200 dosing), TID (three times a day) drugs, or drugs with very narrow therapeutic windows (e.g., warfarin)
8. Fasting blood glucose > 190 mg/dL at screening
9. Diagnosis or treatment of any clinically symptomatic biochemical or structural abnormality of the GI tract or active disease within 12 months prior to screening; including but not limited to inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), gastroesophageal reflux disease (GERD), GI cancer, diverticulitis, duodenal ulcer, erosive esophagitis, gastric ulcer, pancreatitis, symptomatic gallbladder disease, ileus, intestinal/pyloric stenosis/obstruction, fistula, malabsorption, esophageal anatomic abnormalities, or other severe GI disease, particularly those that may impact digestion, nutrient absorption, intestinal motility, and/or gastric emptying
10. History of any previous abdominal or intestinal surgery including endoscopic, open or laparoscopic thoracic or abdominal surgery, surgical resection of the stomach, small or large intestine (excluding appendectomy, cholecystectomy, or resection of benign polyps), or of any

	other risk of abdominal adhesions or obstruction. Appendectomy ≥ 3 months prior to screening is allowed. 11. Clinically significant symptoms (as determined by the Investigator) of nausea, vomiting, bloating, diarrhea, flatulence, constipation, or abdominal pain in the last 30 days prior to screening 12. Use of systemic corticosteroids by oral, intravenous, or intramuscular route; or potent, inhaled, or intrapulmonary steroids known to have a high rate of systemic absorption within 3 months of screening 13. Clinically significant medical condition as judged by the Investigator that could potentially affect study participation and/or personal well-being, including but not limited to the following conditions: a. Uncontrolled hypertension b. Uncontrolled hyperlipidaemia c. Hepatic disease d. Renal disease (eGFR value of <60 mL/min/1.73 m^2) based on the Modification of Diet in Renal Disease equation. e. Endocrine disorder other than diabetes f. Cardiovascular event in the last 12 months g. Central nervous system diseases h. Organ transplantation i. Chronic or acute infection j. Orthostatic hypotension, fainting spells, or blackouts 14. Any evidence of or treatment of malignancy (other than localized basal cell, squamous cell skin cancer, non-aggressive prostate cancer, or cancer in situ that has been resected) within the previous year 15. Clinically significant electrocardiogram (ECG) abnormalities or vital sign abnormalities as determined by the Investigator 16. Clinical laboratory test (clinical chemistry, hematology, coagulation parameters, or urinalysis) abnormality, other than that related to T2D, judged by the Investigator to be clinically significant 17. Alanine aminotransferase or aspartate aminotransferase result >2.5 × upper limit of normal (ULN) or a total bilirubin result >1.5 × ULN 18. Physical, psychological, or historical finding that, in the Investigator's opinion, would make the subject unsuitable for the study 19. Severe infections, injuries, or major surgeries (as determined by the Investigator) within 4 weeks prior to screening or intend to undergo any surgery during the trial 20. Use of a live vaccine within 30 days prior to screening or anticipated need for a live vaccine during the study or for 30 days following the last dose of study drug

	21. History of bleeding associated with procedures (e.g., endoscopy or phlebotomy) or with use of medications (e.g., nonsteroidal anti-inflammatory drugs [NSAIDs] or aspirin) within 28 days prior to screening or planned use during the study 22. Positive test for human immunodeficiency virus (HIV), hepatitis B surface antigen (HBsAg), or hepatitis C antibodies (HCV) 23. History of severe allergy to any drug, food, toxin, or other exposure 24. History of heavy alcohol use within 1 year prior to screening, as defined by: a. Exceeding 4 standard drinks on any day or more than 14 standard drinks per week for men, or more than 3 standard drinks on any day or more than 7 standard drinks per week for women; one standard drink=10 grams of alcohol (equivalent to 12.5 mL of pure alcohol, 285 mL of full-strength beer, 375 mL of mid-strength beer, 425 mL of low-strength beer, 100 mL of wine, or 30 mL of 40% alcohol spirits). 25. History of illicit or prescription drug abuse or addiction within 1 year of screening, or positive urine drug screen at screening and Day -1. The urine drug screen may be repeated at the discretion of the Investigator and the reason for repeat needs to be documented clearly (e.g., suspicion of false positive due to diet). 26. Participation in a clinical trial involving the administration of an investigational or marketed drug or device within 90 days of screening 27. Donation of plasma within 7 days prior to the first administration or donation or loss of blood (excluding volume drawn at screening) of 50 mL to 499 mL of blood within 30 days, or more than 499 mL within 56 days prior to the first administration. 28. Subjects who are unlikely to comply with the study protocol OR those who would not be suitable candidates for participation in the opinion of the Investigator including any reason which, in the opinion of the Investigator, would prevent the subject from completing all study procedures and complying with study restrictions. 29. Any other conditions (e.g., not suitable for venous access) or laboratory abnormality that may increase the risk associated with study participation or study drug administration or interfere with the interpretation of study results and, at the discretion of the investigator, makes the subject inappropriate for entry into this study
Study Assessments:	*Safety and Tolerability:* • Medical history, including evaluation of any on-study AEs and concomitant medication use • Height and weight • Physical examination

	• Vital signs (supine systolic and diastolic blood pressure, heart rate, respiratory rate, body temperature) • 12-lead ECGs • Analysis of laboratory safety markers (including hematology, chemistry, coagulation, and urinalysis) *Exploratory Pharmacodynamics:* Blood (serum and plasma), stool/fecal samples, and urine samples will be collected for analysis and identification of GLY-200-induced PD marker changes.
Statistical Considerations:	*Overview:* In general, clinical data will be summarized for each dose group using descriptive statistics. *Safety and Tolerability:* The primary analysis will assess the safety and tolerability of GLY-200 in patients. The sample size chosen for this study was selected without statistical considerations. <u>Safety Analysis</u> All subjects that are randomised and receive at least one dose of study drug will be included in the Safety population. The safety endpoint includes evaluation of AEs, clinical laboratory assessments, vital signs, 12-lead ECGs, and the use of concomitant medications. Safety data will be aggregated and tabulated based on the safety dataset for each treatment including but not limited to: • Analysis of withdrawal or suspension of medication due to AEs • Incidence and severity of AEs • Analysis of the correlation between AEs and study drug • Analysis of AE outcome • Analysis of SAEs • Descriptive statistical summary of laboratory tests, vital signs, and ECG data • Incidence of abnormal laboratory results. *Exploratory Pharmacodynamics:* PD marker parameters will be summarized using descriptive statistics and graphs (mean ± SEM) for each treatment at each timepoint.

1.2 STUDY SCHEMATIC



*Capsule number and frequency to match active groups.

1.3 SCHEDULE OF ACTIVITIES (SOA)

The study SoA provides an overview of each visit. Additional, unplanned visits may be arranged as deemed necessary by the Investigator.

STUDY SCHEDULE OF ACTIVITIES (SoA)												
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)	**Day 1** Pre-dose	**Day 1** Post-dose	**Days 2-6** (Each day)	**Day 7**	**Days 8-13** (Each day)	**Day 14** (Check-out)	**Day 21** (±1 day)	
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											

STUDY SCHEDULE OF ACTIVITIES (SoA)												
STUDY PERIOD	**Screening**	**Washout**		**Confinement/Inpatient**							**Follow-Up/EOS**	**Early Termination (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)	**Day 1** Pre-dose	**Day 1** Post-dose	**Days 2-6** (Each day)	**Day 7**	**Days 8-13** (Each day)	**Day 14** (Check-out)	**Day 21** (±1 day)	
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							
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	X
Blood Sampling (Exploratory PD: Hormones, Bile acids, Nutrients) [16]				X	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 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 Section 1.3.1).
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 Section 1.3.1 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 Section 1.3.1 for further details.

1.3.1 MIXED MEAL TOLERANCE TEST SCHEDULE

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.

2. INTRODUCTION

2.1 STUDY RATIONALE

T2D is a chronic condition with the fundamental pathophysiological abnormalities of insulin resistance and pancreatic beta cell dysfunction, resulting in reduced insulin secretion and eventually insulin deficiency. Eighty-four (84%) percent of diabetes patients are on oral medications and/or injectables such as insulin [1–3]. However, insulin as a management therapy requires significant lifestyle changes and can result in weight gain, contributing to even greater insulin resistance. Multiple therapies are also required, as no single therapy has been able to treat all the pathophysiological abnormalities of T2D. The persistent hyperglycemia of T2D increases the risk of various macro- and micro-vascular complications such as ischemic heart and cerebrovascular disease, retinopathy, nephropathy, and neuropathy (which can result in limb amputations). Therefore, patients with T2D require long-term medical care (i.e., medications, testing, procedures, etc.) at considerable cost.

There is strong evidence that obesity management is highly beneficial in the treatment of T2D. Surgical procedures for obesity treatment, generally referred to as metabolic surgery, can strongly improve glycemic control and promote significant and durable weight loss, remission of diabetes, improved quality of life, improved cardiovascular outcomes, and reduced mortality [4]. Given its profound effects, metabolic surgery was added to the American Diabetes Association (ADA) treatment algorithm for T2D in 2017. Notably, some metabolic surgeries have been shown to also induce an immediate and significant improvement in glucose homeostasis and diabetes symptoms, well in advance of eventual weight loss [5,6]. One such procedure is the Roux-en-Y gastric bypass (RYGB), in which the stomach is made smaller and attached to a distal part of the small intestine, known as the jejunum. The surgically excluded stomach portion and the duodenum remain in the body but are removed from the path of nutrient flow. Excluding the duodenum from contact with intraluminal chyme ("duodenal exclusion"), triggers profound changes in glucose homeostasis, including improvement in systemic and hepatic insulin resistance and improved pancreatic beta cell function, leading to enhanced insulin responses particularly postprandially. A duodenal exclusion device in development, EndoBarrier® (a gastrointestinal liner inserted in the duodenum via endoscopy), has replicated many of the benefits of metabolic surgery [7] but has had safety issues such as hepatic abscess that have delayed development.

GLY-200 is an orally-dosed, non-absorbed synthetic polymer comprised of a chemically modified derivative of poly(allylamine hydrochloride) and has a molecular weight of approximately 57 kDa, which exceeds the threshold for oral absorption. GLY-200 is being developed as an adjunct to diet and exercise to improve glycemic control in individuals with T2D.

GLY-200 is a soluble molecule that irreversibly crosslinks in a pH-dependent manner with mucin, a major component of the mucus layer that lines the gastrointestinal tract. Specifically, upon delivery to the stomach, GLY-200 dissolves rapidly in the low pH environment (pH < 5.5). As the dissolved polymer passes through the pylorus, the higher pH environment of the duodenum (pH > 5.5) facilitates rapid crosslinking of the polymer with endogenous mucin.

When GLY-200 complexes with mucin, it enhances the natural mucus barrier in the duodenum to provide a temporary pharmacological duodenal exclusion intended to safely reproduce many of the beneficial effects of metabolic surgery and duodenal exclusion devices. The polymer-mucus complex is shed via continuous mucus turnover and is expected to be eliminated in the feces within 24 hours. Twice-daily dosing is expected to provide a continuous duodenum barrier achieving steady-state in approximately 3 days.

This clinical study is being conducted to assess the safety and tolerability of GLY-200 in T2D patients.

2.2 BACKGROUND

GLY-200 has been evaluated in a number of pre-clinical R&D and GLP toxicology studies. Nonclinical animal studies demonstrate that oral administration of high doses of GLY-200 results in no systemic exposure, no local GI tract irritation, and little evidence of GI-related tolerability issues. Moreover, in 3 different rat models of T2D, a significant improvement was observed in fasting and post-prandial hyperglycemia, a reduction in fasting and post-prandial insulin, a parallel reduction in body weight, a normal beta cell response to insulin secretion and improvement in insulin sensitivity. Refer to the Investigator's Brochure (IB) for further details.

First-in-Human Study GLY-200-01

A randomized Phase 1, single-center, placebo-controlled, double-blind study was conducted to assess the safety and tolerability of oral GLY-200 in healthy volunteers. The study consisted of 2 parts: a single ascending dose (SAD) escalation and a multiple ascending dose (MAD) escalation. Study conduct has completed, and a clinical study report is pending.

The SAD study enrolled 32 subjects across 4 cohorts (n=8 each, 6:2 active to placebo). The single oral doses administered were 0.5 g, 2.0 g, 4.0 g, and 6.0 g. There were no serious adverse events (SAEs) and no dose-limiting toxicities in the SAD study. Treatment-emergent adverse events (TEAEs) were primarily gastrointestinal, with most being mild.

The MAD study enrolled 32 subjects across 4 cohorts (n=8, 6:2 active to placebo). Subjects were dosed for 5 days and received either 1.0 g BID, 1.0 g TID, 2.0 g BD, 2.0 g TID, or 3.0 g BID. There were no SAEs in the MAD study, and most of the TEAEs were mild dose-dependent gastrointestinal events.

In summary, administration of GLY-200 was well-tolerated across dose levels tested, supporting the development of GLY-200 for the treatment of T2D.

2.3 BENEFIT/RISK ASSESSMENT

2.3.1 KNOWN POTENTIAL RISKS

Study Drug

The study drug GLY-200 is an investigational product (IP) and therefore has treatment related risks. Refer to the IB for a complete description of known potential risks associated with GLY-200.

Findings in animal toxicity studies demonstrated the lack of systemic toxicity and no clearly adverse local effects in the GI tract. The potential adverse effects from GLY-200 in humans are therefore anticipated to be based on local tolerance effects to the GI tract and decreased nutritional uptake. In the Phase 1 MAD study where subjects were dosed for up to 5 days, the most common reported AEs were:

- Nausea
- Decreased appetite
- Vomiting
- Abdominal distension
- Flatulence
- Diarrhea
- Constipation
- Headache
- Abdominal discomfort
- Eructation
- Dry mouth
- Abdominal pain upper
- Dyspepsia

Allergic Reactions

As with any drug, there is the chance of an allergic reaction that may include difficulty breathing, rash, flushing, weakness, lightheadedness, dizziness, and swelling.

Blood Sampling

There is some risk of pain or local bruising and infection at the site where blood is drawn for laboratory tests. There is also a small risk of a fainting episode, which can occur as a reaction to giving blood.

The total amount of blood that will be collected over the course of screening and the 21-day study is ≤ 600 mL. The total amount of blood that will be collected is less than 1.5 standard blood donations. There is a risk that subjects may experience anemia.

Electrocardiograms

Up to 12 self-adhesive electrodes will be attached to the subject's skin on their arms, legs, and chest. The areas where the electrodes will be placed will be cleaned; some areas may need to be shaved. Some skin irritation can occur where the electrodes are placed.

2.3.2 KNOWN POTENTIAL BENEFITS

Information from this study may help Investigators and the Sponsor learn more about GLY-200 and its ability to improve the management of uncontrolled glycemia and increased weight in patients with T2D. This information may help other subjects in future studies.

2.3.3 OVERALL BENEFIT: RISK CONCLUSION

The results of Glyscend's preclinical study program strongly supports the conclusion that GLY-

200 is non-absorbed, non-toxic, and non-genotoxic. Taken together, the nonclinical package supports an attractive risk-benefit profile for GLY-200 as a novel treatment for T2D.

3. OBJECTIVES AND ENDPOINTS

Primary Objectives	*Primary Endpoints*
• To assess the safety and tolerability of GLY-200 over the 14-day treatment period	• 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
Secondary Objectives	*Secondary Endpoints*
• 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	• 3-hour plasma glucose and insulin profiles • Fasting plasma glucose and insulin
Exploratory Objectives	*Exploratory Endpoints*
• 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	The preliminary PK and pharmacodynamics (PD) will include (but will not be limited to): • boron analysis on urine and feces • CGM glucose parameters • Blood: hormones and bile acids (fractionated and total) • Urinalysis (oxalate, phosphate, uric acid) • Macronutrients, Calories, hunger, and satiety • Changes from baseline in nutritional parameters Additional exploratory markers may be analyzed from stool, plasma, and urine samples.

4. STUDY DESIGN

4.1 OVERALL DESIGN

This study is a Phase 2, randomized, double-blind, placebo-controlled, single-center study in adult patients with T2D. 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. Refer to Section 1.2 for the study schematic.

The dose levels planned are:

- Active Group 1: 0.5 g GLY-200 (1 x 0.5 g capsule) BID (n=12)
- Active Group 2: 1.0 g GLY-200 (2 x 0.5 g capsules) BID (n=12)
- Active Group 3: 2.0 g GLY-200 (4 x 0.5 g capsules) BID (n=12)
- Placebo Group 1: 1 placebo capsule BID (n=4)
- Placebo Group 2: 2 placebo capsules BID (n=4)
- Placebo Group 3: 4 placebo capsules BID (n=4)

Subjects will participate in a ≤ 18-day screening period followed by a metformin washout period of approximately 14 days, and an inpatient period of 16 days. Dosing will occur for 14 days. A follow-up clinic visit will occur on Day 21 [End of Study (EOS)] or Early Termination (ET). Specific timing of protocol procedures is described in the Schedule of Activities (Section 1.3).

This study will be conducted at one investigational site in the US.

4.2 SCIENTIFIC RATIONALE FOR STUDY DESIGN

This randomized, double-blind, placebo-controlled study will evaluate the safety and tolerability of GLY-200 administered orally for 14 consecutive days. The placebo used in this study will be visually indistinguishable from active GLY-200 and placebo group dosing regimens (i.e., capsule counts) will match the active control groups to preserve the blind. A placebo-controlled, double-blind design is considered appropriate for this study as it allows for evaluation of the safety and tolerability of GLY-200 through an unbiased comparison of the active study drug and placebo.

4.3 JUSTIFICATION FOR DOSE

In the GLY-200-01 study, single doses of up to 6 g GLY-200 were administered in the SAD part of the study and were well tolerated in healthy subjects. In the MAD part of the study, healthy subjects received up to 5 days dosing of 1 g BID, 1 g TID, 2 g BID, 2 g TID, or 3 g BID GLY-200 resulting in total daily doses of 2 to 6 g. The most common AEs observed were mild nausea, decreased appetite, and vomiting that infrequently resulted in dose holidays for some subjects that received the highest total daily dose of 6 g. Gastrointestinal AEs appeared to be dose-dependent and BID dosing was better tolerated than TID dosing. There were no deaths, SAEs, or AEs leading to withdrawal in the active groups and no safety signals were identified based on AEs, clinical labs, vital signs, ECGs, and physical exams.

Doses of 0.5 to 2 g BID were selected for this Phase 2 study as they were well tolerated in the MAD/SAD study and are predicted to have positive effects on postprandial glucose based on

nonclinical rodent studies.

4.4 END OF STUDY DEFINITION

A subject is considered to have completed the study if he or she has completed all phases of the study including the last visit shown in the Schedule of Activities (Section 1.3).

5. STUDY POPULATION

Eligibility of subjects will be determined by the following inclusion and exclusion criteria. Subjects should meet all the inclusion criteria and none of the exclusion criteria.

5.1 PARTICIPANT INCLUSION CRITERIA

In order to be eligible for the study, subjects must fulfill all of the following criteria:

1. Patients diagnosed with type 2 diabetes:
    a. treated with a stable dose of metformin ≥750 mg/day for at least 3 months prior to screening
    b. willing to come off current metformin treatment during washout and treatment period
    c. HbA1c ≥ 6.0 and ≤ 8.5% at screening
    d. completed at least 12 days of metformin washout without meeting rescue criteria prior to dosing
2. Male or female, ≥ 30 and ≤ 70 years old at the time of screening
3. BMI ≥ 18 and ≤ 40 at screening
4. Capable of consent, and written informed consent signed prior to entry into the study
5. Willing to consume standard meals provided by the study center
6. Able and willing to attend the necessary visits to the study center
7. Willing to stop antacid use at screening
8. Male subjects with female partners of childbearing potential must comply with the contraception requirements as described in Appendix 3 from the time of first dose of study medication until 90 days after the last dose of study medication (i.e., one sperm cycle)
9. Female subjects must comply with contraception requirements as described in Appendix 3 from the screening period through the end of study or be of non-childbearing potential, defined as meeting at least one of the following criteria:
    a. Achieved postmenopausal status, defined as: cessation of regular menses for at least 12 consecutive months with no alternative pathological or physiological cause; and have a serum follicular stimulating hormone (FSH) level within the laboratory's reference range for postmenopausal women
    b. Have undergone a documented hysterectomy and/or bilateral oophorectomy
    c. Have medically confirmed ovarian failure

    All other female subjects (including women with tubal ligations and women who do NOT have a documented hysterectomy, bilateral oophorectomy and/or ovarian failure) will be considered to be of childbearing potential.

PLEASE NOTE: ORAL CONTRACEPTION IS **NOT ALLOWED** (and is exclusionary) IN THIS STUDY AS THE STUDY DRUG (GLY-200) MAY REDUCE THE EFFECTIVENESS OF ORAL CONTRACEPTION DUE TO POTENTIAL DISRUPTION ON GI ABSORPTION.

10. Fully vaccinated against COVID-19 based on the recommendations of the region at least 14 days prior to dosing

5.2 PARTICIPANT EXCLUSION CRITERIA

Any potential subject who meets one or more of the following criteria will not be included in this study:

1. Women who are pregnant (as confirmed by a serum or urine human chorionic gonadotrophin [hCG] test) or lactating
2. Women of childbearing potential who are using oral contraception
3. Vegetarian or vegan
4. Treated with any prescription medication for the treatment of type 2 diabetes or weight loss other than metformin in the last 3 months prior to screening
5. Use of any drug treatment that affects gastric pH (prescription or over-the counter), such as H2-receptor antagonists and proton pump inhibitors for 3 months prior to screening (e.g., famotidine, ranitidine, esomeprazole, dexlansoprazole, omeprazole, pantoprazole)
6. Use of any drug treatment that affects gastrointestinal motility including but not limited to cholinergic agonists and antagonists, prokinetic agents, opioid antagonists, antidiarrheals, and antibiotics in the last 3 months prior to screening (e.g., cevimeline, pilocarpine, loperamide, erythromycin, metoclopramide, domperidone, cisapride, mosapride, sincalide)
7. Any chronic condition that requires oral drug treatment that would violate concomitant medication use restrictions described in Section 6.8 (i.e., all orally administered concomitant medications must be administered at least 2 hours before and 4 hours after GLY-200 dosing), TID (three times a day) drugs, or drugs with very narrow therapeutic windows (e.g., warfarin)
8. Fasting blood glucose > 190 mg/dL at screening
9. Diagnosis or treatment of any clinically symptomatic biochemical or structural abnormality of the GI tract or active disease within 12 months prior to screening; including but not limited to inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), gastroesophageal reflux disease (GERD), GI cancer, diverticulitis, duodenal ulcer, erosive esophagitis, gastric ulcer, pancreatitis, symptomatic gallbladder disease, ileus, intestinal/pyloric stenosis/obstruction, fistula, malabsorption, esophageal anatomic abnormalities, or other severe GI disease, particularly those that may impact digestion, nutrient absorption, intestinal motility, and/or gastric emptying
10. History of any previous abdominal or intestinal surgery including endoscopic, open or laparoscopic thoracic or abdominal surgery, surgical resection of the stomach, small or large intestine (excluding appendectomy, cholecystectomy, or resection of benign polyps), or of any other risk of abdominal adhesions or obstruction. Appendectomy ≥ 3 months prior to screening is allowed.

11. Clinically significant symptoms (as determined by the Investigator) of nausea, vomiting, bloating, diarrhea, flatulence, constipation, or abdominal pain in the last 30 days prior to screening
12. Use of systemic corticosteroids by oral, intravenous, or intramuscular route; or potent, inhaled, or intrapulmonary steroids known to have a high rate of systemic absorption within 3 months of screening
13. Clinically significant medical condition as judged by the Investigator that could potentially affect study participation and/or personal well-being, including but not limited to the following conditions:
    a. Uncontrolled hypertension
    b. Uncontrolled hyperlipidaemia
    c. Hepatic disease
    d. Renal disease (eGFR value of <60 mL/min/1.73 m^2) based on the Modification of Diet in Renal Disease equation.
    e. Endocrine disorder other than diabetes
    f. Cardiovascular event in the last 12 months
    g. Central nervous system diseases
    h. Organ transplantation
    i. Chronic or acute infection
    j. Orthostatic hypotension, fainting spells, or blackouts
14. Any evidence of or treatment of malignancy (other than localized basal cell, squamous cell skin cancer, non-aggressive prostate cancer, or cancer in situ that has been resected) within the previous year
15. Clinically significant electrocardiogram (ECG) abnormalities or vital sign abnormalities as determined by the Investigator
16. Clinical laboratory test (clinical chemistry, hematology, coagulation parameters, or urinalysis) abnormality, other than that related to T2D, judged by the Investigator to be clinically significant
17. Alanine aminotransferase or aspartate aminotransferase result >2.5 × upper limit of normal (ULN) or a total bilirubin result >1.5 × ULN
18. Physical, psychological, or historical finding that, in the Investigator's opinion, would make the subject unsuitable for the study
19. Severe infections, injuries, or major surgeries (as determined by the Investigator) within 4 weeks prior to screening or intend to undergo any surgery during the trial
20. Use of a live vaccine within 30 days prior to screening or anticipated need for a live vaccine during the study or for 30 days following the last dose of study drug
21. History of bleeding associated with procedures (e.g., endoscopy or phlebotomy) or with use of medications (e.g., nonsteroidal anti-inflammatory drugs [NSAIDs] or aspirin) within 28 days prior to screening or planned use during the study
22. Positive test for human immunodeficiency virus (HIV), hepatitis B surface antigen (HBsAg), or hepatitis C antibodies (HCV)

23. History of severe allergy to any drug, food, toxin, or other exposure
24. History of heavy alcohol use within 1 year prior to screening, as defined by:
    a. Exceeding 4 standard drinks on any day or more than 14 standard drinks per week for men, or more than 3 standard drinks on any day or more than 7 standard drinks per week for women; one standard drink=10 grams of alcohol (equivalent to 12.5 mL of pure alcohol, 285 mL of full-strength beer, 375 mL of mid-strength beer, 425 mL of low-strength beer, 100 mL of wine, or 30 mL of 40% alcohol spirits).
25. History of illicit or prescription drug abuse or addiction within 1 year of screening, or positive urine drug screen at screening and Day -1. The urine drug screen may be repeated at the discretion of the Investigator and the reason for repeat needs to be documented clearly (e.g., suspicion of false positive due to diet).
26. Participation in a clinical trial involving the administration of an investigational or marketed drug or device within 90 days of screening
27. Donation of plasma within 7 days prior to the first administration or donation or loss of blood (excluding volume drawn at screening) of 50 mL to 499 mL of blood within 30 days, or more than 499 mL within 56 days prior to the first administration.
28. Subjects who are unlikely to comply with the study protocol OR those who would not be suitable candidates for participation in the opinion of the Investigator including any reason which, in the opinion of the Investigator, would prevent the subject from completing all study procedures and complying with study restrictions.
29. Any other conditions (e.g., not suitable for venous access) or laboratory abnormality that may increase the risk associated with study participation or study drug administration or interfere with the interpretation of study results and, at the discretion of the investigator, makes the subject inappropriate for entry into this study

5.3 LIFESTYLE CONSIDERATIONS

5.3.1 CONTRACEPTION

FEMALE SUBJECTS

Female subjects of non-childbearing potential must meet at least one of the following criteria:

a. Achieved postmenopausal status, defined as: cessation of regular menses for at least 12 consecutive months with no alternative pathological or physiological cause; and have a serum follicular stimulating hormone (FSH) level within the laboratory's reference range for postmenopausal women
b. Have undergone a documented hysterectomy and/or bilateral oophorectomy
c. Have medically confirmed ovarian failure

All other female subjects (including women with tubal ligations and women who do NOT have a documented hysterectomy, bilateral oophorectomy, and/or ovarian failure) are considered to be of childbearing potential and must be using effective forms of contraception from the screening period through the end of study. Refer to Appendix 3.

NOTE: ORAL CONTRACEPTION IS NOT ALLOWED IN THIS STUDY AS GLY-200 MAY REDUCE THE EFFECTIVENESS OF ORAL CONTRACEPTION DUE TO POTENTIAL DISRUPTION ON GI ABSORPTION.

MALE SUBJECTS

Male subjects with female partners of childbearing potential must comply with the contraception requirements as described in Appendix 3 from the time of first dose of study medication until 90 days after the last dose of study medication (i.e., one sperm cycle).

5.3.2 MEALS AND DIETARY RESTRICTIONS

Subjects must fast for at least 8 hours prior to clinical laboratory blood sample collection timepoints (Days 1, 7, 14, and 21 [EOS] or ET).

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.

Subjects should limit their consumption of foods high in boron (e.g., avocado, dried fruits, and nuts/nut butters) starting after the Day -7 Washout visit until EOS or ET. Subjects should also limit their consumption of foods containing whole corn kernels starting after the Day -7 Washout visit until EOS or ET as it could interfere with fecal PK assessments.

Meals

On Days -1, 1, and 13, an ad lib meal will be provided for dinner. Refer to Section 8.2.12 for further details regarding Food Intake and Appetite Assessments.

Ensure Plus (100 g carb, 700 kcal in 16 fl ounce) will be provided for MMTTs and for breakfast on Day 13. Refer to Section 8.2.13 for further details regarding the MMTT.

For all other meals during the inpatient period, subjects will be provided a weight-maintaining diet with standardized meals (see Operational Manual). Food intake will be documented as described in Section 8.2.11.

5.3.3 OTHER RESTRICTIONS

Additional restrictions during the study period include no use of tobacco products, alcohol, or illicit drugs. Caffeine cannot be consumed during the time subjects are confined to the clinical unit. Refer to Section 6.8 for restrictions related to concomitant therapies.

5.4 SCREEN FAILURES

5.4.1 SCREEN FAILURES

Screen failures are defined as subjects who consent to participate in the clinical trial but are not subsequently dosed or entered into the study.

5.4.2 SUBJECT RESCREENING

Subjects are allowed to be rescreened once. Rescreens are limited to subjects who did not meet inclusion/exclusion criteria due to a transient reason. Transient refers to self-limiting and predictably resolving conditions or acute events, reversible medical conditions that are successfully treated, and/or being unable to comply with study procedures due to administrative convenience (e.g., family issues or attending to a private matter).

Subjects who failed any entry criteria for which no further treatment or spontaneous resolution is expected are not allowed to be rescreened.

Any rescreened subject must be re-consented and will be issued a new subject number. All screening procedures and assessments must be performed at rescreen.

5.5 STRATEGIES FOR RECRUITMENT AND RETENTION

A detailed recruitment and retention plan will be maintained by the Sponsor and/or study site.

6. STUDY INTERVENTION

6.1 STUDY INTERVENTION ADMINISTERED

6.1.1 STUDY INTERVENTION(S) DESCRIPTION

GLY-200

GLY-200 is an amorphous polymer containing 4-8 wt.% water. The polymer is inert and is provided in opaque gelatine capsules (Capsugel size 00) that contain 0.5 g active study drug. Capsules are packaged in high-density polyethylene bottles with induction seals and child-resistant polypropylene plastic caps.

Placebo

Placebo capsules are opaque 00 shells filled with microcrystalline cellulose to a weight comparable to the active capsules. The appearance of the placebo and active is identical, the same shells are used for both.

6.1.2 DOSING AND ADMINISTRATION

The dose levels planned are:

- Active Group 1: 0.5 g GLY-200 (1 x 0.5 g capsule) BID (n=12)
- Active Group 2: 1.0 g GLY-200 (2 x 0.5 g capsules) BID (n=12)
- Active Group 3: 2.0 g GLY-200 (4 x 0.5 g capsules) BID (n=12)
- Placebo Group 1: 1 placebo capsule BID (n=4)
- Placebo Group 2: 2 placebo capsules BID (n=4)
- Placebo Group 3: 4 placebo capsules BID (n=4)

Each subject will be randomly assigned to a group at Day -1 or Day 1.

During the inpatient period, delegated study site personnel will supervise and instruct patients on the administration of the Investigational Products. Study drug should be administered 60 minutes prior to breakfast and dinner. The exact time/date of IP administration will be recorded in the eCRF.

All capsules will be consumed orally, with water as required. The subject will be instructed to avoid food for 2 hours prior to and 1 hour following each dose administration.

6.2 PREPARATION/HANDLING/STORAGE/ACCOUNTABILITY

6.2.1 ACQUISITION

A designated manufacturer/distributor of the Sponsor will provide the site (or designated Pharmacy) with a sufficient quantity of IP (GLY-200 capsules and placebo capsules). The site Pharmacy must ensure that deliveries of IP are correctly received by a responsible person, that all receipts of drug shipments are recorded on the appropriate Drug Accountability forms prepared by the site Pharmacy, and that all IP are stored in a secure area under recommended storage conditions, as listed in this protocol (for GLY-200 capsules and placebo capsules).

The Investigator or designee must confirm appropriate temperature conditions have been maintained during transit for all study intervention received and any discrepancies are reported and resolved before use of the study intervention.

6.2.2 PREPARATION

Multiple capsules will be used to vary dosing as part of the trial design. Refer to Section 6.1.2.

6.2.3 HANDLING AND STORAGE

Study drug supplies will be stored securely at the site and/or designated Pharmacy according to applicable local and country regulations.

GLY-200 and placebo capsules should be stored between 15 to 25 °C in the original manufacturer's container. Shelf-life stability testing of the intact bottles is on-going.

6.2.4 STUDY INTERVENTION ACCOUNTABILITY PROCEDURES

The Investigator and/or designee is responsible for study intervention accountability, reconciliation, and record maintenance (i.e., receipt, reconciliation, and final disposition records).

All used and unused study medication received, dispensed, and returned must be accounted for in the study medication dispensing and accountability logs. The log includes, but is not limited to the following:

- Subject number and initials
- Date study medication was dispensed
- Quantity dispensed

- Date and quantity of study medication returned
- Capsule counts for doses taken

The study medication dispensing log and remaining drug inventory will be reviewed by the Sponsor-designated clinical monitor.

Only participants enrolled in the study may receive study intervention and only authorized site staff may supply or administer study intervention. All study intervention must be stored in a secure, environmentally controlled, and monitored (manual or automated) area in accordance with the labeled storage conditions with access limited to the investigator and authorized site staff.

6.3 MEASURES TO MINIMIZE BIAS: RANDOMIZATION AND BLINDING

An unblinded, trained pharmacist will be responsible for the dispensing of study treatments in order to maintain blinding.

A statistician will be responsible for generating a randomization schedule before the study starts. Participants will be blinded to treatment assignment (GLY-200 or placebo). Study drug will be prepared for administration by an unblinded pharmacist and administered by blinded personnel at the study site (when applicable). The randomization schedules will be maintained under controlled access. If the blind is unintentionally broken at the study center, the breaking of the blind should be reported to the Sponsor as soon as possible.

In the event of a medical emergency in which knowledge of the identity of the study medication is critical to the management of the subject's immediate course of treatment, the blind may be broken by a qualified physician who is an Investigator in the study. If deemed necessary to break the blind for a subject, the Medical Monitor should be contacted to obtain concurrence. If it is not possible to contact the Medical Monitor beforehand, he or she should be contacted as soon as possible after breaking the blind for a subject.

6.4 STUDY INTERVENTION COMPLIANCE

Not applicable.

6.5 DOSE MODIFICATION/ADJUSTMENTS/DELAYS

In rare instances, it may be necessary for a subject to temporarily discontinue study drug at the direction of the Investigator. Dosing may be reinitiated at the Investigator's discretion, but dose adjustments are not allowed.

6.6 CONTINUED ACCESS TO STUDY INTERVENTION AFTER THE END OF THE STUDY

Not applicable.

6.7 TREATMENT OF OVERDOSE

There is no specific antidote for GLY-200 but overdose is not expected to require specific action beyond observation and conservative measures, as compound excretion should be complete over

a period of 24 to 48 hours.

6.8 CONCOMITANT THERAPY

All concomitant therapies taken within 7 days of the Screening Visit up until the closeout visit will be captured in the source documents and CRF. "All therapies" should include prescription, over-the-counter, supplements, as well as herbal or alternative medications.

Any allowed oral concomitant medications must be taken at least 2 hours before and 4 hours after GLY-200 dosing. In addition:

- It is preferable that QD medications be taken in the morning, at least 2 hours before GLY-200 dosing.
- For BID medications, it is preferable that the first dose be taken in the morning, at least 2 hours before GLY-200 dosing, and the second dose be taken at least 4 hours after the evening GLY-200 dose.

See Section 6.8.1 for exceptions.

6.8.1 PROHIBITED MEDICATIONS, TREATMENTS, AND PROCEDURES

The following medications, treatments, and procedures are prohibited for the duration of the study:

- Any prescription medication for the treatment of type 2 diabetes or weight loss
- Any medication that requires TID (three times a day) dosing
- Any drug treatment that affects gastric pH (prescription or over-the counter), such as H2-receptor antagonists and proton pump inhibitors (e.g., famotidine, ranitidine, esomeprazole, dexlansoprazole, omeprazole)
- Any drug treatment that affects gastrointestinal motility including but not limited to cholinergic agonists, prokinetic agents, opioid antagonists, antidiarrheals, and antibiotics (e.g., cevimeline, pilocarpine, loperamide, erythromycin, metoclopramide, domperidone, cisapride, mosapride, sincalide), unless necessary to treat an AE as determined by the Investigator in consultation with the Sponsor
- Systemic corticosteroids by oral, intravenous, or intramuscular route; or potent, inhaled, or intrapulmonary steroids known to have a high rate of systemic absorption
- Participation in a clinical trial involving the administration of an investigational or marketed drug or device

The following medications and treatments are allowed during the study and can be taken at any time relative to GLY-200 dosing at the discretion of the Investigator:

- Oral medication necessary to treat an AE (e.g., intake of paracetamol is allowed, however, only after consulting the Investigator and obtaining approval).
- Antiemetic treatment (in order of preference: odansetron [Zofran], prochlorpereazine [Compazine], or promethazine [Phenergan]) as deemed appropriate by the Investigator for subjects who develop nausea or vomiting. Counseling and non-pharmaceutical treatments should be utilized as first- and second-line treatments, respectively, before antiemetics are prescribed.

6.8.2 RESCUE MEDICINE

A subject with 2 consecutive fasting blood glucose values > 240 mg/dL is considered to have met protocol-specified criteria for Loss of Glucose Control unless there is a subsequent value below 240 mg/dL. Subjects meeting protocol-specified criteria for Loss of Glucose Control may initiate rescue medication per the judgment of the clinical Investigator in consultation with the Sponsor. Subjects may be rescued with metformin or insulin.

Fasting blood glucose will be measured by the subject using a home glucose meter daily from Day -7 to inpatient check-in. If a subject meets criteria for rescue during the washout period, the subject will not be randomized.

7. DISCONTINUATION OF STUDY INTERVENTION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 DISCONTINUATION OF STUDY INTERVENTION

In rare instances, it may be necessary for a participant to permanently discontinue study intervention. If study intervention is permanently discontinued, the participant should remain in the study and continue with scheduled study visits if possible to be evaluated for safety.

7.2 PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

7.2.1 REASONS FOR DISCONTINUATION/WITHDRAWAL

Subjects have the right to withdraw from the study at any time for any reason. The Investigator also has the right to decide whether a subject should be removed from the study. Treatment will be discontinued, and the subject may be discontinued from study under the following circumstances:

- Any unacceptable AEs, in the judgement of the Investigator
- Subject non-compliance
- Physician decision
- Request by a regulatory authority
- Lost to follow-up
- Death
- Withdrawal by subject
- Study terminated by Sponsor
- Other

7.2.2 HANDLING OF PARTICIPANT DISCONTINUATION/WITHDRAWAL

Subjects who discontinue from the trial should always be asked about the reason(s) for their discontinuation and about the presence of any AEs. The date the subject is withdrawn from the study and the reason for the discontinuation should be recorded on the CRF. If possible, the subjects should be seen and assessed by an Investigator and have an ET Visit. AEs should be followed up until resolved or stable and determined to be chronic.

Subjects who are randomized but who withdraw prior to dosing will be replaced. Subjects who withdraw after dosing or subjects that were nonevaluable due to significant protocol deviations may be replaced to ensure adequate numbers to complete the study. The decision to replace or not after dosing will be made on a case-by-case basis by the Sponsor.

7.3 LOST TO FOLLOW-UP

A subject will be considered lost to follow-up if he or she repeatedly fails to return for scheduled visits and is unable to be contacted by the study site.

The following actions must be taken if a subject fails to return to the clinic for a required study visit:

- The site must attempt to contact the subject and reschedule the missed visit as soon as possible and counsel the subject on the importance of maintaining the assigned visit schedule and ascertain whether or not the subject wishes to and/or should continue in the study.
- Before a subject is deemed lost to follow up, the Investigator or designee must make every effort to regain contact with the subject (where possible, 3 telephone calls and, if necessary, a certified letter to the subject's last known mailing address or local equivalent methods). These contact attempts should be documented in the subject's medical record.
- Should the subject continue to be unreachable, he/she will be considered to have withdrawn from the study.

8. STUDY ASSESSMENTS AND PROCEDURES

Written informed consent must be obtained prior to any study-specific assessments.

All screening evaluations must be completed and reviewed to confirm that potential participants meet all eligibility criteria. The Investigator will maintain a screening log to record details of all participants screened and to confirm eligibility or record reasons for screening failure, as applicable.

Clinical staff are required to perform assessments at the nominated timepoints within the time windows indicated in SoA. Actual times of procedures for each patient may vary depending on scheduling and will be recorded in the eCRF. In the event of multiple procedures scheduled at the same time, the order, where possible, will be (1) vital signs, (2) 12-lead ECG, (3) safety laboratory blood sampling, and (4) PD sampling. Repeat or unscheduled samples may be taken for safety reasons or for technical issues with the samples.

8.1 EFFICACY ASSESSMENTS

Not applicable.

8.2 SAFETY ASSESSMENTS

The following safety assessments will be collected at the timepoints provided in the SoA (Section 1.3).

8.2.1 MEDICAL HISTORY

A medical history will be obtained and will include demographic data (e.g., age, race/ethnicity). In addition, medical information will also be recorded, including a detailed oncologic history with specifics of previous cancer treatments, other medical and surgical history, medication history and drug allergies and any other medical conditions and disease states that, in the opinion of the Investigator, are relevant to the subject's study participation.

8.2.2 HEIGHT AND WEIGHT

Body height and weight will be measured, and body mass index (BMI) will be calculated using the height recorded at screening. Height should be measured on a wall-mounted stadiometer. Weight should be measured in light-weight clothing, without shoes.

8.2.3 PHYSICAL EXAMINATION

A complete physical examination will be performed at Screening and at the EOS or ET visit. The complete physical examination will include, at a minimum, assessment of the following systems: HEENT, Mouth/Dental (if required), Neck (including Thyroid & Nodes), Cardiovascular, Respiratory, Gastrointestinal, Renal, Neurological, Musculoskeletal, Skin, Other. All other scheduled examinations will be symptom-directed.

All physical examinations are to be conducted by a medically qualified Investigator.

8.2.4 ABDOMINAL EXAMINATION

Abdominal examinations are to include at a minimum palpation, auscultation (noting presence and frequency of bowel sounds), and percussion (looking for signs of peritonitis or localized tenderness). Abdominal examinations are to be conducted at the study visits indicated in the SoA prior to dosing.

Besides local gastrointestinal tolerability effects, such as nausea, vomiting, diarrhea, and constipation, there is a theoretical risk of small or large bowel obstruction. To mitigate this risk, particular attention will be applied for abdominal examination, with particular interest in signs of obstruction (reduced bowel sounds, decreased stool, bilious vomiting, and abdominal discomfort and distension). If an obstruction is suspected, the subject will be transferred to a medical facility and an abdominal X-ray obtained as well as other imaging that may be appropriate.

8.2.5 VITAL SIGNS

Vital signs will be measured by a qualified staff member. Vital signs assessments will include systolic and diastolic blood pressure, heart rate, respiratory rate, and body temperature. Patients should be resting in a supine position for at least 5 minutes prior to and during vital signs measurements.

8.2.6 ELECTROCARDIOGRAMS

Twelve-lead ECGs will be assessed (including but not limited to the measurements of ventricular

heart rate (HR), PR interval, respiratory rate (RR), QRS duration, QT interval and QT interval corrected by the Fridericia's formula [QTcF]). Screening and prior to first dose, triplicate 12-lead ECGs each separated by at least 1 minute will be taken to establish eligibility at baseline. The average value for the triplicate will be utilized for assessing QT interval corrected using Fridericia's formula (QTcF) exclusion criteria. 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 (separated by at least 1 minute).

ECG normal ranges are as follows:

- PR interval: 120 msec – 220 msec (inclusive);
- QRS duration: < 120 msec;
- QTcF ≤450 msec (males); QTcF ≤470 msec (females);
- HR 45-100 beats/min (inclusive)

If the "re-check" triplicate's average is still above these parameters, the PI shall be notified for a determination on further action. The same model of ECG machine should be used for Screening, pre-dose and post-dose readings for all patients. ECGs will be taken after at least 5 minutes in a supine quiet rest. ECGs will be interpreted, signed and dated by the PI, or medically qualified designee. The ECGs will be classified as normal, not having a clinically significant abnormality or having a clinically significant abnormality. In addition, ECG parameters of HR, PR interval, QT interval, PR interval, RR interval, QRS duration, and QTcF will be noted on the eCRF. All clinically significant findings will be recorded as AEs. Clinical staff are required to perform ECG assessments at the nominated time-points within the time windows indicated in the SoA.

8.2.7 CLINICAL LABORATORY EVALUATIONS

Subjects will be required to fast for at least 8 hours prior to clinical laboratory blood sample collection. Samples for local clinical laboratory analysis will be collected by a qualified staff member. All safety laboratory assessments will be assessed by a certified local laboratory, using the relevant laboratory's internal and standard normal range values.

At a minimum, the following tests will be conducted:

- *Hematology:* hemoglobin, hematocrit, red blood cell indices, thrombocyte count (platelets), reticulocyte count, white blood cell count with differential (including neutrophils, eosinophils, basophils, lymphocytes and monocytes).
- *Coagulation:* international normalized ratio, prothrombin time and activated partial thromboplastin time.
- *Chemistry:* sodium, potassium, magnesium, chloride, bicarbonate, phosphate, calcium, amylase, lipase, uric acid, albumin, globulins, protein, lactate dehydrogenase, creatine kinase, creatinine (estimated glomerular filtration rate), blood urea nitrogen, alkaline phosphatase, ALT, AST, GGT, total bilirubin, conjugated and unconjugated bilirubin, total cholesterol, high-density lipoproteins, low-density lipoproteins, triglycerides, glucose. Vitamin and mineral panels will be evaluated per the SoA.
- *Urinalysis:* pH, specific gravity, protein, glucose, ketones, bilirubin, blood, nitrites, urobilinogen, leukocyte esterase. Urine sediment microscopy will be conducted in the

instance of abnormal urinalysis findings.

Refer to Appendix 4 for a table of clinical laboratory assessments.

The Investigator or designee must review the results of each subject's screening clinical laboratory test results prior to first dose. The subject must not be treated on Day 1 if the subject has any clinically significant laboratory abnormalities as defined in the eligibility criteria.

The Investigator must review the laboratory report, document this review, and record any clinically relevant changes occurring during the study in the AE section of the eCRF.

8.2.8 PREGNANCY TEST

Blood samples for serum pregnancy analysis will be collected from all female subjects by a qualified staff member.

Serum and urine human chorionic gonadotropin pregnancy testing will be performed.

Any positive urine pregnancy test will be confirmed with a serum pregnancy test.

8.2.9 OTHER LABORATORY TESTS

- Serology: HIV, HBsAg, HCV antibodies
- Follicle-stimulating hormone (FSH) and estradiol levels (for post-menopausal females only)
- Drugs of abuse screen: The urine screen will include methamphetamines, opiates, cocaine, phencyclidine, benzodiazepines, barbiturates, methadone, amphetamines.

8.2.10 FLUID BALANCE

Urine samples will be taken for volume over a 24-hour period and compared with intake of fluids to provide the fluid balance.

8.2.11 DOCUMENTATION OF FOOD INTAKE

During the inpatient period, food intake (date, time, and quantity [% consumed based on visual estimation]) will be documented daily.

8.2.12 FOOD INTAKE AND APPETITE ASSESSMENTS

Food intake and appetite assessments will be performed at breakfast and dinner on Days -1, 1, and 13 to assess changes in food intake, kcals, and macronutrients based on food weight consumed and nutrient analysis.

For appetite assessments on these days, subjects will complete appetite visual analog scale (VAS) questionnaires.

8.2.13 MIXED MEAL TOLERANCE TEST

The MMTT is performed in the morning. Subjects should fast at least 8 hours prior to the MMTT. The mixed meal used in this protocol is Ensure Plus (100 g carb, 700 kcal in 16 fl

ounce) and should be consumed within 10 minutes.

Refer to the MMTT Test Schedule in Section 1.3.1 for further details.

An intravenous (IV) catheter should be placed for collection of PD blood samples. Plasma samples will be collected to evaluate glucose and insulin profiles.

8.3 ADVERSE EVENTS, SERIOUS ADVERSE EVENTS, AND OTHER SAFETY REPORTING

The definitions of AEs and SAEs can be found in Section 10.2.

AEs will be reported by the subject (or, when appropriate, by a caregiver, surrogate, or the subject's legally authorized representative).

The Investigator and any qualified designees are responsible for detecting, documenting, and recording events that meet the definition of an AE or SAE and remain responsible for following up as described in Section 8.3.3.

SAEs should be reported to the Sponsor and/or CRO within 24 hours from the discovery of the event.

The method of recording, evaluating, and assessing causality of AEs and SAEs and the procedures for completing and transmitting SAE reports are provided in Section 10.2.

8.3.1 TIME PERIOD AND FREQUENCY FOR COLLECTING AE AND SAE INFORMATION

Data regarding AEs will be collected in this study. AEs are events that occur during the course of the study that are not present prior to informed consent, or, if present at the time of informed consent, have worsened in severity during the course of the study. AEs will be assessed at each study visit after the informed consent document is signed through EOS.

TEAEs are events that occur during the course of the study that are not present prior to study medication administration, or, if present at the time of study medication administration, have worsened in severity during the course of the study.

8.3.2 METHOD OF DETECTING AES AND SAES

Each subject will be observed and queried by the Investigator or designee at each study visit for any continuing AEs or new AEs since the previous visit. Care will be taken not to introduce bias when detecting AEs and/or SAEs. Open-ended and non-leading verbal questioning of the participant is the preferred method to inquire about AE occurrences.

8.3.3 FOLLOW-UP OF AES AND SAES

The subject may be asked to return to the site for an unscheduled visit if an AE occurs between study visits, and if, in the opinion of the Investigator, the AE requires a study visit for full evaluation. Any AE reported by the subject or noted by the Investigator or designee will be recorded within the eCRF.

AEs that are not serious and are ongoing at the subject's last visit will be followed for a maximum of 30 days. SAEs that are not resolved or stabilized during this time period will be followed until resolution or stabilization.

8.3.4 REGULATORY REPORTING REQUIREMENTS FOR SAES

The Investigator is responsible for reporting SAEs to the Sponsor and IRB according to 21 CFR part 50, part 56, and part 312. Refer to Section 10.2.5 for further details.

8.3.5 PREGNANCY

If the subject or partner of the subject becomes pregnant, the pregnancy is to be followed until the outcome is known. An IRB-approved Pregnant Subject or Pregnant Partner Data Release Form should be completed by the subject or the subject's pregnant partner in order to obtain consent to follow the progress of the pregnancy and birth, and the health of the infant.

Any pregnancy will be collected on a Pregnancy Report Form. Information will be collected for any pregnancy in a female subject or the pregnant female partner of a male subject (if consenting), which occurs during the study, including perinatal and neonatal outcome. Infants resulting from such pregnancies should be followed for up to 12 weeks.

8.3.6 DISEASE-RELATED EVENTS AND/OR DISEASE-RELATED OUTCOMES NOT QUALIFYING AS AES OR SAES

Not applicable.

8.3.7 ADVERSE EVENTS OF SPECIAL INTEREST

The following are considered AEs of special interest in this study:

- **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

Events of special interest will be reported to the Sponsor in the same way as SAEs.

If the Sponsor identifies other AEs that qualify as events of special interest, sites will be notified.

8.4 EXPLORATORY PHARMACODYNAMICS AND PHARMACOKINETICS

Blood, urine, and fecal samples will be collected to assess the exploratory endpoints of GLY-200 as indicated in the SoA (Section 1.3).

A variety of exploratory markers will be analyzed from stool, urine, and plasma. These include (but will not be limited to):

Exploratory PD:

- Urine analysis (phosphate, oxalate, uric acid)
- Plasma (GLP-1, GIP, PYY, ghrelin, glucagon, fractionated and total bile acids, cholesterol, HDL/LDL)

Exploratory PK:

- Stool/Fecal analysis (boron analysis)
- Urine analysis (boron analysis)

PD blood samples will be collected via intravenous (IV) catheter.

Serum and plasma samples will be collected as indicated in the SoA (Section 1.3) and will be stored for future evaluation of GLY-200 metabolites.

Blood, urine, and fecal samples collected during the study will be stored for potential future exploratory analyses including but not limited to evaluating metabolites and PK and PD markers of GLY-200.

8.4.1 CONTINUOUS GLUCOSE MONITORING

The CGM device to be used in this protocol is the Dexcom G6. The device will be placed/replaced on the study days indicated in the SoA and will be removed on Day 14 prior to check-out.

9. STATISTICAL CONSIDERATIONS

This section describes the planned statistical analyses in general terms. A complete description of the statistical analyses will be specified in a statistical analysis plan (SAP), finalized before database lock.

9.1 STATISTICAL HYPOTHESES

No formal hypotheses are being tested in this study.

9.2 SAMPLE SIZE DETERMINATION

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

9.3 ANALYSIS SETS

Safety Analysis Set: All subjects who were randomized and took at least 1 dose of the study medication.

Full Analysis Set: All subjects who were randomized and took at least 1 dose of the study medication. This population is identical to the Safety Analysis Set.

Per-Protocol (PP) Analysis Set: Subjects in the Full Analysis Set who did not have any major protocol deviations.

9.4 STATISTICAL ANALYSES

9.4.1 GENERAL CONSIDERATIONS

Detailed methodology for summaries and statistical analyses will be documented in a SAP that will be finalized before database lock. Any changes to the methods described in the final SAP will be described and justified as needed in the Clinical Study Report (CSR).

All available data will be included in data listings. Data tabulations will be performed for specific analysis populations. Continuous data will be summarized with descriptive statistics including but not limited to arithmetic mean, standard deviation (SD), median, minimum, and maximum. Categorical endpoints will be summarized using frequencies and percentages. All summaries will present the data by dose group.

9.4.2 ANALYSIS OF PRIMARY SAFETY AND TOLERABILITY ENDPOINTS

Safety endpoints will include AEs, clinical laboratory assessments, vital signs, 12-lead ECGs, and the use of concomitant medications. No inferential statistical analyses are planned.

AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA, latest version), and data will be summarized by System Organ Class (SOC) and Preferred Term (PT). The number and percent of subjects reporting each AE will be summarized for dose level/treatment, as appropriate.

TEAEs are defined as AEs that occurred or worsened following the first administration of study drug. Only TEAEs will be included in the AE summary tables, which will present data by dose level/treatment.

Furthermore, TEAEs by severity status, relationship to study drug, and serious AEs will be summarised. All AEs regardless of treatment emergence will be listed.

Absolute laboratory values, changes in laboratory values, and graded laboratory abnormalities will be displayed for each subject and dose level/treatment and summarized over time. Laboratory values will be compared to normal ranges, and values that fall outside of the normal ranges will be flagged as: H (High) and L (Low) in the data listings.

ECGs, vital signs and physical examination findings data will be similarly displayed by dose level/treatment, using descriptive statistics.

9.4.3 ANALYSIS OF SECONDARY AND EXPLORATORY PHARMACODYNAMIC ENDPOINTS

PD and PK profiles and parameters (e.g., C_{max}, T_{max}, AUC) will be summarized using descriptive statistics and graphs (mean ± SEM) for each treatment at each timepoint. Where appropriate both observed data and change from baseline we be summarized and will be described in the SAP.

9.4.4 BASELINE DESCRIPTIVE STATISTICS

The baseline value for each analysis will be defined in the SAP. In most cases, baseline will be

defined as the last value recorded for any given parameter prior to study medication administration on Day 1.

9.5 INTERIM ANALYSES

Not applicable to this study.

10. SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 APPENDIX 1: REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

10.1.1 REGULATORY AND ETHICAL CONSIDERATIONS

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

- Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines
- Applicable ICH Good Clinical Practice (GCP) Guidelines
- Applicable laws and regulations

The protocol, protocol amendments, ICF, IB, and other relevant documents (e.g., advertisements) must be submitted to an IRB/IEC by the Investigator and reviewed and approved by the IRB/IEC before the study is initiated.

Any amendments to the protocol will require IRB/IEC approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study participants.

Protocols and any substantial amendments to the protocol will require health authority approval prior to initiation except for changes necessary to eliminate an immediate hazard to study participants.

The Investigator will be responsible for the following:

- Providing written summaries of the status of the study to the IRB/IEC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/IEC
- Notifying the IRB/IEC of SAEs or other significant safety findings as required by IRB/IEC procedures
- Providing oversight of the conduct of the study at the site and adherence to requirements of 21 CFR, ICH guidelines, the IRB/IEC, European regulation 536/2014 for clinical studies (if applicable), European Medical Device Regulation 2017/745 for clinical device research (if applicable), and all other applicable local regulations.

10.1.2 INFORMED CONSENT PROCESS

The Investigator or designee will explain the nature of the study to the participant and answer all questions regarding the study.

Participants must be informed that their participation is voluntary. Participants will be required to sign a statement of informed consent that meets the requirements of 21 CFR 50, local regulations, ICH guidelines, Health Insurance Portability and Accountability Act (HIPAA)

requirements, where applicable, and the IRB/IEC or study center.

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

Participants must be re-consented to the most current version of the ICF(s) during their participation in the study.

A copy of the ICF(s) must be provided to the participant.

10.1.3 DATA PROTECTION

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

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

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

10.1.4 COMMITTEES STRUCTURE

Not applicable.

10.1.5 SAFETY OVERSIGHT

Clinical safety oversight will be performed by centralized review conducted by Medical Monitors per the Medical Monitoring Plan. In addition, on-site review of all data including safety data will be conducted routinely by clinical monitors /clinical research associates.

10.1.6 DISSEMINATION OF CLINICAL STUDY DATA

This study will be posted on the US National Institutes of Health's website *www.clinicaltrials.gov*.

10.1.7 DATA QUALITY ASSURANCE

As defined by ICH guidelines, the CRF is a printed, optical, or electronic document designed to record all protocol-required information to be reported to the Sponsor on each study subject.

All participant data relating to the study will be recorded on printed or electronic CRF unless transmitted to the Sponsor or designee electronically (e.g., laboratory data). The Investigator is responsible for verifying that data entries are accurate and correct by physically or electronically signing the CRF.

Data collection on the CRF will follow the instructions described in the CRF Completion Guidelines. Copies of the completed eCRFs will be retained by the investigational center as well as the Sponsor.

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

10.1.8 SOURCE DOCUMENTS

The Investigator must maintain required records for all study subjects. Source data are all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data for this study will be recorded in the subject's source documents and on the eCRFs, unless otherwise noted. All data on these eCRFs should be recorded completely and promptly. A copy of the completed eCRFs for each subject will be retained by the investigational center.

The Investigator must maintain adequate and accurate source documents upon which eCRFs for each subject are based. They are to be separate and distinct from eCRFs, except for cases in which the Sponsor has predetermined that direct data entry into specified pages of the subject's eCRF is appropriate.

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

10.1.9 STUDY RECORDS RETENTION

During this study, the Investigator is required to prepare and maintain adequate and accurate case histories designed to record all observations and other data pertinent to the investigation on each individual treated with the IP or entered as a control in the investigation.

Records and documents, including signed ICFs, pertaining to the conduct of this study must be retained by the Investigator for the maximum period required by (1) applicable regulations and guidelines or institution procedures or (2) for the period specified by the Sponsor, whichever is longer. The Investigator must contact the Sponsor before destroying any records associated with the study. No records may be destroyed during the retention period without the written approval of the Sponsor.

The Sponsor will notify the Investigator when the study records are no longer needed.

In the event the Investigator withdraws from the study (e.g., relocation, retirement), the records shall be transferred to a mutually agreed-upon designee (e.g., another Investigator). Notice of such transfer will be given in writing to the Sponsor.

The Sponsor will maintain correspondence with the Investigator after study closeout to ensure that study documentation is retained for the appropriate amount of time.

10.1.10 QUALITY ASSURANCE AND QUALITY CONTROL

This study will be organized, performed, and reported in compliance with the protocol, SOPs, site/Investigator training, and applicable regulations and guidelines. Clinical Investigator sites will be trained at the Investigator Meeting(s) and/or at on-site visits. All aspects of the study will be monitored carefully by the Sponsor's designees with respect to current GCP and SOPs for compliance with applicable government regulations. It is the responsibility of the Investigator to provide all study records, including CRFs and source documents, among other records, for review and inspection by the clinical monitor, representatives of the Sponsor, and regulatory authorities, as needed.

Clinical monitoring will be performed per the Clinical Monitoring Plan. Clinical monitors/clinical research associates will periodically evaluate the progress of the study, including the verification of appropriate consent form procedures and the verification of the accuracy and completeness of eCRFs. Clinical monitors/clinical research associates will also ensure that all protocol requirements, applicable US FDA regulations, other regulatory requirements, and the Investigator's obligations are being fulfilled.

10.1.11 PROTOCOL DEVIATIONS

A protocol deviation is any noncompliance with the clinical trial protocol or ICH GCP. The noncompliance may be either on the part of the participant, the Investigator, or the study site staff.

The Investigator and study staff will apply due diligence to avoid protocol deviations. If protocol deviations do occur, the Investigator or study staff must report them to the local IRB/IEC per their policies.

10.1.12 PREMATURE TERMINATION OR STUDY SUSPENSION

The Sponsor reserves the right to prematurely terminate the study or specific groups within the study at any time for administrative, safety, or treatment-related benefit-risk reasons. Written notification documenting the reason for study suspension or termination will be provided to the Investigator and regulatory authorities as appropriate. If the study is prematurely terminated or suspended, the Investigator will promptly inform the IRB and will provide the reason(s) for the termination or suspension.

10.1.13 PUBLICATION POLICY

The results of this study may be published or presented at scientific meetings. If this is foreseen, the Investigator agrees to submit all manuscripts or abstracts to the Sponsor before submission. This allows the Sponsor to protect proprietary information and to provide comments.

The Sponsor will comply with the requirements for publication of study results. In accordance with standard editorial and ethical practice, the Sponsor will generally support publication of multicenter studies only in their entirety and not as individual site data. In this case, a coordinating Investigator will be designated by mutual agreement.

Authorship will be determined by mutual agreement and in line with International Committee of

Medical Journal Editors authorship requirements.

10.1.14 FUTURE USE OF STORED SPECIMENS AND DATA

Data and biospecimens collected in this study may be useful for other research studies.

Samples (blood, urine, and fecal) will be stored for future metabolite analysis and may be used to assess additional PK and PD markers of GLY-200.

10.2 APPENDIX 2: AES AND SAES: DEFINITIONS AND PROCEDURES FOR RECORDING, EVALUATING, FOLLOW-UP, AND REPORTING

10.2.1 DEFINITION OF AE

AE Definition

- An AE is any unfavorable and unintended sign, symptom, or disease temporally associated with the use of an investigational (medicinal) product or other protocol-imposed intervention, regardless of attribution.
- An AE may include intercurrent illnesses or injuries that represent an exacerbation (increase in frequency, severity, or specificity) of preexisting conditions (e.g., worsening of asthma).
- Whenever possible, it is preferable to record a diagnosis as the AE term rather than a series of symptoms relating to a diagnosis. In order to classify AEs and diseases, preferred terms will be assigned by the Sponsor to the original terms entered on the eCRF, using the Medical Dictionary for Regulatory Activities (MedDRA).

Events Meeting the AE Definition

- Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (e.g., ECG, radiological scans, vital signs measurements), including those that worsen from baseline, considered clinically significant in the medical and scientific judgment of the investigator (i.e., not related to progression of underlying disease).
- Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition.
- New conditions detected or diagnosed after study intervention administration even though it may have been present before the start of the study.
- Signs, symptoms, or the clinical sequelae of a suspected intervention-intervention interaction.
- Signs, symptoms, or the clinical sequelae of a suspected overdose of either study intervention or a concomitant medication. Overdose per se will not be reported as an AE/SAE unless it is an intentional overdose taken with possible suicidal/self-harming intent. Such overdoses should be reported regardless of sequelae.

Events NOT Meeting the AE Definition

- Any clinically significant abnormal laboratory findings or other abnormal safety assessments which are associated with the underlying disease, unless judged by the investigator to be more severe than expected for the participant's condition.
- The disease/disorder being studied or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the participant's condition.
- Medical or surgical procedure (e.g., endoscopy, appendectomy): the condition that leads to the procedure is the AE.
- Situations in which an untoward medical occurrence did not occur (social and/or convenience admission to a hospital).
- Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.

AEs of Special Interest
The following are considered AEs of special interest in this study: • **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

10.2.2 DEFINITION OF SAE

An SAE is defined as any serious adverse event that, at any dose:
1. Results in death
2. Is life-threatening • The term 'life-threatening' in the definition of 'serious' refers to an event in which the subject was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.
3. Requires inpatient hospitalization or prolongation of existing hospitalization • In general, hospitalization signifies that the participant has been admitted (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious. • Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE.
4. Results in persistent or significant disability/incapacity • The term disability means a substantial disruption of a person's ability to conduct normal life functions. • This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (e.g., sprained ankle) which may interfere with or prevent everyday life functions but do not constitute a substantial disruption.
5. Is a congenital anomaly/birth defect
6. Is an important medical event, defined as an event that does not fit one of the other outcomes, but may jeopardize the subject and may require medical or surgical intervention (treatment) to prevent one of the other outcomes • Examples of such events include intensive treatment for allergic bronchospasm (a serious problem with breathing) requiring treatment in an emergency room, serious blood dyscrasias (blood disorders), seizure/convulsion that does not result in hospitalization or development of intervention dependency or intervention abuse.

10.2.3 DEFINITION OF UNANTICIPATED PROBLEMS (UP)

UP Definition

- An unanticipated problem is defined as, in general, any incident, experience, or outcome that meets all of the following criteria:
  - Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;
  - Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
  - Suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

10.2.4 RECORDING AND FOLLOW-UP OF AE AND/OR SAE

AE and SAE Recording

- **Serious Adverse Events (SAEs) should be reported to the Sponsor and/or CRO within 24 hours from the discovery of the event.**
- When an AE/SAE occurs, it is the responsibility of the investigator to review all documentation (e.g., hospital progress notes, laboratory reports, and diagnostics reports) related to the event.
- The Investigator will then record all relevant AE/SAE information.
- The Investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. Whenever possible, the diagnosis (not the individual signs/symptoms) will be documented as the AE/SAE.

Assessment of Severity

The investigator will make an assessment of severity for each AE and SAE reported during the study and assign it to 1 of the following categories:

- **Mild:** An event that is easily tolerated by the participant, causing minimal discomfort and not interfering with everyday activities.
- **Moderate:** An event that causes sufficient discomfort to interfere with normal everyday activities.
- **Severe:** An event that prevents normal everyday activities. An AE that is assessed as severe should not be confused with an SAE. Severe is a category utilized for rating the intensity of an event; and both AEs and SAEs can be assessed as severe.

An event is defined as 'serious' when it meets at least 1 of the predefined outcomes as described in the definition of an SAE, NOT when it is rated as severe.

Assessment of Relationship to Study Agent

The relationship of the study treatment to an AE will be determined by the Investigator based on the following definitions:

1. Not Related

The AE is not related if (1) administration of the study medication has not occurred **or** (2) the occurrence of the AE is not reasonably related in time **or** (3) the AE is considered related to another event, medical

Assessment of Relationship to Study Agent

condition or product not associated with the study medication (such as disease progression, environmental factors, unrelated trauma, etc.)

2. Unlikely Related

The AE is unlikely related if (1) the AE is unlikely related in time **or** (2) the AE is considered unlikely to be related to use of the study medication (i.e., there are no facts [evidence] or arguments to suggest a causal relationship), **or** the AE is considered possibly related to another event, medical condition or product not associated with the study medication.

3. Possibly Related

The AE is possibly related if (1) the study medication and AE are considered reasonably related in time **and** (2) the AE could equally be explained by causes other than exposure to the study medication.

4. Probably Related

The AE is probably related if (1) the study medication and AE are considered reasonably related in time and (2) the study medication is more likely than other causes to be responsible for the AE or is the most likely cause of the AE.

- The Investigator will use clinical judgment to determine the relationship.
- Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to study intervention administration will be considered and investigated.
- There may be situations in which an SAE has occurred and the Investigator has minimal information to include in the initial report (refer to Section 10.2.5). However, it is very important that the Investigator always make an assessment of causality for every event before the initial transmission of the SAE data (refer to Section 10.2.5).
- The Investigator may change his/her opinion of causality in light of follow-up information and send an SAE follow-up report with the updated causality assessment.
- The causality assessment is one of the criteria used when determining regulatory reporting requirements.

Assessment of Expectedness

- The Sponsor will be responsible for determining whether an AE/SAE is expected or unexpected.
- An AE/SAE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study agent in the IB or is not listed in the IB at the specificity or severity that has been observed.

10.2.5 REPORTING OF SAES

SAE Reporting to Sponsor and IRB

- Any AE that meets any criterion for an SAE requires the completion of an SAE Report Form in addition to being recorded on the AE page/screen of the eCRF.
- All SAEs must be reported by email to Sponsor Drug Safety or its designee within 24 hours of the Investigator's knowledge of the event.
  - Sponsor Drug Safety Contact Information: SAE@glyscend.com
  - ProSciento Safety Team Email: pvsafety@prosciento.com
- The Investigator is required to ensure that the data on these forms is accurate and consistent. This requirement applies to all SAEs (regardless of relationship to IP) that occur during the study (from the time the participant signs informed consent until post-treatment follow up visit, or any SAE made known to the

SAE Reporting to Sponsor and IRB

Investigator at any time thereafter that are suspected of being related to IP). Serious adverse events occurring prior to treatment (after signing the ICF) will be captured.

- The SAE report should provide a detailed description of the SAE and include a concise summary of hospital records and other relevant documents. If a participant died and an autopsy has been performed, copies of the autopsy report and death certificate are to be sent to Sponsor Drug Safety or its designee as soon as these become available. Any follow-up data should be detailed in a subsequent SAE Report Form, or approved equivalent form, and sent to Sponsor Drug Safety or its designee.
- Where required by local legislation, the Investigator is responsible for informing the IRB/EC of the SAE and providing them with all relevant initial and follow-up information about the event. The Investigator must keep copies of all SAE information on file including correspondence with Sponsor and the IRB/EC.
- Details will be provided in the Safety Management Plan (SMP).
- There may be instances when copies of medical records for certain cases are requested by the Sponsor. In this case, all subject identifiers, with the exception of the subject number, will be redacted on the copies of the medical records before submission to Sponsor.

10.2.6 REPORTING OF UPS

Unanticipated Problem Reporting

- Incidents or events that meet the criteria for unanticipated problem require the creation and completion of an UP report. It is the site Investigator's responsibility to report UPs to their IRB and to the Sponsor. The UP report will include the following information:
    - Protocol identifying information: protocol title and number, PI's name, and the IRB project number;
    - A detailed description of the event, incident, experience, or outcome;
    - An explanation of the basis for determining that the event, incident, experience, or outcome represents an UP; and
    - A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UP.
- To satisfy the requirement for prompt reporting, UPs will be reported using the following timeline:
    - UPs that are SAEs will be reported to the IRB and to the Sponsor within 24 hours of the Investigator becoming aware of the event.
    - Any other UP will be reported to the IRB and to the Sponsor within the IRB-required reporting timeframe.
    - All UPs should be reported to appropriate institutional officials (as required by an institution's written reporting procedures) and IRB within the timeframe specified by the institution procedures and IRB.

10.3 APPENDIX 3: CONTRACEPTIVE AND BARRIER GUIDANCE

An acceptable method of contraception for **female subjects of childbearing potential** is one of the following:

1. Total abstinence from heterosexual intercourse (acceptable only if it is the subject's usual form of birth control/lifestyle choice)

-or-

2. Have a biologically or surgically sterile partner (i.e., a female subject's male partner has undergone effective surgical sterilization, such as vasectomy, before entering the clinical trial and has obtained documentation of absence of sperm in his ejaculate, and he is the sole sexual partner of the female volunteer during the clinical trial)

-or-

3. Use of a combination of a condom for the female subject's partner, and one of the following effective methods of contraception:
   a. Intravaginal or transdermal combined (estrogen and progestogen containing) hormonal contraceptives
   b. Injectable or implantable progestogen-only hormonal contraceptives
   c. Intrauterine device or intrauterine hormone-releasing system
   d. Bilateral tubal ligation/occlusion

PLEASE NOTE: ORAL CONTRACEPTION IS **NOT ALLOWED** (and is exclusionary) IN THIS STUDY AS THE STUDY DRUG (GLY-200) MAY REDUCE THE EFFECTIVENESS OF THE ORAL CONTRACEPTION DUE TO POTENTIAL DISRUPTION ON GI ABSORPTION.

An acceptable method of contraception for **male subjects** is one of the following:

1. Total abstinence from heterosexual intercourse (acceptable only if it is the subject's usual form of birth control/lifestyle choice)

-or-

2. Biologically or surgically sterile (i.e., the male volunteer has undergone effective surgical sterilization, such as vasectomy, before entering the clinical trial and has obtained documentation of absence of sperm in his ejaculate)

-or-

3. Use of a combination of a condom in addition to having a female partner of childbearing potential use one the following effective methods of contraception:
   a. Oral, intravaginal, or transdermal combined (estrogen and progestogen containing) hormonal contraceptives
   b. Oral, injectable, or implantable progestogen-only hormonal contraceptives
   c. Intrauterine device or intrauterine hormone-releasing system
   d. Bilateral tubal ligation/occlusion

Notes:

- Total abstinence is defined as the strict avoidance of all forms of heterosexual activity. Periodic abstinence (e.g., calendar, ovulation, symptothermal post-ovulation methods) and withdrawal are not acceptable methods of contraception.
- When it is their preferred and usual lifestyle, WOCBP or males who have a same-sex partner (i.e., abstain from heterosexual intercourse) are not required to use an acceptable method of contraception.

10.4 APPENDIX 4: CLINICAL LABORATORY ASSESSMENTS

Refer to Section 1.3 Schedule of Activities for clinical laboratory assessment timepoints.

Hematology	**Chemistry, Including Liver Function**	**Coagulation**	**Urinalysis** [1]
Complete Blood Count (CBC) and Differential such as but not limited to: -Hemoglobin -Hematocrit -RBC indices -Thrombocyte count (platelets) -Reticulocyte count -White blood cell (WBC) count with differential: Neutrophil count Eosinophil count Basophil count Lymphocyte count Monocyte count	Sodium Potassium Chloride Bicarbonate Phosphate Calcium Magnesium Glucose Lipase Uric acid Albumin Globulins Protein LDH Creatine kinase Creatinine and eGFR Urea ALP ALT AST GGT Total bilirubin Conjugated and unconjugated bilirubin Total cholesterol, HDL LDL Triglycerides *Day 1 predose and Day 14 only:* -Vitamin A, E, β-Carotene -Vitamin B12 and Folates -Vitamin D -Iron, TIBC, and Ferritin	PT INR aPTT	pH Specific gravity Glucose Protein Ketones Bilirubin Blood Nitrites Urobilinogen, Leukocyte esterase. Phosphate, Oxalate, Uric acid, Boron [1] *Urine sediment microscopy will be conducted in the instance of abnormal urinalysis findings.*
Serology and Virology	**Pregnancy Test**	**Follicle-Stimulating Hormone**	**Other Serum & Plasma Tests**
HIV HBsAg HCV RNA PCR	Urine hCG [2] Serum hCG [2] *If the urine hCG is positive, pregnancy will be confirmed by a serum hCG test*	FSH	Thyroid panel and CRP *(only at Screening to assess exclusion criteria)*

Abbreviations: RBC = red blood cell; WBC = white blood cell; PT = prothrombin time; INR = international normalized ratio; aPTT = activated partial prothrombin time; HIV = Human Immunodeficiency Virus; HBsAg = Hepatitis B surface antigen; LDH = lactate dehydrogenase; ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; GGT = gamma-glutamyl transferase; HDL = high-density lipoprotein; LDL = low-density lipoprotein; TIBC = total iron binding capacity; HCV = hepatitis C virus; hCG = human chorionic gonadotropin; CRP = C-reactive protein.

10.5 APPENDIX 5: ABBREVIATIONS

Abbreviation	Term
ADA	American Diabetes Association
AE(s)	adverse event(s)
ALT	alanine transaminase
AST	aspartate transaminase
BID	twice a day
BMI	body mass index
BP	blood pressure
CGM	continuous glucose monitor
CIOMS	Council for International Organizations of Medical Sciences
CRO	contract research organization
CSR	clinical study report
DJBS	duodenal-jejunal bypass sleeve
DKA	diabetic ketoacidosis
DMR	Duodenal Mucosal Resurfacing
ECG(s)	electrocardiogram(s)
eCRF	electronic case report form
EOS	End of Study
ET	Early Termination
FSH	follicular stimulating hormone
GCP	Good Clinical Practice
GERD	gastroesophageal reflux disease
GGT	gamma-glutamyl transferase
GIP	gastric inhibitory polypeptide
GLP	Good Laboratory Practice
GLP-1	glucagon-like peptide-1
HbA1c	hemoglobin A1c
HBsAG	hepatitis B surface antigen
hCG	human chorionic gonadotropin
HCV	hepatitis C virus

Abbreviation	Term
HDL	high-density lipoprotein
HIPAA	Health Insurance Portability and Accountability Act
HR	heart rate
IB	Investigator's Brochure
IBD	inflammatory bowel disease
IBS	irritable bowel syndrome
ICF	informed consent form
ICH	International Council for Harmonisation
IP	investigational product
IRB	Institutional Review Board
IV	intravenous
LDL	low-density lipoprotein
MAD	multiple ascending dose
MedDRA	Medical Dictionary for Regulatory Activities
MMTT	mixed meal tolerance test
NSAIDs	nonsteroidal anti-inflammatory drugs
PD	pharmacodynamic(s)
PK	pharmacokinetic(s)
PT	preferred term
PYY	peptide YY
RR	respiratory rate
RYGB	Roux-en-Y gastric bypass
SAD	single ascending dose
SAE(s)	serious adverse event(s)
SAP	statistical analysis plan
SD	standard deviation
SEM	standard error of the mean
SoA	Schedule of Activities
SOC	system organ class
SOP	standard operating procedure
T2D	type 2 diabetes

Abbreviation	Term
TEAE(s)	treatment-emergent adverse event(s)
TID	three times a day
ULN	upper limit of normal
UP	Unanticipated Problem
VAS	visual analog scale
wt.	weight

11. LITERATURE REFERENCES

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12. AMENDMENTS

Amendment 02 Version 01 Summary of Changes

Purpose: The primary purpose of this amendment is to refine and clarify the study procedures.

Summary of Changes

Change	Section(s) Affected	Rationale
Updated amendment version/date	Throughout	To reflect protocol Amendment 02
Removed leptin from exploratory PD measures.	Section 1.1, Section 1.3.1, Section 3, Section 8.4	Deletion was made to refine exploratory PD analyses.
Revised inclusion criterion #1c: "HbA1c ≥ ~~6.5~~ **6.0** and ≤ 8.5% at screening"	Section 1.1 and Section 5.1	Lower end of acceptable HbA1c range was reduced to aid in subject recruitment.
The following was added to footnote 19 of the Schedule of Activities: "VAS assessments may be performed within a ± 10-minute window."	Section 1.3	Text was added to clarify window for VAS assessments.
The following was added to footnote 20 of the Schedule of Activities: "For the Day -1 visit, the CGM can be placed on Day -2 (check-in)."	Section 1.3	Text was added to clarify acceptable CGM replacement timing.
The first row was of the MMTT test schedule was updated: "To be completed prior to MMTT timepoints **(if applicable)**"	Section 1.3.1	"If applicable" was added as the listed procedures are not performed on all MMTT days.
"cholesterol, HDL/LDL" were removed from footnote 3 of the MMTT schedule.	Section 1.3.1	Edit was made to correct an error; separate blood samples for cholesterol and HDL/LDL need not be collected during MMTT.
The following was updated in footnote 3 of the MMTT Schedule: "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	Section 1.3.1	Details regarding timing of fractionated bile acid analysis were added to refine the exploratory PD testing strategy.

Change	Section(s) Affected	Rationale
Day 14."		
Section 6.1.2 was updated: "Each subject will be randomly assigned to a group at **Day -1 or** Day 1."	Section 6.1.2	Update was made to allow for randomization on Day -1.
Tetrahydrocannabinol was removed from the "Drugs of abuse" screen.	Section 8.2.9	THC was deleted from the drug screen list as it is not an exclusionary drug.
"Clinically significant hyperglycemia" and "clinically significant hypoglycemia" (and definitions) were added to as AEs of special interest.	Section 8.3.7 and Section 10.2.1	Additions were made per FDA recommendation.
"Fasting Lipids" was removed from the "Day 1 predose and Day 14 only" clinical laboratory assessments.	Section 10.4	Deletion was made because the lipid panel done as part of the standard chemistry lab is sufficient.

Amendment 01 Version 01 Summary of Changes

Purpose: The primary purpose of this amendment is to address FDA comments received in response to the protocol submission as part of the original IND. Additional edits are also made to refine and clarify the study procedures.

Summary of Changes

Change	Section(s) Affected	Rationale
Added amendment version/date and Summary of Changes	Throughout and Section 12	To reflect protocol Amendment 01
Sponsor Signature Pages were combined into one page.	Sponsor Signature Page	Administrative edit
Glucagon was added to pharmacodynamic measures in the exploratory objectives	Synopsis, Section 1.3.1, Section 3, and Section 8.4	To refine exploratory PD tests
Inclusion criterion 1c was revised: "HbA1c ≥~~7~~ **6.5** and ≤ ~~9.6~~ **8.5**% at screening"	Synopsis, Section 5.1	Per FDA recommendation
Inclusion criterion 1d was added: "d. completed at least 12 days of metformin washout without meeting	Synopsis, Section 5.1	To clarify that subjects meeting criteria for rescue during the washout period

Change	Section(s) Affected	Rationale
rescue criteria prior to dosing"		will not be randomized
Inclusion criterion 3 was revised: BMI ≥ 18 and ≤ 40 *at screening*	Synopsis, Section 5.1	To clarify timing of inclusion/exclusion criteria assessment
Exclusion criterion 8 was revised: Fasting blood glucose > ~~210~~ **190** mg/dL at screening~~; can be reassessed once during the screening period~~	Synopsis, Section 5.2	Per FDA recommendation
"Urine Dipstick (ketones)" row was removed from the SoA.	Section 1.3	Ketones are assessed as part of clinical laboratory urinalysis.
"FSH Test" row was added to the SoA.	Section 1.3	To reflect FSH testing performed on postmenopausal women at screening
The following was added to the SoA: "Note: Prescreening laboratory values can be used to determine subject eligibility if they were collected within the screening window."	Section 1.3	To clarify screening procedures
Footnote 9 was updated in the SoA: "ECGs will be taken after at least ~~10~~ **5** minutes resting quietly…"	Section 1.3	For consistency with Section 8.2.6 Electrocardiograms
The following was added to SoA footnote 15: "Approximate weights of fecal collections should be recorded by site staff."	Section 1.3	To refine study procedures
Footnote 18 was updated in the SoA: "Recording of water and fluids in and output (urine volume out) over 24 hours daily ~~during confinement~~."	Section 1.3	To clarify that fluid balance is only being performed on a subset of inpatient days as noted in the SoA
The following was added to the protocol: "Fractionated bile acids will be analyzed in a subset of subjects/doses based on initial total bile acid data."	Section 1.3.1 and Section 8.4	To refine exploratory PD analysis testing strategy
The following was added to Section 6.8.2 Rescue Medicine: "Fasting blood glucose will be measured by the subject using a home glucose meter	Section 6.8.2	To clarify that subjects meeting criteria for rescue during the washout period will not be randomized

Change	Section(s) Affected	Rationale
daily from Day -7 to inpatient check-in. If a subject meets criteria for rescue during the washout period, the subject will not be randomized."		
Section 8.2.6 Electrocardiograms was edited: "~~Day 1 pre-dose and~~ all other post-first dose ECGs will be single readings."	Section 8.2.6	To enhance accuracy; Day 1 pre-dose ECG will be in triplicate.
Alcohol breath test was removed from Section 8.2.9 Other Laboratory Tests.	Section 8.2.9	Alcohol breath test is part of the site's prescreening process.
Section 8.4 was reorganized, and calprotectin (fecal analysis) and calcium (urine analysis) were deleted.	Section 8.4	To clarify exploratory PD vs PK markers and to delete assays that were included in error
Corrected section references in Section 10.2.4 Recording and Follow-up of AE and/or SAE	Section 10.2.4	Administrative edit; section references in Assessment of Relationship to Study Agent were incorrect
Added sponsor drug safety contact information.	Section 10.2.5	Previously omitted in error
Appendix 4: Clinical Laboratory Assessments was edited: • Clarification was made to refer to the SoA for timepoints • Full iron panel was defined • Timing of "Other Serum & Plasma tests" was clarified • Abbreviations were updated for completeness.	Section 10.4	Edits were made to clarify clinical laboratory assessments