Protocol (a): I8F-MC-GPHU

The Impact of Tirzepatide on Gastric Emptying (GE) in Overweight/Obese Non-diabetic Subjects and in Overweight/Obese Subjects with Type 2 Diabetes Mellitus

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Protocol I8F-MC-GPHU (a)
The Impact of Tirzepatide on Gastric Emptying (GE) in Overweight/Obese Non-diabetic Subjects and in Overweight/Obese Subjects with Type 2 Diabetes Mellitus

Tirzepatide (LY3298176)

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Clinical Pharmacology Protocol Electronically Signed and Approved by Lilly on 30 Apr 2020.
Amendment (a) Electronically Signed and Approved by Lilly on approval date provided below

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Table of Contents

The Impact of Tirzepatide on Gastric Emptying (GE) in Overweight/Obese Non-diabetic Subjects and in Overweight/Obese Subjects with Type 2 Diabetes Mellitus

Section **Page**

List of Tables

Table **Page**

List of Figures

Figure **Page**

List of Appendices

1. Protocol Synopsis

Title of Study:
The Impact of Tirzepatide on Gastric Emptying (GE) in Overweight/Obese Non-diabetic Subjects and in Overweight/Obese Subjects with Type 2 Diabetes Mellitus

Rationale:
Tirzepatide (LY3298176) is a dual glucose-dependent insulinotropic peptide (GIP) and glucagon-like peptide 1 (GLP-1) receptor agonist being developed as an adjunct to diet and exercise for chronic weight management in adult patients with or without type 2 diabetes mellitus (T2DM) who have an initial body mass index (BMI) of 30 kg/m^2 or greater (obese) or 27 kg/m^2 or greater (overweight). The impact of tirzepatide on gastric emptying (GE) has been assessed in healthy subjects and in patients with T2DM using acetaminophen pharmacokinetics (PK) as a surrogate (Study I8F-MC-GPGA). However, data on the GE effects of tirzepatide in overweight/obese non-diabetic subjects are presently lacking.

Study I8F-MC-GPHU (GPHU) will assess the effect of multiple doses of tirzepatide (5 to 15 mg/0.5 mL) on the PK of acetaminophen to determine the impact on gastric emptying (GE), and the pharmacodynamics (PD), safety and tolerability of tirzepatide in overweight/obese non-diabetic subjects and overweight/obese T2DM subjects when administered using a prefilled syringe (PFS).

Objectives/Endpoints:

Objectives	**Endpoints**
Primary	
To assess the effect of tirzepatide on the PK profile of acetaminophen, a marker for GE	Acetaminophen area under the concentration versus time curve from time zero to time t, where t is the last time point with a measurable concentration (AUC0-t_{last}), time of maximum observed drug concentration (t_{max}), maximum observed drug concentration (C_{max})
Secondary	
To evaluate tirzepatide PK and PD effects following multiple doses of SC tirzepatide in overweight/obese non-diabetic subjects and overweight/obese T2DM subjects	Glycemic control (plasma glucose, HbA1c), and tirzepatide area under the concentration versus time curve from time zero to infinity ($AUC_{0-\infty}$), AUC0-t_{last}, t_{max}, C_{max}
To evaluate tirzepatide safety and tolerability following multiple doses of SC tirzepatide in overweight/obese non-diabetic subjects and overweight/obese T2DM subjects	Adverse events and hypoglycemic events

Summary of Study Design:

This is an open-label, fixed sequence study in overweight/obese non-diabetic subjects and overweight/obese T2DM subjects.

Potential subjects will be screened to assess their eligibility to enter the study within 28 days prior to Day 1. Subjects will be admitted to the CRU on Day -3 and will establish baseline measurements during the Baseline Period from Days -3 to -1.

Subjects will receive subcutaneous tirzepatide 5 mg on Days 1 and 8; 10 mg on Days 15, 22, and 29; and 15 mg on Day 36, and will receive oral acetaminophen on Days -1, 2, and 37. Doses will be administered at approximately the same time on each dosing day.

Subjects may be permitted to leave the CRU on Day 4 of the Treatment Period following completion of study procedures. A second inpatient period will take place from Days 35 to 39. Outpatient visits will take place on Days 8 (±1 day), 15 (±1 day), 22 (±1 day), 29 (±1 day), and 43 (±2 days).

A final follow-up visit will occur on Day 64 ± 1 day (i.e., 28 days ± 1 day after the final tirzepatide dose).

Safety will be assessed by vital signs, ECGs, CCI [redacted], safety laboratory tests, and the recording of AEs. Pharmacokinetic blood samples for acetaminophen and tirzepatide will be collected.

Treatment Arms and Planned Duration for an Individual Subject:

The study duration for individual subjects, inclusive of screening is expected to be approximately 13 weeks, divided as follows:

- Screening: up to 28 days prior to Day 1
- Treatment Period: Day 1 to Day 43 (±2 days)
- Follow-up: Day 64 (±1 day) will be considered as the final follow-up visit

Number of Subjects:

Approximately 18 overweight or obese non-diabetic subjects and 18 overweight or obese T2DM subjects will be enrolled so that a minimum of 12 subjects in each group completes the study.

Statistical Analysis:

The primary parameters for analysis will be C_{max}, $AUC_{0-\infty}$, $AUC0\text{-}t_{last}$, and t_{max}.

Pharmacokinetic parameters will be evaluated to estimate the impact of tirzepatide on GE and on the PK of acetaminophen. Log-transformed C_{max} and AUC will be evaluated in a linear mixed-effects model, with day (-1, 2, 37) as a fixed effect and subject as a random effect. The differences in C_{max} and AUC between acetaminophen + tirzepatide (Test; Days 2 and 37) and acetaminophen alone (Reference; Day -1) will be back-transformed to present the ratios of geometric least squares means and the corresponding 90% CI. Other parameters may be analyzed in this way as needed.

The t_{max} will be analyzed using a Wilcoxon signed rank test. Estimates of the median difference based on the observed medians and 90% CIs from the Wilcoxon test will be calculated.

Planned PK parameters will also be summarized with descriptive statistics.

Pharmacodynamic parameters that will be assessed include body weight, appetite Visual Analog Scale (VAS) scores and HbA1c. The parameters will be listed and summarized using standard descriptive statistics. Additional analysis will be performed if warranted upon review of the data.

Safety parameters that will be assessed include safety laboratory parameters, vital signs, CCI [redacted] TEAEs (including TEAEs of special interest), and SAEs. The parameters will be listed and summarized using standard descriptive statistics. Additional analysis will be performed if warranted upon review of the data. All AEs related to study or protocol procedure will be listed, and if the frequency of events allows, will be also summarized using descriptive methodology. Hypoglycemic events will be summarized and listed.

Physical examinations and ECGs will be performed for safety monitoring purposes and will not be presented.

If warranted, additional analyses will be performed upon review of the data.

2. Schedule of Activities

Study Schedule Protocol I8F-MC-GPHU Screening and Baseline

	Screening	Baseline			Comments
Procedure	D-28 to -4	D-3	D-2	D-1	
Informed Consent	X				
Admission to CRU		X			Enrollment will occur on Day-2 after clearing medical assessment.
Outpatient visits to CRU	X				
Review and confirm inclusion / exclusion criteria	X				
Medical History	X				
Physical Exam /Medical Assessment	X		X		Full physical examination at screening. Thereafter, assessments performed to include medical review and targeted examination, as appropriate.
Height, Weight, and BMI	X				Refer to Section 9.4.3 on how body weight should be measured. Body weight at screening must be assessed to conform to Inclusion Criterion [2].
Waist Circumference	X		X		Refer to Section 9.4.3 on how waist circumference should be measured.
Temperature	X				
Distribution of glucose meters, test strips, and diaries / conduct SMPG and hypoglycemia training		Any time from Day -3 to Day -1			Applicable to T2DM subjects only, with exception of how to recognize the signs and symptoms of hypoglycemia which is applicable to all subjects.
6-point plasma glucose profile				X	30 minutes pre-meals, 2 hours post meals. Meals = breakfast, lunch and dinner.
C-SSRS (baseline / screening form) + SHSF + SHFF	X				The SHFF is only required only if triggered by the SHSF, per instructions in the form.
PHQ-9	X				
Safety 12-lead ECG	X		X		Single safety ECG at site. Subjects must be supine for approximately 5 to 10 minutes before ECG collection and remain supine but awake during ECG collection.
Supine Vital Signs	X		X		Additional time points may be added at the investigator's clinical judgment.
Clinical Laboratory Tests	X		X		See Appendix 2 for details. Baseline may be collected anytime from D-3 to D-1 as long as results are reviewed prior to first tirzepatide dosing on D1. Performed by a local laboratory.
CCI					
Meal				-0.5	A meal will be provided and should be completed within 20 minutes. Acetaminophen dosing will occur within 15 minutes of meal completion. Exact time of sampling must be recorded. Acetaminophen PK sampling times are relative to acetaminophen dosing (0 hours).
Acetaminophen 1g dose				0	
Acetaminophen PK sampling (hours)				-0.25, 0.5, 0.75, 1, 2, 3, 4, 6, 9, 12	
Pregnancy Test	X			X	Female subjects only. See Appendix 2 for details.
Drug Screen	X				
AEs/Concomitant Medications	X	X	X	X	

Study Schedule Protocol I8F-MC-GPHU Treatment and Follow-up

	Treatment and Follow Up														Comments
Procedure	D1	D2	D3	D4	D8 ±1	D15 ±1	D22 ±1	D29 ±1	D35	D36	D37	D38	D43 ±2	FU/ ED[a]	
Admission to CRU									X						
Discharge from CRU				X								D39			CRU stay may be extended at the investigator's discretion.
Outpatient Visit					X	X	X	X					X	X	
Pregnancy test														X	
Medical Assessment	P				P	P	P	P						X	
Weight and BMI	P				P	P	P	P					X	X	Refer to Section 9.4.3 on how body weight should be measured.
Waist circumference							X						X	X	Refer to Section 9.4.3 on how waist circumference should be measured.
Temperature	P				P	P	P	P						X	
Safety 12-lead ECG (hours)	P, 12	X	X	X	P	P	P	P		P, 12	X	X	X	X	Refer to screening and baseline comments.
Supine Vital Signs (hours)	P, 12	X	X	X	P	P	P	P		P, 12	X	X	X	X	Additional time points may be added at the investigator's clinical judgment.
CCI															
Clinical Laboratory Tests						P		P					X	X	Refer to screening and baseline comments
Plasma glucose				X	P	P	P	P		P		X	X	X	
6-point plasma glucose profile			X								X				Refer to screening and baseline comments.
SMPG	Performed pre-breakfast, pre-lunch and pre-dinner and before bedtime for at least 3 days per week and whenever subject experiences hypoglycemic symptoms. On weeks where subjects are admitted to CRU, SMPG should be performed at least 2 days per week (as 6-point plasma glucose profile will be performed during inpatient visits).														Applicable to T2DM subjects only. Results should be captured in the subject diaries.
AEs/Conmeds	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Appetite Analysis by Visual Analog Scale (VAS)	P	X	X							P	X	X		X	Refer to Section 9.9. To be performed prior to dosing and/or breakfast.
Meal		-0.5									-0.5				Refer to screening and baseline comments.
Acetaminophen 1g dose		0									0				

	Treatment and Follow Up														Comments
Procedure	D1	D2	D3	D4	D8 ±1	D15 ±1	D22 ±1	D29 ±1	D35	D36	D37	D38	D43 ±2	FU/ ED[a]	
Acetaminophen PK sampling (hours)	24	-0.25, 0.5, 0.75, 1, 2, 3, 4, 6, 9, 12	24, 36								-0.25, 0.5, 0.75, 1, 2, 3, 4, 6, 9, 12	24, 36			Exact time of sampling must be recorded. Acetaminophen PK sampling times are relative to acetaminophen dosing (0 hours). 24-hr acetaminophen PK on Day 1 must be taken prior to tirzepatide dosing.
Tirzepatide dosing	X				X	X	X	X		X					Days 1 and 8 = 5 mg; Days 15, 22, and 29 = 10 mg; Day 36 = 15 mg. All tirzepatide doses will be administered subcutaneously as detailed in Section 7.1.
Tirzepatide PK Sampling (hours)	P, 8, 12	24, 36	48	72	P	P	P	P		P, 8, 12	24, 36	48	X	X	Exact time of sampling must be recorded. PK sampling times are relative to tirzepatide dosing.
Immunogenicity Samples	P					P				P				X	
Pharmacogenetics	P														

Abbreviations: CRU = clinical research unit; C-SSRS = Columbia-Suicide Severity Rating Scale; ECG = electrocardiogram; ED = early discontinuation; P = predose; PHQ-9 = Patient Health Questionnaire-9; PD = pharmacodynamics; PK = pharmacokinetics; SHFF = Self-Harm Follow-Up Form; SHSF = Self-Harm Supplementary Form; SMPG = self-monitoring of plasma glucose.

Note: Site should schedule procedures per as appropriate. However, if multiple procedures take place at the same time point, the following order of the procedure should be use: ECG, vital signs, PK/PD samples, glucose samples, immunogenicity, clinical laboratory samples and storage samples. Unless otherwise specified, predose samples and procedure may be collected or performed any time on the same day prior to dosing. A 10% time allowance variance will be considered acceptable for acetaminophen and tirzepatide PK sampling.

[a] Final follow-up will occur on Day 64 ± 1 day (i.e. 28 days ± 1 day after final tirzepatide dose). Early discontinuation visit should take place within 14 days upon confirmation of early discontinuation. Tirzepatide PK and immunogenicity samples need not be drawn if the subject discontinues prior to receiving a dose of tirzepatide.

3. Introduction

3.1. Study Rationale

Tirzepatide (LY3298176) is a dual glucose-dependent insulinotropic peptide (GIP) and glucagon-like peptide 1 (GLP-1) receptor agonist being developed as an adjunct to diet and exercise for chronic weight management in adult patients with or without type 2 diabetes mellitus (T2DM) who have an initial body mass index (BMI) of 30 kg/m^2 or greater (obese) or 27 kg/m^2 or greater (overweight). The impact of tirzepatide on gastric emptying (GE) has been assessed in healthy subjects and in patients with T2DM using acetaminophen pharmacokinetics (PK) as a surrogate (Study I8F-MC-GPGA). However, data on the GE effects of tirzepatide in overweight/obese non-diabetic subjects are presently lacking.

Study I8F-MC-GPHU (GPHU) will assess the effect of multiple doses of tirzepatide (5 to 15 mg) on the PK of acetaminophen to determine the impact on gastric emptying, and the pharmacodynamics (PD), safety and tolerability of tirzepatide in overweight/obese non-diabetic subjects and overweight/obese T2DM subjects when administered using a prefilled syringe (PFS).

3.2. Background

The available preclinical and clinical data indicate that co-stimulation of GIP and GLP-1 receptors may enhance insulin secretion, improve insulin sensitivity, and reduce body weight beyond the effect of selective GLP-1 receptor stimulation (Frias et al. 2018; Coskun et al. 2018).

Tirzepatide, a dual agonist of GIP and GLP-1, is a 39-amino acid synthetic peptide. It is administered once weekly by subcutaneous (SC) injection. Its structure is based on the GIP sequence and includes a C20 fatty di-acid moiety that prolongs the duration of action. It has a chemical structure and pharmacologic profile that is distinct from the GLP-1 receptor agonists due to the addition of GIP, which is unique among the marketed incretin mimetics.

In a Phase I study (Coskun et al. 2018) that included single and multiple ascending dose (SAD, MAD) parts, tirzepatide has been administered as single SC doses up to 8 mg in healthy subjects. In the MAD part, higher doses up to 10 mg were attained in healthy subjects via dose escalation. Doses up to 15 mg were achieved in patients with T2DM via dose escalation. In this study, gastrointestinal (GI) adverse events (AEs) (nausea, vomiting, diarrhea, abdominal distension) and decreased appetite were the most frequently reported events by both healthy subjects and also by patients with T2DM and were dose related. Most AEs were mild in severity, a few were moderate, and none were reported as severe. During the single ascending dose study, the high incidence of GI AEs, notably vomiting, were considered to be dose limiting at the 8-mg dose; therefore, the 5-mg dose was considered the maximum tolerated dose. A dose-dependent increase in heart rate was detected for both healthy subjects and patients with T2DM who received tirzepatide, similar to what was observed with selective GLP-1 receptor agonists. Immunogenicity assessments in Phase I showed low treatment-emergent antidrug antibody (TE-ADA) rate in tirzepatide-treated subjects (2.6%). Highest ADA titers in TE-ADA+ subjects ranged from 1:40 to 1:160 and showed no impact on tolerability or PK profile of tirzepatide. A

few subjects experienced transient elevations in lipase and/or amylase levels, but these episodes were not associated with any relevant outcomes. Once-weekly doses of 1, 5, 10, and 15 mg with titration have been further investigated in a Phase 2 study (Frias et al, 2018). An additional dose level of 12 mg and alternate dose escalation schemes were investigated in a 12-week Phase 2 study. The Phase 1 and 2 clinical data have not shown AEs different from that of available GLP receptor agonists currently marketed.

Based on the properties of tirzepatide CCI [redacted] t is expected to be metabolized via proteolytic cleavage of peptide components and is not anticipated to be metabolized by any single organ or by any cytochrome P450 enzymes contributing significantly to its metabolism. Hence, the risk of drug-drug interactions (DDIs) that may be noted could be largely attributed to the influence of tirzepatide on GE which in turn could alter the absorption of commonly co-administered medications administered via oral route.

In healthy subjects and patients with T2DM, changes in the mean concentration-time profiles of acetaminophen suggested a dose-dependent delay in GE following a single dose of tirzepatide. The delay in GE resulted in a 50 % decrease in C_{max} and approximately a 1-hour delay in t_{max}. The overall AUC was not affected. The delay in GE was greatest following the first dose of tirzepatide (at dose level ≥1.5 mg) and appeared to show tachyphylaxis after repeated dosing. Further details can be found in the Investigator's Brochure (IB).

The impact of tirzepatide on GE delay and tachyphylaxis after repeated dosing was similar between healthy volunteers and patients with T2DM (including some who were overweight) based on Phase 1 study. However, the impact of tirzepatide on GE delay in non-diabetic subjects who are obese/overweight has not been determined.

3.3. Benefit/Risk Assessment

Risks of tirzepatide have been consistent with risks associated with other GLP-1 receptor agonists currently marketed. Potential risks include, but are not limited to, GI effects, acute pancreatitis (very rare), increases in heart rate, and hypoglycemic events (GLP-1 receptor agonist class effect).

No clinically significant safety or tolerability concerns have been identified during clinical investigation of tirzepatide up to the single dose level of 5 mg or multiple weekly doses, when escalated up to 15 mg. Based on this information, the 6 weekly doses of 5 to 15 mg/0.5 mL tirzepatide (5/5/10/10/10/15 mg) to be administered in this study are reasonably anticipated to be tolerable in this group of subjects.

Based on the known pharmacology of tirzepatide, overweight/obese non-diabetic subjects may benefit from weight reduction. In addition, the reference subjects with T2DM are also expected to experience benefits of the study treatments, including improved glycemic control and reduced body weight.

More information about the known and expected benefits, risks, serious adverse events (SAEs) and reasonably anticipated AEs of tirzepatide are to be found in the Investigator's Brochure IB.

4. Objectives and Endpoints

Table GPHU.1 shows the objectives and endpoints of the study.

Table GPHU.1. Objectives and Endpoints

Objectives	Endpoints
Primary To assess the effect of tirzepatide on the PK profile of acetaminophen, a marker for GE	Acetaminophen area under the concentration versus time curve from time zero to time t, where t is the last time point with a measurable concentration (AUC0-t_{last}), time of maximum observed drug concentration (t_{max}), maximum observed drug concentration (C_{max})
Secondary To evaluate tirzepatide PK and PD effects following multiple doses of SC tirzepatide in overweight/obese non-diabetic subjects and overweight/obese T2DM subjects To evaluate tirzepatide safety and tolerability following multiple doses of SC tirzepatide in overweight/obese non-diabetic subjects and overweight/obese T2DM subjects	Glycemic control (plasma glucose, HbA1c), and tirzepatide area under the concentration versus time curve from time zero to infinity ($AUC_{0-\infty}$) AUC0-t_{last}, t_{max}, C_{max} Adverse events and hypoglycemic events
Exploratory	
CCI	
To evaluate the PD effects of tirzepatide on appetite and weight	Body weight, waist circumference, appetite VAS scores

Abbreviations: $AUC_{0-\infty}$= area under the drug concentration-time curve from zero to infinity; C_{max} = maximum observed drug concentration; PK = pharmacokinetics; SC = subcutaneous dose; t_{max} = time to maximum concentration; VAS = Visual Analog Scale.

5. Study Design

5.1. Overall Design

This is an open-label, fixed sequence study in overweight/obese non-diabetic subjects and overweight/obese T2DM subjects.

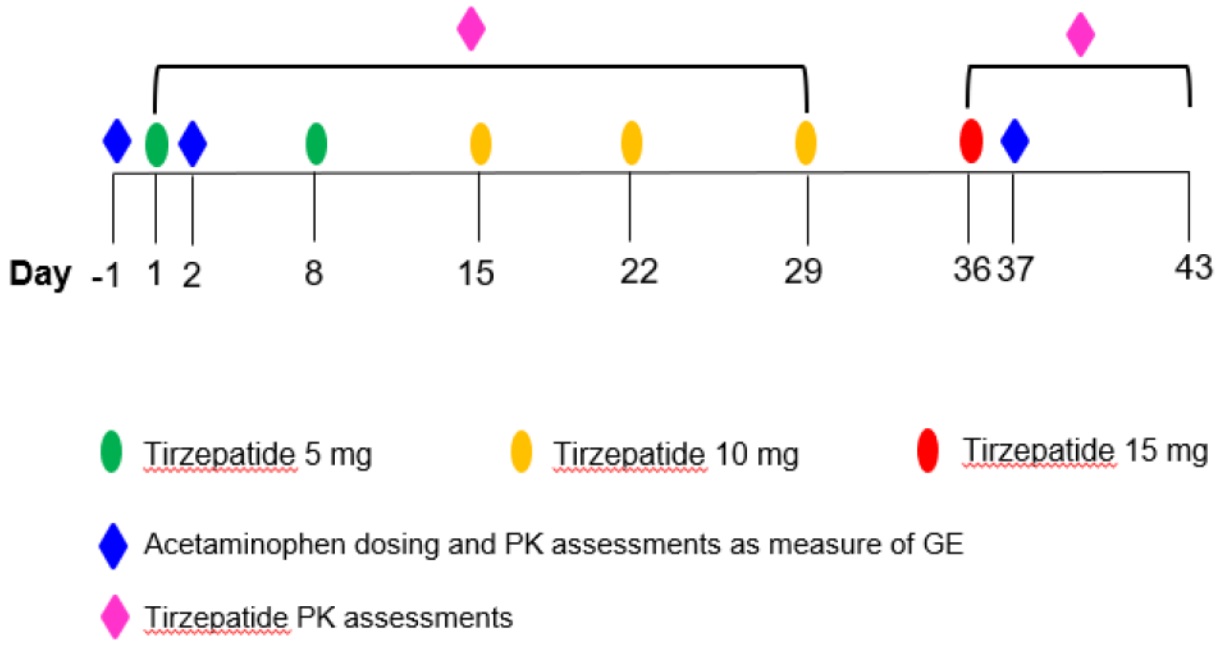
Potential subjects will be screened to assess their eligibility to enter the study within 28 days prior to Day 1. Subjects will be admitted to the CRU on Day -3 and will establish baseline measurements during the Baseline Period from Days -3 to -1.

Subjects may be permitted to leave the CRU on Day 4 of the Treatment Period following completion of study procedures. A second inpatient period will take place from Days 35 to 39. Outpatient visits will take place on Days 8 (±1 day), 15 (±1 day), 22 (±1 day), 29 (±1 day), and 43 (±2 days).

A final follow-up visit will occur on Day 64 ± 1 day (i.e. 28 days ± 1 day after the final tirzepatide dose).

Study governance considerations are described in detail in Appendix 3.

Figure GPHU.1 illustrates the study design.



Abbreviations: GE = gastric emptying; PK = pharmacokinetics.

Figure GPHU.1. Illustration of study design for Protocol I8F-MC-GPHU.

Safety will be assessed by vital signs, ECGs, CCI [redacted], safety laboratory tests, and the recording of AEs according to the schedule of activities (Section 2).

The PK blood samples for acetaminophen and tirzepatide will be collected according to the schedule of activities (Section 2).

5.2. Number of Participants

Approximately 18 overweight or obese non-diabetic subjects and 18 overweight or obese T2DM subjects will be enrolled so that a minimum of 12 subjects in each group completes the study. For purposes of this study, a patient completes the study when all scheduled procedures shown in the Schedule of Activities have been finished.

5.3. End of Study Definition

End of the study is the date of the last visit or last scheduled procedure shown in the Schedule of Activities (Section 2) for the last subject/patient.

5.4. Scientific Rationale for Study Design

This study will be open-label as the study primary endpoint PK measures are objective rather than subjective. This study is designed to assess the effect of multiple doses of tirzepatide (5 to 15 mg/0.5 mL) on gastric emptying and the PD, safety and tolerability of tirzepatide in both overweight/obese nondiabetic subjects and overweight/obese T2DM subjects. Acetaminophen is selected as the study probe drug as it is a well-established marker for the rate and extent of gastric emptying (Young 2005). A delay in gastric emptying is reflected in the alterations to the concentration-time profile of acetaminophen

The dose justification for tirzepatide is provided in Section 5.5 (Justification of Dose).

The rationale for the sample size is provided in Section 10.1 (Sample Size Determination).

5.5. Justification for Dose

The tirzepatide doses of 5 to 15 mg administered SC QW have been selected based on current preclinical pharmacology, toxicology, and clinical data; 15 mg SC QW is the highest maintenance dose planned for evaluation in the Phase 3 programs.

Tirzepatide dosing will start at a low dose of 5 mg followed by 5-mg, 10-mg, 10-mg, 10-mg every week to finally achieve the target dose of 15-mg. The stepwise increments are expected to permit adequate time for development of tolerance to GI events and are expected to minimize GI tolerability concerns. As shown in previous studies, the delay in GE is greatest following the first tirzepatide dose and appeared to show tachyphylaxis, and hence, the acetaminophen tests are planned after first dose (5 mg on Day 1) and after the first maximum dose (15 mg on Day 36) of tirzepatide. The highest dose of 15 mg tirzepatide is predicted to maintain an exposure multiple of 1.6 to 2.4 to the no-observed-adverse-effect level doses in 6-month monkey and rat toxicology studies, respectively. Additional information can be found in the IB.

6. Study Population

Eligibility of subjects for the study will be largely based on the results of medical history, physical examination, vital signs, clinical laboratory tests and 12-lead electrocardiogram (ECG).

The nature of any conditions present at the time of the physical examination and any preexisting conditions will be documented.

Screening may occur up to 28 days prior to Day 1. If the investigator decides not to administer the dose to a subject or not to enroll a subject on a particular day, the subject's visit may be rescheduled, and any assessments or procedures performed up to that point may be repeated to confirm their eligibility.

Prospective approval of protocol deviations to recruitment and enrollment criteria, also known as protocol waivers or exemptions, are not permitted.

6.1. Inclusion Criteria

Subjects are eligible for inclusion in the study only if they meet all of the following criteria at screening:

Subject Characteristics

[1] Are overweight or obese (body mass index [BMI] of 27 kg/m^2 to 45 kg/m^2, inclusive)

[1a] nondiabetic male or female subjects, as determined by medical history, physical examination, and safety laboratory assessments at screening

[1b] diabetic male or female subjects as determined by medical history

- Have a diagnosis of T2DM for at least 1 year
- Have T2DM controlled with diet and exercise alone or are on a stable dose of metformin for at least 3 months
- Have a hemoglobin A1c value at screening of ≥7.0% and ≤10.0 %
- Have a self-reported change in body weight of not more than ±5 kg within 3 months prior to screening

[2] Have safety laboratory test results within normal reference range or with abnormalities deemed clinically insignificant by the investigator

[3] Have venous access sufficient to allow for blood sampling as per the protocol

[4] Male or female subjects between the ages of 18 to 65 years, inclusive;

[4a] Male subjects:

- men, regardless of their fertility status, with nonpregnant woman of childbearing potential (WOCBP) partners must agree to either remain abstinent (if this is their preferred and usual lifestyle) or use condoms plus 1 additional highly effective (less than 1% failure rate) method of contraception (such as combination oral contraceptives, implanted contraceptives, or intrauterine device) or effective method of contraception, (such as diaphragms with spermicide or cervical sponge) for the duration of the study and for 5 half-lives of study drug plus 90 days, corresponding to 4 months after the last injection .
    - men and their partners may choose to use a double-barrier method of contraception. (Barrier protection methods without concomitant use of a spermicide are not an effective or acceptable method of contraception. Thus, each barrier method must include use of a spermicide. It should be noted however that the use of male and female condoms as a double-barrier method is not considered acceptable due to the high failure rate when these barrier methods are combined.)
    - periodic abstinence (e.g., calendar, ovulation, symptothermal, postovulation methods), declaration of abstinence just for the duration of a study, and withdrawal are not acceptable methods of contraception.
- men with pregnant partners should use condoms during intercourse for the duration of the study and until the end of estimated relevant potential exposure in WOCBP, which corresponds to 4 months following last injection.
- men must agree to refrain from sperm donation for the duration of the study and until their plasma concentrations are below the level that could result in a relevant potential exposure to a possible fetus, predicted to be 4 months following last injection.
- men who are in exclusively same-sex relationships (as their preferred and usual lifestyle) are not required to use contraception.

[4b] Female subjects:

- women of childbearing potential (WOCBP) who are abstinent (if this is complete abstinence, as their preferred and usual lifestyle) or in a same-sex relationship (as part of their preferred and usual lifestyle) must agree to either remain abstinent or stay in a same-sex relationship without sexual relationships with males. Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods), declaration of abstinence just for the duration of a study, and withdrawal are not acceptable methods of contraception.

- otherwise, WOCBP participating must agree to use effective contraception, where at least 1 form is highly effective (less than 1% failure rate), for the entirety of the study. Contraception must continue following completion of IP administration for 30 days.
    - women of childbearing potential participating must test negative for pregnancy prior to initiation of treatment as indicated by a negative serum pregnancy test at the screening visit followed by a negative urine pregnancy test within 24 hours prior to exposure
    - two forms of effective contraception, where at least 1 form is highly effective (less than 1% failure rate, such as combination oral contraceptives, implanted contraceptives or intrauterine devices) will be used. Effective contraception (such as male or female condoms with spermicide, diaphragms with spermicide or cervical sponges) may be used as the second therapy. Barrier protection methods without concomitant use of a spermicide are not an effective or acceptable method. Thus, each barrier method must include use of a spermicide (that is, condom with spermicide, diaphragm with spermicide, female condom with spermicide). It should be noted that the use of male and female condoms as a double barrier method is not considered acceptable due to the high failure rate when these methods are combined.
    - Must not be breastfeeding
- women who are not of childbearing potential may participate and include those who are:
    - infertile due to surgical sterilization (hysterectomy, bilateral oophorectomy, or tubal ligation), congenital anomaly such as mullerian agenesis, or
    - postmenopausal – defined as either
        i. a woman at least 40 years of age with an intact uterus, not on hormone therapy, who has cessation of menses for at least 1 year without an alternative medical cause, AND a follicle-stimulating hormone (FSH) >40 mIU/mL; women in this category must test negative in pregnancy test prior to study entry
        ii. a woman 55 years or older not on hormone therapy, who has had at least 12 months of spontaneous amenorrhea; or
        iii. a woman 55 years or older with a diagnosis of menopause prior to starting hormone replacement therapy.

Informed Consent

[5] Are reliable and willing to make themselves available for the duration of the study and are willing to follow study procedures

[6] Are willing and able to participate in the dietary intervention part of the study, in the opinion of the investigator

[7] Have given written informed consent approved by Eli Lilly and Company (Lilly) and the institutional review board (IRB) governing the site

6.2. Exclusion Criteria

Subjects will be excluded from study enrollment if they meet any of the following criteria at screening:

Medical Conditions – General

[8] Have a history or current CV (for example, acute myocardial infarction, congestive heart failure, unstable angina, uncontrolled hypertension [systolic blood pressure ≥ 160 mmHg and/or diastolic blood pressure ≥ 100 mmHg], cerebrovascular accident [including transient ischemic attack], venous thromboembolis, etc), respiratory, hepatic, renal, GI, endocrine, hematological (including history of thrombocytopenia), or neurological disorders capable of significantly altering the absorption, metabolism, or elimination of drugs; or constituting a risk when taking the IP; or may interfere with the interpretation of data

[9] Have obesity induced by other endocrinologic disorders (for example, Cushing Syndrome) or diagnosed monogenetic or syndromic forms of obesity (for example, Melanocortin 4 Receptor deficiency or Prader Willi Syndrome)

[10] Have acute or chronic pancreatitis or a history of acute idiopathic pancreatitis; or have other GI disorders (for example, relevant esophageal reflux or gall bladder disease) that could be aggravated by GLP-1 analogs; subjects who had cholecystolithiasis (gall stones) and/or cholecystectomy (removal of gall bladder) in the past, with no long-term complications, are eligible for participation

[11] Have a known clinically significant gastric emptying abnormality (e.g., gastric outlet obstruction), have undergone weight loss surgery such as gastric bypass (bariatric) surgery or restrictive bariatric surgery (e.g., Lap-Band®), or have endoscopic and/or device-based therapy for obesity or have had device removal within the last 6 months (for example, mucosal ablation, gastric artery embolization, intragastric balloon and duodenal-jejunal bypass sleeve)

[12] Have a personal or family history of medullary thyroid carcinoma (MTC), have multiple endocrine neoplasia syndrome type 2 (MEN 2), or calcitonin ≥20 pg/mL at screening

[13] Have confirmed type 1 diabetes mellitus, or a history of ketoacidosis, or hyperosmolar state/coma

[14] Have findings in the 12-lead ECG at screening that, in the opinion of the investigator, may increase the risks of potentially clinically relevant worsening associated with participation in the study

[15] Have an active or untreated malignancy or have been in remission from a clinically significant malignancy (other than basal or squamous cell skin cancer, in situ carcinomas of the cervix, or in situ prostate cancer) for <5 years prior to screening

[16] Have evidence of human immunodeficiency virus (HIV) and/or positive HIV antibodies at screening

[17] Have evidence of hepatitis B or positive hepatitis B surface antigen and/or evidence of hepatitis C virus (HCV) or hepatitis C antibody with confirmed presence of hepatitis C virus ribonucleic acid (RNA) at screening (a positive HCV antibody at screening will need an additional HCV RNA assay – detectable HCV RNA means a subject will meet exclusion criteria)

- Subjects with a previous diagnosis of HCV who have been treated with antiviral therapy and achieved a sustained virological response may be eligible for inclusion in the study, provided they have no detectable HCV RNA on the screening HCV polymerase chain reaction test. A sustained virological response is defined as an undetectable HCV RNA level 24 weeks after completion of a full, documented course of an approved antiviral therapy for HCV.
- Subjects who have spontaneously cleared HCV infection, defined as (1): a positive HCV antibody test and (2): a negative HCV RNA test, with no history of anti-HCV treatment, may be eligible for inclusion in the study, provided they have no detectable HCV RNA on screening for this study.

[18] Have serum aspartate aminotransferase (AST) or alanine aminotransferase (ALT) >2.0 × the upper limit of normal (ULN) or total bilirubin (TBL) >1.5 × ULN; (except for subjects with Gilbert's syndrome, which can be enrolled with TBL <2.0× ULN), or alkaline phosphatase (ALP) >1.5× ULN;

- subjects with nonalcoholic fatty liver disease are allowed to participate

[19] Have had a blood donation of 450 mL or more in the last 3 months or any blood donation within the last month prior to screening

[20] Have a history of drug or alcohol abuse or a positive drug screen (unless the positive testing is due to a drug prescribed to the subject by a health care professional); and/or smoke >10 cigarettes per day or the equivalent; or are unable or unwilling to refrain from nicotine use during CRU admission

[21] Have had a blood transfusion or severe blood loss within the last 3 months or have known hemoglobinopathy, hemolytic anemia, sickle cell anemia, or have a hemoglobin value <11 g/dL (males) or <10 g/dL (females), or any other condition known to interfere with hemoglobin A1c measurement

[22] Have an average weekly alcohol intake that exceeds 21 units per week (males) and 14 units per week (females) (1 unit = 12 oz or 360 mL of beer; 5 oz or 150 mL of wine; 1.5 oz or 45 mL of distilled spirits), or are unwilling to stop alcohol consumption from admission to the CRU and until discharge from the study or completion of all study procedures

[23] Have a history of atopy or clinically significant multiple or severe drug allergies, or severe posttreatment hypersensitivity reactions (including, but not limited to, erythema multiforme major, linear immunoglobulin A dermatosis, toxic epidermal necrolysis, or exfoliative dermatitis)

[24] Impaired renal estimated glomerular filtration rate <60 mL/min/1.73 m^2 calculated by Chronic Kidney Disease-Epidemiology. One retest may be performed in case of an initial borderline result <60 mL/min/1.73 m^2. The highest value from the 2 tests will be accepted

Prior/Concomitant Therapy – General

[25] Have been treated with prescription drugs that promote weight loss (e.g., sibutramine, mazindol, phentermine, naltrexone/bupropion, liraglutide) or similar other BW loss medications, including over-the-counter medications (e.g., Alli®) within 6 months prior to screening or between screening and enrollment

[26] Have received chronic (lasting >14 consecutive days) systemic glucocorticoid therapy (excluding topical, intra-articular, and inhaled preparations) within 1 month before screening, or between screening and enrollment

[27] Have been treated with:

[27a] Non-diabetic subjects: any glucose-lowering agent during the last 3 months prior to screening or between screening and enrollment

[27b] T2DM subjects: have taken any glucose-lowering medications other than metformin any time during the last 3 months before screening or during the screening/lead-in period; short-term use of insulin (<14 days) for treatment of acute conditions is allowed in the 3-month period prior to entry and after enrollment

[28] Have received treatment with a drug that has not received regulatory approval for any indication within 30 days or 5 half-lives (whichever is longer) of screening

Prior/Concurrent Clinical Trial Experience

[29] Are persons who have previously completed or withdrawn from this study

[30] Have previous exposure or known allergies to tirzepatide or related compounds

[31] Are currently enrolled in a clinical study involving an IP or any other type of medical research judged not to be scientifically or medically compatible with this study.

Exclusions Applicable Only to T2DM Subjects

[32] Have had more than 1 episode of severe hypoglycemia, as defined by the American Diabetes Association criteria, within 6 months before screening or has a history of hypoglycemia unawareness or poor recognition of hypoglycemic symptoms; any patient that cannot communicate an understanding of hypoglycemic symptoms and the appropriate treatment of hypoglycemia prior to the first dose of study drug should also be excluded

[33] Have a history of proliferative retinopathy or maculopathy as determined by the investigator based on a recent (<6 months) ophthalmologic examination

Other Exclusions

[34] Have any lifetime history of a suicide attempt

[35] Have a Patient Health Questionnaire-9 (PHQ-9) score of 15 or more at screening

[36] On the Columbia-Suicide Severity Rating Scale (C-SSRS) at screening:

- a "yes" answer to either Question 4 (Active Suicidal Ideation with Some Intent to Act, Without Specific Plan) or
- a "yes" answer to Question 5 (Active Suicidal Ideation with Specific Plan and Intent) on the "Suicidal Ideation" portion of the C-SSRS, or
- a "yes" answer to any of the suicide-related behaviors (Actual Attempt, Interrupted Attempt, Aborted Attempt, Preparatory Act or Behavior) on the "Suicidal Behavior" portion of the C-SSRS, and
- the ideation or behavior occurred within the past month

[37] Are investigative site personnel directly affiliated with this study and their immediate families. Immediate family is defined as a spouse, parent, child, or sibling, whether biological or legally adopted;

[38] Are Eli Lilly and Company employees;

[39] Are deemed unsuitable by the investigator for any other reason.

6.3. Lifestyle and/or Dietary Requirements

Throughout the study, subjects may undergo medical assessments and review of compliance with requirements before continuing in the study.

6.3.1. Meals and Dietary Restrictions

Subjects will be required to fast overnight for at least 8 hours before tirzepatide and when undergoing specific procedures, and also when clinical laboratory test samples are taken (see Schedule of Activities [Section 2]). A meal will be offered to study subjects after about 2 hours post tirzepatide dose. On the day of gastric emptying evaluation, a meal will be given about half an hour before the scheduled acetaminophen dose in order to assess gastric emptying (see Schedule of Activities [Section 2]). The macronutrient composition (i.e. number of grams of carbohydrate, fat and protein) of this meal for each subject should be consistent on all gastric emptying procedure days and the amount consumed must be recorded in the subject's CRF. For T2DM patients, the morning dose of metformin may be taken together with this meal if applicable. During inpatient stays, subjects may not consume any food other than that provided by the CRU, although water may be consumed freely.

6.3.2. Caffeine, Alcohol, and Tobacco

No alcohol and nicotine use will be permitted while the subject is resident in the CRU. While not resident in the CRU, subjects must consume no more than 10 cigarettes or the equivalent per day and alcohol consumption should be limited to approximately 3 units daily for males and 2 units daily for females, as defined in Exclusion Criteria [22].

Subjects will be allowed to maintain their regular caffeine consumption throughout the study period (except during specific fasting time periods).

6.3.3. Activity

No strenuous physical activity will be allowed for 48 hours prior to tirzepatide dosing on Day 1 until completion of all study procedures.

6.4. Screen Failures

Screening tests such as clinical laboratory tests and vital signs/ECGs may be repeated at the discretion of the investigator. Individuals who do not meet the criteria for participation in this study (screen failure) may not be re-screened. Subject numbers/enrollment numbers are assigned on Day -2 of the Baseline Period to ensure only eligible subjects enter the study.

7. Treatment

7.1. Treatment Administered

Subjects will receive tirzepatide SC QW alone for 6 weeks on Days 1, 8, 15, 22, 29, and 36, and will receive oral acetaminophen on Days -1, 2, and 37. Acetaminophen may be administered with water and dosing cups or syringes should be flushed with water to ensure the complete dose is delivered.

Table GPHU.2 shows the treatments administered.

Table GPHU.2. Treatments Administered

Treatment Name	Tirzepatide	Acetaminophen
Dosage Formulation	Prefilled syringe	Liquid
Dosage strength and volume	5 mg / 0.5 ml; 10 mg/0.5 ml; 15 mg/0.5 ml	160 mg/5 mL
Route of Administration	SC	Oral
Dosing instructions	SC QW into abdominal quadrant	Single dose of 31.3 mL to be taken orally

Abbreviations: QW = once weekly; SC = subcutaneous.

The investigator or designee is responsible for:

- explaining the correct use of the investigational product (IP) to the site personnel
- verifying that instructions are followed properly
- maintaining accurate records of IP dispensing and collection
- and returning all unused medication to Lilly or its designee at the end of the study

Note: In some cases, sites may destroy the material if, during the investigative site selection, the evaluator has verified and documented that the site has appropriate facilities and written procedures to dispose of clinical materials.

Site staff will administer all injections. Detailed instructions for will be provided by the sponsor. All injections will be administered into the SC tissue of the abdominal wall, with injection sites alternated weekly between 4 sites, that is, right and left upper quadrants and right and left lower quadrants. Only a limited number of individuals will perform SC administration for consistency reasons. Dosing will ideally occur at approximately the same time of day in all dose cohorts. The actual time of dosing will be recorded in the patient's electronic case report form (eCRF).

7.1.1. Packaging and Labeling

Tirzepatide will be supplied by Lilly. Tirzepatide will be provided as prefilled syringes containing 0.5 mL solution and provided in individual cartons to be dispensed.

The IP will be labeled according to the country's regulatory requirements.

Commercially available liquid acetaminophen will be site sourced.

7.2. Method of Treatment Assignment

All subjects will receive the same treatment

7.2.1. Selection and Timing of Doses

The doses will be administered at approximately the same time on each dosing day. The actual time of all dose administrations will be recorded in the subject's eCRF.

7.3. Blinding

This is an open-label study.

7.4. Dose Modification

Dose modification is not permitted in this study.

7.5. Preparation/Handling/Storage/Accountability

The investigator or designee must confirm appropriate temperature conditions have been maintained, as communicated by Sponsor, during transit for all IP received and any discrepancies are reported and resolved before use of the study treatment.

Only participants enrolled in the study may receive IP or study materials, and only authorized site staff may supply or administer IP. All IP should be stored in an 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.

The investigator is responsible for study treatment accountability, reconciliation, and record maintenance (such as receipt, reconciliation and final disposition records).

7.6. Treatment Compliance

Tirzepatide and acetaminophen will be administered at the study site and documentation of treatment administration will occur at the site.

7.7. Concomitant Therapy

In general, concomitant medication should be avoided. If the need for concomitant medication arises (for example, to treat an AE), inclusion or continuation of the subject may be at the discretion of the investigator, preferably after consultation with a Lilly Clinical Pharmacologist (CP) or CRP or designee. Any medication used during the course of the study must be documented.

All subjects on stable concomitant medication at the time of study entry, other than those that are prohibited, should continue their regular, unchanged dose throughout the study.

Subjects with T2DM may be on a stable dose of metformin at screening should continue to receive metformin for the duration of the study. In addition, T2DM subjects may have received or be permitted to receive short-term (<14 days) insulin within 3 months prior to study entry and/or after enrollment.

The tirzepatide dose escalation schemes have been designed to reduce the development of intolerable GI symptoms. During the dose escalation period, every effort should be made by the investigator to be able to escalate and maintain subjects on the tirzepatide dosage.

To mitigate GI symptoms and manage subjects with poorly tolerated GI AEs, the investigator should:

- Advise subjects to eat smaller meals, for example, splitting 3 daily meals into 4 or more smaller meals, and to stop eating when they feel full.
- Prescribe symptomatic medication (for example, approved anti-emetic or anti-diarrheal medication)

7.8. Treatment after the End of the Study

Not applicable.

8. Discontinuation Criteria

Subjects discontinuing from the treatment or study prematurely for any reason should complete adverse event and other follow-up procedures per Section 2 (Schedule of Activities).

8.1. Discontinuation from Study Treatment

8.1.1. Permanent Discontinuation from Study Treatment

Possible reasons leading to permanent discontinuation of IP:

- The subject/patient requests to discontinue IP
- If a subject/patient is inadvertently enrolled and it is determined that continued treatment with study drug would not be medically appropriate (Section 8.1.2 Discontinuation of Inadvertently Enrolled Subjects)
- If a subject is diagnosed with acute or chronic pancreatitis after enrollment
- If a subject is confirmed to have developed T1DM after enrollment
- If a subject is diagnosed with MTC after enrollment
- If a subject is diagnosed with an active or untreated malignancy (other than basal or squamous cell skin cancer, in situ carcinomas of the cervix, or in situ prostate cancer) after enrollment
- If the investigator, after consultation with the sponsor-designated medical monitor, determines that a systemic hypersensitivity reaction has occurred related to study drug administration, the participant should be permanently discontinued from the investigational drug.
- If a subject is diagnosed with any other TEAE, SAE, or clinically significant laboratory value for which the investigator believes that permanent IP discontinuation is the appropriate measure to be taken
- If female subject becomes pregnant

Discontinuation of the IP for abnormal liver tests **should be considered** by the investigator when a patient meets 1 of the following conditions after consultation with the Lilly designated medical monitor:

- ALT or AST >8× ULN
- ALT or AST >5× ULN sustained for more than 2 weeks
- ALT or AST >3× ULN **and** (TBL >2× ULN **or** international normalized ratio >1.5)
- ALT or AST >3× ULN, with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia (>5%).Drug-related vomiting requiring intravenous (IV) hydration treatment or causing severe distress (prevents daily

activities and results in no appetite, or requires an emergency department visit, or hospitalization), that cannot be resolved by temporary interruption of IP.

Subjects who discontinue IP early will be also discontinued from the study after performing the ED visit and safety follow-up visit procedures, as specified in Section 2 (Schedule of Activities).

8.1.2. Discontinuation of Inadvertently Enrolled Subjects

If the Sponsor or investigator identifies a subject who did not meet enrollment criteria and was inadvertently enrolled, a discussion must occur between the Lilly clinical pharmacologist/CRP and the investigator to determine if the subject may continue in the study. If both agree it is medically appropriate to continue, the investigator must obtain documented approval from the Lilly clinical pharmacologist/CRP to allow the inadvertently enrolled subject to continue in the study with or without continued treatment with IP.

8.2. Discontinuation from the Study

In addition to the situations that result in IP discontinuation described in Section 8.1.1 (Permanent Discontinuation from Study Treatment), subjects will be discontinued in the following circumstances:

- Enrollment in any other clinical study involving an IP or enrollment in any other type of medical research judged not to be scientifically or medically compatible with this study
- Participation in the study needs to be stopped for medical, safety, regulatory, or other reasons consistent with applicable laws, regulations, and Good Clinical Practice
- Investigator Decision
    - the investigator decides that the subject should be discontinued from the study
    - if the subject, for any reason, requires treatment with another therapeutic agent that has been demonstrated to be effective for treatment of the study indication, discontinuation from the study occurs prior to introduction of the new agent
- Subject Decision
    - the subject, or legal representative, requests to be withdrawn from the study.

8.3. Subjects 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. Site personnel are expected to make diligent attempts to contact subjects who fail to return for a scheduled visit or were otherwise unable to be followed up by the site.

9. Study Assessments and Procedures

Section 2 lists the Schedule of Activities, detailing the study procedures and their timing (including tolerance limits for timing).

Appendix 2 lists the laboratory tests that will be performed for this study.

Appendix 5 provides a summary of the maximum number and volume of invasive samples, for all sampling, during the study.

The specifications in this protocol for the timings of safety and sample collections are given as targets to be achieved within reasonable limits. Modifications may be made to the time points based upon emerging clinical information. The scheduled time points may be subject to minor alterations; however, the actual time must be recorded correctly in the eCRF. Failure or delays (i.e., outside stipulated time allowances) in performing procedures or obtaining samples, including those due to legitimate clinical issues (e.g., equipment technical problems, venous access difficulty, or subject defaulting or turning up late on an agreed scheduled procedure) must be documented in writing via a file note but will not be considered as a protocol deviation.

Unless otherwise stated in subsections below, all samples collected for specified laboratory tests will be destroyed within 60 days of receipt of confirmed test results. Certain samples may be retained for a longer period, if necessary, to comply with applicable laws, regulations, or laboratory certification standards.

9.1. Efficacy Assessments

This section is not applicable for this study.

9.2. Adverse Events

Investigators are responsible for monitoring the safety of subjects who have entered this study and for alerting Lilly or its designee to any event that seems unusual, even if this event may be considered an unanticipated benefit to the subject.

The investigator is responsible for the appropriate medical care of subjects during the study.

Investigators must document their review of each laboratory safety report.

The investigator remains responsible for following, through an appropriate health care option, AEs that are serious or otherwise medically important, considered related to the IP or the study, or that caused the subject to discontinue the IP before completing the study. The subject should be followed until the event resolves, stabilizes with appropriate diagnostic evaluation, or is reasonably explained. The frequency of follow-up evaluations of the AE is left to the discretion of the investigator.

The investigator will record all relevant AE and SAE information in the eCRF. After the ICF is signed, study site personnel will record, via eCRF, the occurrence and nature of each subject's preexisting conditions, including clinically significant signs and symptoms of the disease under

treatment in the study. Additionally, site personnel will record any change in the condition(s) and the occurrence and nature of any AEs.

The investigator will interpret and document whether or not an AE has a reasonable possibility of being related to study treatment, or a study procedure, taking into account the disease, concomitant treatment or pathologies.

A "reasonable possibility" means that there is a potential cause and effect relationship between the IP, and/or study procedure and the AE.

Planned surgeries should not be reported as AEs unless the underlying medical condition has worsened during the course of the study.

If a subject's IP is discontinued as a result of an AE, study site personnel must report this to Lilly or its designee via eCRF.

9.2.1. Serious Adverse Events

An SAE is any AE from this study that results in one of the following:

- death
- initial or prolonged inpatient hospitalization
- a life-threatening experience (that is, immediate risk of dying)
- persistent or significant disability/incapacity
- congenital anomaly/birth defect
- important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above.
- when a condition related to the PFS necessitates medical or surgical intervention to preclude either permanent impairment of a body function or permanent damage to a body structure, the serious outcome of "required intervention" will be assigned.

Study site personnel must alert the Lilly CRP/CP, or its designee, of any SAE as soon as practically possible.

Additionally, study site personnel must alert Lilly Global Patient Safety, or its designee, of any SAE within 24 hours of investigator awareness of the event via a sponsor-approved method. If alerts are issued via telephone, they are to be immediately followed with official notification on study-specific SAE forms. This 24-hour notification requirement refers to the initial SAE information and all follow-up SAE information.

Investigators are not obligated to actively seek AEs or SAEs in subjects once they have discontinued from and/or completed the study (the patient summary eCRF has been completed). However, if the investigator learns of any SAE, including a death, at any time after a subject has

been discharged from the study, and he/she considers the event reasonably possibly related to the study treatment or study participation, the investigator must promptly notify Lilly.

Pregnancy (maternal or paternal exposure to IP) does not meet the definition of an adverse event. However, to fulfill regulatory requirements any pregnancy should be reported following the SAE process to collect data on the outcome for both mother and fetus.

9.2.1.1. Suspected Unexpected Serious Adverse Reactions

Suspected unexpected serious adverse reactions (SUSARs) are serious events that are not listed in the IB and that the investigator reports as related to IP or procedure. Lilly has procedures that will be followed for the recording and expedited reporting of SUSARs that are consistent with global regulations and the associated detailed guidances.

9.2.2. Adverse Events of Special Interest

9.2.2.1. Hypoglycemia

Tirzepatide is an incretin which acts on the pancreatic β cell as a non-secretagogue; therefore, the risk of hypoglycemia is very low.

If hypoglycemia occurs, each episode should be treated according to the standards of care by the investigator and additional monitoring of glucose levels may be requested at the investigator's discretion. As a general safety precaution, subjects will be trained during the baseline period about signs and symptoms of hypoglycemia, how to treat hypoglycemia, and how to collect appropriate information for each episode of hypoglycemia.

Hypoglycemia may be identified by spontaneous reporting of symptoms from participants (whether confirmed or unconfirmed by simultaneous glucose values) or by blood/plasma glucose samples collected during study visits.

Subjects with T2DM will be given glucometers (plus consumables such as test strips, lancets, alcohol swabs etc) by the site staff to assist in the evaluation of reported symptoms consistent with hypoglycemia. Subjects receiving glucometers will record relevant information (for example, glucose values, symptoms) in a site developed diary. Subjects without T2DM may, at the investigator's discretion, be given glucometers to assist in the evaluation of reported symptoms consistent with hypoglycemia. Subjects receiving glucometers will record relevant information (for example, glucose values, symptoms) in a diary.

Investigators should use the following definitions and criteria when diagnosing and categorizing an episode considered to be related to hypoglycemia (the PG values in this section refer to values determined by a laboratory or International Federation of Clinical Chemistry and Laboratory Medicine plasma-equivalent glucose meters and strips) (American Diabetes Association 2019):

Glucose Alert Value (Level 1):

- **Documented symptomatic hypoglycemia** is defined as any time a subject feels that he or she is experiencing symptoms and/or signs associated with hypoglycemia, and has a PG level of ≤70 mg/dL (≤3.9 mmol/L).

- **Documented asymptomatic hypoglycemia** is defined as any event not accompanied by typical symptoms of hypoglycemia, but with a measured PG ≤70 mg/dL (≤3.9 mmol/L).
- **Documented unspecified hypoglycemia** is defined as any event with no information about symptoms of hypoglycemia available, but with a measured PG ≤70 mg/dL (≤3.9 mmol/L).

Clinically Significant Hypoglycemia (Level 2):

- **Documented symptomatic hypoglycemia** is defined as any time a subject feels that he/she is experiencing symptoms and/or signs associated with hypoglycemia, and has a PG level of <54 mg/dL (<3.0 mmol/L).
- **Documented asymptomatic hypoglycemia** is defined as any event not accompanied by typical symptoms of hypoglycemia, but with a measured PG <54 mg/dL (<3.0 mmol/L).
- **Documented unspecified hypoglycemia** is defined as any event with no information about symptoms of hypoglycemia available, but with a measured PG <54 mg/dL (<3.0 mmol/L).

Severe Hypoglycemia (Level 3):

- **Severe hypoglycemia** is defined as an episode with severe cognitive impairment requiring the assistance of another person to actively administer carbohydrate, glucagon, or other resuscitative actions. These episodes may be associated with sufficient neuroglycopenia to induce seizure or coma. Blood glucose measurements may not be available during such an event, but neurological recovery attributable to the restoration of BG to normal is considered sufficient evidence that the event was induced by a low BG concentration.

Other Hypoglycemia Categories:

- **Nocturnal hypoglycemia** is defined as any hypoglycemic event that occurs between bedtime and waking.

If a hypoglycemic event meets the criteria of severe, the investigator must record the event as serious on the AE pages of the eCRF and reported to Lilly as an SAE.

To avoid duplicate reporting, all consecutive plasma glucose values ≤70 mg/dL (3.9 mmol/L) occurring within a 1-hour period may be considered to be a single hypoglycemic event (Weinberg et al. 2010; Danne et al. 2013).

In each case of suspected or confirmed hypoglycemia, it is important that the event be properly categorized, the effect of the intervention be assessed, and the frequency of hypoglycemia be evaluated. The role of dietary changes and physical exercise (or any other contributing factor) in the development of an event should be established. The subject should receive additional education, if deemed appropriate.

9.2.2.2. Pancreatitis

Acute pancreatitis is defined as an AE of interest in all studies with tirzepatide including this study. Acute pancreatitis is an acute inflammatory process of the pancreas that may also involve peripancreatic tissues and/or remote organ systems (Banks and Freeman 2006). The diagnosis of acute pancreatitis requires 2 of the following 3 features:

- abdominal pain, characteristic of acute pancreatitis (generally located in the epigastrium and radiates to the back in approximately half the cases [Banks and Freeman 2006; Koizumi et al. 2006]; the pain is often associated with nausea and vomiting);
- serum amylase (total and/or pancreatic) and/or lipase ≥3 × ULN
- characteristic findings of acute pancreatitis on computed tomography (CT) scan or magnetic resonance imaging (MRI).

If acute pancreatitis is suspected, appropriate laboratory tests (including levels of pancreatic amylase and lipase) should be obtained via the local laboratory. Imaging studies, such as abdominal CT scan with or without contrast, MRI, or gallbladder ultrasound, should be performed. If laboratory values and/or abdominal imaging support the diagnosis of acute pancreatitis, the subject must discontinue IP, and will be discontinued from the study after completing all ED and follow-up procedures. The most appropriate diabetes therapeutic regimen will be decided by the investigator, based on the subject's clinical status. A review of the subject's medical data, including concomitant medications, should be conducted to assess potential causes of pancreatitis.

Each case of AE of pancreatitis must be reported. If typical signs and/or symptoms of pancreatitis are present and confirmed by laboratory values (lipase or amylase [total and/or pancreatic]) and imaging studies, the event must be reported as an SAE. For a potential case that does not meet all of these criteria, it is up to the investigator to determine the seriousness of the case (AE or SAE) and the relatedness of the event to IP.

Each subject will have measurements of amylase and lipase, part of safety laboratory tests as shown in Section 2 (Schedule of Activities) to assess the effects of study treatment on pancreatic enzyme levels. Serial measures of pancreatic enzymes have limited clinical value for predicting episodes of acute pancreatitis in asymptomatic subjects (Nauck et al. 2017; Steinberg et al. 2017a; Steinberg et al. 2017b). Thus, further diagnostic follow-up of cases of asymptomatic pancreatic hyperenzymemia (lipase and/or pancreatic amylase ≥3 × ULN) is not mandated but may be performed based on the investigator's clinical judgment and assessment of the subject's overall clinical condition. If further diagnostic assessment due to asymptomatic hyperenzymemia will be warranted, it should follow Lilly standard algorithm for the monitoring of pancreatic enzymes (refer to Appendix 6).

9.2.2.3. Thyroid Malignancies and C-Cell Hyperplasia

Individuals with personal or family history of MTC and/or MEN 2 will be excluded from the study, as well as those with calcitonin ≥20 pg/mL at screening. Subjects who are diagnosed with

MTC and/or MEN-2 during the study will have study drug stopped and should continue follow-up with an endocrinologist.

9.2.2.4. Supraventricular Arrhythmias and Cardiac Conduction Disorders

Treatment-emergent cardiac conduction disorders should be further evaluated. Subjects who develop any event from this group of disorders should undergo an ECG. Additional diagnostic tests to determine exact diagnosis should be performed, as needed. The specific diagnosis will be recorded as an AE in the eCRF. Events that meet criteria for serious conditions as described in Section 9.2.1 (Serious Adverse Events) must be reported as SAEs.

9.2.2.5. Hypersensitivity Events

Many drugs, but particularly biologic agents, carry the risk of systemic hypersensitivity reactions. If such a reaction occurs, additional data describing each symptom should be provided to the sponsor in the eCRF.

Sites should have appropriately trained medical staff and appropriate medical equipment available when study subjects are receiving study drug. It is recommended that subjects who experience a systemic hypersensitivity reaction be treated per the local standard of care.

In the case of generalized urticaria or anaphylaxis, additional blood samples should be collected as described in Section 9.6.3 (Immunogenicity Assessments). Laboratory results are provided to the sponsor via the central or a referral laboratory.

9.2.2.6. Injection-Site Reactions

Injection-site assessments for local tolerability will be conducted, when reported as:

- an AE from a subject, or
- a clinical observation from an investigator.

Reported injection-site reactions will be characterized within the following categories:

- edema
- erythema
- induration
- itching
- pain

Injection-site reactions will be collected on the eCRF created for these events. At the time of AE occurrence, unscheduled samples may be collected for measurement of tirzepatide ADA and tirzepatide PK if the event is suspected to be immune related by the investigator.

All injection-site reactions reported as AEs will be closely monitored until resolution. The report of a clinically significant AE of injection-site reaction may prompt notification of the sponsor, clinical photography, and referral for dermatologic evaluation and consideration of a skin biopsy

and laboratory evaluations (ALT, AST, complete blood count with percent eosinophils, and additional immunogenicity testing).

Site staff will be provided with separate instructions/training on how to evaluate injection-site reactions and their severity in a consistent manner. Photographs of injection-site reactions may be taken if deemed necessary by the investigator for subject monitoring and record-keeping purposes; however, the photographs will not be used to evaluate the severity of injection-site reaction.

9.2.2.7. Hepatobiliary Disorders

All events of treatment-emergent biliary colic, cholecystitis, or other suspected events related to gallbladder disease should be evaluated and additional diagnostic tests performed, as needed. In cases of elevated liver markers, hepatic monitoring should be initiated as outlined in Section 9.4.10.1 (Hepatic Safety) and Appendix 4.

9.2.2.8. Severe Gastrointestinal Adverse Events

Tirzepatide may cause severe GI AEs, such as nausea, vomiting, and diarrhea. Information about severe GI AEs as well as antiemetic/antidiarrheal use will be collected in the AE form of the eCRF.

9.2.2.9. Acute Renal Events

Renal safety will be assessed based on routine laboratory renal functional assessment as well as assessment of AEs suggestive of acute renal failure or worsening of chronic renal failure. Subjects with GI AEs, including nausea, diarrhea, and vomiting are at increased risk of developing dehydration. Dehydration may cause a deterioration in renal function, including acute renal failure. Subjects should be advised to notify investigators in case of severe nausea, frequent vomiting, or symptoms of dehydration.

9.2.3. Complaint Handling

Lilly collects product complaints on IPs and drug delivery systems used in clinical trials in order to ensure the safety of study participants, monitor quality, and to facilitate process and product improvements.

Subjects should be instructed to contact the investigator as soon as possible if he or she has a complaint or problem with the IP or PFS so that the situation can be assessed.

9.3. Treatment of Overdose

For the purposes of this study, an overdose of tirzepatide is considered any dose higher than the protocol-specified dose. There is no specific antidote. The subject should be watched for GI symptoms and hypoglycemia. Treatment is supportive, depending on the subject's symptoms. For detailed information, refer to the IB for tirzepatide.

9.4. Safety

9.4.1. Laboratory Tests

For each subject, laboratory tests detailed in Appendix 2 should be conducted according to the Schedule of Activities (Section 2).

Any clinically significant findings from laboratory tests that result in a diagnosis and that occur after the subject receives the first dose of the IP should be reported to Lilly, or its designee, as an AE via eCRF.

9.4.2. Vital Signs

For each subject, vital signs measurements should be conducted according to the Schedule of Activities (Section 2).

Blood pressure and pulse rate should be measured after at least 5 minutes in the supine position.

Unscheduled orthostatic vital signs should be assessed, if possible, during any AE of dizziness or posture-induced symptoms. Additional vital signs may be measured during each study period if warranted.

9.4.3. Body Weight and Waist Circumference

Weight and waist circumference will be measured according to the schedule provided in the Schedule of Activities (Section 2).

Subjects will be weighed at approximately the same time in the morning, before dosing and after an overnight fast and evacuation of the bowel and bladder, if possible. Weight will be measured twice on each scheduled occasion, with the subject stepping off the scale between measurements. A third measurement will be made if the first 2 measurements are >0.5 kg apart. The 2 closest measurements will be recorded. Wherever possible, the same scale will be used for all weight measurements throughout the study, and every effort will be made to ensure the scale will not be moved or need to be recalibrated. Subjects will be weighed in light clothing. The mean weight will be captured in the eCRF.

Waist circumference will be measured at the midpoint between the inferior border of the rib cage and the superior aspect of the iliac crest. The subject will stand in a straight, upright position with feet together and arms at the side. The area measured will be cleared of all clothing other than undergarments. The measurement should be taken at the end of normal expiration. Two separate measures will be taken and steps are repeated until 2 measurements are obtained within 0.5 cm of each other. The mean waist circumference will be captured in the eCRF.

9.4.4. Electrocardiograms

For each subject, a single 12-lead ECG should be collected according to the Schedule of Activities (Section 2).

Electrocardiograms must be recorded before collecting any blood samples. Subjects must be supine for approximately 5 to 10 minutes before ECG collection and remain supine but awake

during ECG collection. Electrocardiograms may be obtained at additional times, when deemed clinically necessary. All ECGs recorded should be stored at the investigational site.

Electrocardiograms will be interpreted by a qualified investigator (a physician or qualified designee) at the site as soon after the time of ECG collection as possible, and ideally while the subject is still present, to determine whether the subject meets entry criteria at the relevant visit(s) and for immediate subject management, should any clinically relevant findings be identified.

If a clinically significant finding is identified (including, but not limited to, changes in QT/QTc interval from baseline) after enrollment, the investigator will determine if the subject can continue in the study. The investigator, or qualified designee, is responsible for determining if any change in subject management is needed and must document his/her review of the ECG printed at the time of collection. Any new clinically relevant finding should be reported as an adverse event. Any clinically significant findings from ECGs that result in a diagnosis and that occur after the subject receives the first dose of the IP, should be reported to Lilly, or its designee, as an AE via eCRF.

9.4.6. Physical Examinations

Physical examinations and routine medical assessments will be conducted as specified in Section 2 (Schedule of Activities) and as clinically indicated.

9.4.7. 6-point Plasma Glucose Profile

Plasma samples will be taken for the profiling of plasma glucose before and after meals, as specified in Section 2. Any plasma glucose results that fulfil the criteria of hypoglycemia will be captured in the eCRF as specified in Section 9.2.2.1.

9.4.8. Self-monitoring of Plasma Glucose (SMPG)

Subjects with T2DM will be educated on the symptoms of hypoglycemia and instructed on how to use the glucometer provided by the site to perform SMPG. The site will provide a subject diary card and instruct T2DM subjects to perform and record SMPG results during the dosing phase of the study and whenever the subject experiences symptoms of hypoglycemia as specified

in the Schedule of Activities (Section 2). Results of the SMPG need not be databased, but investigator review of glucose results clinically indicative of hypoglycemia or symptomatic hyperglycemia is required.

All hypoglycemic events will be captured in the eCRF as specified in Section 9.2.2.1. Clinically significant hyperglycemic events should be reported as AEs.

9.4.9. Suicidal Ideation Assessment

Overweight and obese subjects are at an increased risk of depression (Luppino et al. 2010). Depression can increase the risk of suicidal ideation and behavior. Therefore, study subjects will be screened at trial entry for depression, suicidal ideation and behavior using the C-SSRS and PHQ-9 as specified in the Schedule of Activities (Section 2), and excluded from the study if they meet thresholds defined by Exclusion Criteria [35] and [36].

The C-SSRS (Columbia Lighthouse Project [WWW]) is a scale that captures the occurrence, severity, and frequency of suicidal ideation and/or behavior during the assessment period. The scale includes suggested questions to solicit the type of information needed to determine if suicidal ideation and/or behavior occurred. The tool was developed by the National Institute of Mental Health trial group for the purpose of being a counterpart to the Columbia Classification Algorithm of Suicide Assessment categorization of suicidal events. For this study, the C-SSRS is adapted for the assessment of the ideation and behavior categories only. The Intensity of Ideation and Lethality of Behavior sections are removed.

The PHQ-9 is a validated self-report screening tool that assesses the presence and intensity of depressive symptoms. The PHQ-9, which incorporates the 9 Diagnostic and Statistical Manual-IV depression criteria as “0” (not at all) to “3” (nearly every day), was developed for use in primary care settings (Kroenke et al. 2001).

9.4.10. Safety Monitoring

The Lilly clinical pharmacologist or CRP/scientist will monitor safety data throughout the course of the study.

Lilly will review SAEs within time frames mandated by company procedures. The Lilly clinical pharmacologist or CRP will periodically review the following data:

- trends in safety data
- laboratory analytes including glucose, amylase, and lipase
- serious and nonserious AEs, including AEs of interest (see Section 9.2.2 for full list)

When appropriate, the Lilly clinical pharmacologist or CRP will consult with the functionally independent Global Patient Safety therapeutic area physician or clinical research scientist.

9.4.10.1. Hepatic Safety

Close hepatic monitoring

Laboratory tests (Appendix 4), including ALT, AST, ALP, TBL, direct bilirubin, gamma-glutamyl transferase (GGT), and creatine kinase (CK), should be repeated within 48 to 72 hours to confirm the abnormality and to determine if it is increasing or decreasing, if 1 or more of these conditions occur:

If a participant with baseline results of ...	develops the following elevations:
ALT or AST <1.5× upper limit of normal (ULN)	ALT or AST ≥3× ULN
ALP <1.5× ULN	ALP ≥2× ULN
TBL <1.5× ULN	TBL ≥2× ULN (except for subjects with Gilbert's syndrome)
ALT or AST ≥1.5× ULN	ALT or AST ≥2× baseline
ALP ≥1.5× ULN	ALP ≥2× baseline
TBL ≥1.5× ULN	TBL ≥2× baseline (except for subjects with Gilbert's syndrome)

If the abnormality persists or worsens, clinical and laboratory monitoring, and evaluation for possible causes of abnormal liver tests should be initiated by the investigator in consultation with the Lilly-designated CP/CRP. At a minimum, this evaluation should include physical examination and a thorough medical history, including symptoms, recent illnesses (for example, heart failure, systemic infection, hypotension, or seizures), recent travel, history of concomitant medications (including over-the-counter), herbal and dietary supplements, history of alcohol drinking and other substance abuse.

Initially, monitoring of symptoms and hepatic biochemical tests should be done at a frequency of 1 to 3 times weekly, based on the participant's clinical condition and hepatic biochemical tests. Subsequently, the frequency of monitoring may be lowered to once every 1 to 2 weeks, if the participant's clinical condition and lab results stabilize. Monitoring of ALT, AST, ALP, and TBL should continue until levels normalize or return to approximate baseline levels.

Comprehensive hepatic evaluation

A comprehensive evaluation should be performed to search for possible causes of liver injury if one or more of these conditions occur:

If a participant with baseline results of...	develops the following elevations:
ALT or AST <1.5× ULN	ALT or AST ≥3× ULN with hepatic signs/symptoms*, or ALT or AST ≥5× ULN
ALP <1.5× ULN	ALP ≥3× ULN
TBL <1.5× ULN	TBL ≥2× ULN (except for subjects with Gilbert's syndrome)
ALT or AST ≥1.5× ULN	ALT or AST ≥2× baseline with hepatic signs/symptoms*, or ALT or AST ≥3× baseline
ALP ≥1.5× ULN	ALP ≥2× baseline
TBL ≥1.5× ULN	TBL ≥1.5× baseline (except for subjects with Gilbert's syndrome)

* Hepatic signs/symptoms are severe fatigue, nausea, vomiting, right upper quadrant abdominal pain, fever, rash, and/or eosinophilia >5%.

At a minimum, this evaluation should include physical examination and a thorough medical history, as outlined above, as well as tests for prothrombin time-international normalized ratio

(PT-INR); tests for viral hepatitis A, B, C, or E; tests for autoimmune hepatitis; and an abdominal imaging study (for example, ultrasound or CT scan).

Based on the subject's history and initial results, further testing should be considered in consultation with the Lilly-designated medical monitor, including tests for hepatitis D virus (HDV), cytomegalovirus (CMV), Epstein-Barr virus (EBV), acetaminophen levels, acetaminophen protein adducts, urine toxicology screen, Wilson's disease, blood alcohol levels, urinary ethyl glucuronide, and serum phosphatidylethanol. Based on the circumstances and the investigator's assessment of the participant's clinical condition, the investigator should consider referring the participant for a hepatologist or gastroenterologist consultation, magnetic resonance cholangiopancreatography (MRCP), endoscopic retrograde cholangiopancreatography (ERCP), cardiac echocardiogram, or a liver biopsy.

Additional safety data collection in hepatic safety eCRF should be performed in study participants who meet 1 or more of the following conditions:

- Elevation of serum ALT to ≥5 × ULN on 2 or more consecutive blood tests (if baseline ALT <1.5 × ULN)
    - In participants with baseline ALT ≥1.5 × ULN, the threshold is ALT ≥3 × baseline on 2 or more consecutive tests
- Elevated serum TBL to ≥2× ULN (except for cases of known Gilbert's syndrome)
    - In participants with baseline TBL ≥1.5× ULN, the threshold should be TBL ≥2 × baseline
- Elevation of serum ALP to ≥2× ULN on 2 or more consecutive blood tests
    - In participants with baseline ALP ≥1.5 × ULN, the threshold is ALP ≥2 × baseline on 2 or more consecutive blood tests
- Hepatic event considered to be a SAE
- Discontinuation of study drug due to a hepatic event

Note: the interval between the 2 consecutive blood tests should be at least 2 days.

9.5. Pharmacokinetics

At the visits and times specified in the Schedule of Activities (Section 2), venous blood samples of approximately 3 mL each will be collected to determine the plasma concentrations of tirzepatide. A maximum of 3 unscheduled samples may be collected at additional time points during the study if warranted and agreed upon between both the investigator and sponsor. Instructions for the collection and handling of blood samples will be provided by the sponsor. The actual date and time (24-hour clock time) of each sampling will be recorded.

Failure or being late (i.e., outside stipulated time allowances) to perform obtain samples due to legitimate clinical issues (e.g., equipment technical problems, venous access difficulty, or subject

defaulting or turning up late on an agreed scheduled procedure) will not be considered as protocol deviations but the CRU will have to notify the sponsor in writing via a file note.

9.5.1. Bioanalysis

Samples will be analyzed at a laboratory approved by the sponsor and stored at a facility designated by the sponsor.

Concentrations of tirzepatide and acetaminophen will be assayed using a validated liquid chromatography tandem mass spectrometry method.

Bioanalytical samples collected to measure IP concentrations will be retained for a maximum of 2 years following last subject visit for the study. During this time, samples remaining after the bioanalyses may be used for exploratory analyses such as metabolism, protein binding, or bioanalytical method cross-validation.

9.6. Pharmacodynamics

9.6.1. Gastric Emptying

Acetaminophen is a well-established marker for the rate and extent of gastric emptying. It is rapidly absorbed from the duodenum upon release from the stomach. A delay in gastric emptying is reflected in the alterations to the concentration-time profile of acetaminophen, specifically, decreasing its C_{max} and t_{max} without altering the extent (total drug amount) of absorption. A dose of approximately 1 g acetaminophen is considered to be sufficient for bioanalytical detection and will be administered on Day -1 and about 24 hours after first and sixth tirzepatide doses (Schedule of Activities [Section 2]).

Venous blood samples of approximately 2 mL each will be collected to determine the plasma concentrations of acetaminophen (Schedule of Activities [Section 2]). Up to 3 additional samples may be taken if required.

9.6.2. Body Weight

Weight will be measured according to the schedule provided in Section 2 (Schedule of Activities). Refer to Section 9.4.3 for details.

9.6.3. Immunogenicity Assessments

For immunogenicity testing, venous blood samples of approximately 10 mL will be collected from each subject according to the Schedule of Activities (Section 2), to determine antibody production against tirzepatide. Up to 3 additional samples may be collected if there is a possibility that an AE is immunologically mediated (see Section 9.2.2.5). All samples for immunogenicity testing should have a time-matched sample for PK analysis where relevant. Detailed instructions for the sample collections and handling will be provided by the sponsor. The actual date and 24-hour clock time of each sampling will be recorded.

Immunogenicity will be assessed by a validated assay designed to detect antidrug antibodies (ADA) in the presence of tirzepatide at a laboratory approved by the sponsor. Antibodies may

be further evaluated for their ability to neutralize the activity of tirzepatide on GIP and GLP-1 receptors. Positive tirzepatide ADA samples may also be tested for cross-reactivity against native GIP and GLP-1, and, if positive, may then be tested for neutralizing antibodies against native GIP and/or GLP-1.

All subjects will have an ADA sample measured at early discontinuation or at Day 64 ± 1 day. A risk-based approach will be used to monitor subjects who develop treatment-emergent ADA (TE ADA), defined in Section 10.3.4 (Evaluation of Immunogenicity).

Clinically significant TE ADA will be defined as any TE ADA at the last visit with:

- a high titer (≥1280) or an increasing titer from last measured value
- an association with a moderate-to-severe injection-site reaction

Subjects who have clinically significant TE ADA at early discontinuation or at the safety follow-up should be followed with ADA testing every 3 months until the ADA titers have returned to the baseline ADA titer (defined as ADA titer within 2-fold of baseline) or for up to 1 year, whichever is less. A PK sample may be collected at the follow-up immunogenicity assessment(s), if warranted and agreed upon by the investigator and sponsor.

Every effort should be made to contact subjects for the follow-up immunogenicity assessment; however, if subjects are unwilling or unable to return for the visit, this is not considered a protocol deviation.

Subjects followed for at least 1 year after dosing who have not returned to baseline, as defined above, will be assessed for safety concerns and, if no clinical sequelae are recognized by the clinical team, no further follow-up will be required. Subjects who have clinical sequelae that are considered potentially related to the presence of TE ADA may also be asked to return for additional follow-up testing.

Samples will be retained for a maximum of 15 years after the last subject visit, or for a shorter period if local regulations and ERBs allow, at a facility selected by the sponsor. The duration allows the sponsor to respond to future regulatory requests related to the tirzepatide. Any samples remaining after 15 years will be destroyed.

9.7. Genetics

A blood sample will be collected for pharmacogenetic analysis as specified in the Schedule of Activities (Section 2), where local regulations allow.

Samples will not be used to conduct unspecified disease or population genetic research either now or in the future. Samples will be used to investigate variable exposure or response to tirzepatide and to investigate genetic variants thought to play a role in T2DM. Assessment of variable response may include evaluation of AEs or differences in efficacy.

All samples will be coded with the subject number. These samples and any data generated can be linked back to the subject only by the investigative site personnel.

Samples will be retained for a maximum of 15 years after the last subject visit, or for a shorter period if local regulations and/or ERBs impose shorter time limits, for the study at a facility selected by Lilly or its designee. This retention period enables use of new technologies, response to regulatory questions, and investigation of variable response that may not be observed until later in the development of tirzepatide or after tirzepatide is commercially available.

Molecular technologies are expected to improve during the 15-year storage period and therefore cannot be specifically named. However, existing approaches include whole genome or exome sequencing, genome wide association studies, multiplex assays, and candidate gene studies. Regardless of technology utilized, data generated will be used only for the specific research scope described in this section.

9.8. Biomarkers

Not applicable.

9.9. Appetite Analysis

To explore the effects of tirzepatide on meal intake and appetite sensation, subjects will be asked to rate their appetite sensations using a 100-mm visual analog scale (VAS) for parameters of hunger, fullness, satiety, and prospective food consumption prior to dosing on Day 1, and in the fasted state while inpatient as well as on scheduled outpatient visits. These measurements shall be performed according to the Schedule of Activities (Section 2).

The VAS is a validated tool to assess appetite sensation parameters (Flint et al. 2000). The VAS is presented as a 10-cm (100-mm) line, anchored by verbal descriptors, usually “extremely” and “not at all”. Subjects are required to rate their subjective sensations on four 100-mm scales combined with questions similar to the following:

1. “How hungry do you feel?”
2. “How satisfied do you feel?”
3. “How full do you feel?”
4. “How much do you think you could eat?”

A staff member will use a caliper to measure the distance from 0 to the mark that the subject placed on the VAS and record the measurement in the source document. Overall appetite score is calculated as the average of the 4 individual scores: satiety + fullness + (100-prospective food consumption) + (100-hunger) / 4 (van Can et al. 2014). The higher overall appetite score indicates less appetite, and the lower score indicates more appetite.

9.10. Health Economics

This section is not applicable for this study.

10. Statistical Considerations and Data Analysis

10.1. Sample Size Determination

Approximately 18 overweight or obese non-diabetic subjects and 18 overweight or obese T2DM subjects will be enrolled so that a minimum of 12 subjects in each group completes the study. In total, approximately 36 subjects will be enrolled so that a minimum of 24 subjects complete the study.

For acetaminophen AUC and C_{max}, the intra-subject variability (coefficient of variation [CV]) was estimated to be 15.7% and 21.9%, respectively (derived from study I8F-MC-GPGA). Based on this assumption, 24 subjects will provide a precision of 0.09 and 0.13 on a log-scale for AUC and C_{max}, respectively. This would result in a 90% probability that the half-width of the 90% confidence interval (CI) of the ratio of the geometric least square means for AUC and C_{max} is no larger than 8.8% and 12.0%, respectively.

10.2. Populations for Analyses

10.2.1. Study Participant Disposition

A detailed description of subject disposition will be provided at the end of the study. All subjects who discontinue from the study will be identified, and the extent of their participation in the study will be reported. If known, a reason for their discontinuation will be given.

10.2.2. Study Participant Characteristics

The subject's age, sex, weight, height, BMI, or other demographic characteristics will be recorded and may be used in the PK and safety analyses as quantitative of classification variables.

10.3. Statistical Analyses

Statistical analysis of this study will be the responsibility of Eli Lilly and Company or its designee.

Pharmacokinetic and PD analyses will be conducted on data from all subjects who receive at least one dose of the IP and have evaluable data.

Safety analyses will be conducted for all subjects who receive at least one dose of the IP, whether or not they completed all protocol requirements.

Additional exploratory analyses of the data will be conducted as deemed appropriate. Study results may be pooled with the results of other studies for population PK analysis purposes.

10.3.1. Safety Analyses

10.3.1.1. Clinical Evaluation of Safety

All IP, protocol procedure AEs, and complaints will be listed, and if the frequency of events allows, data will be summarized using descriptive methodology.

The incidence of adverse events will be presented by severity and by association with IP as perceived by the investigator. Adverse events reported to occur prior to enrollment will be distinguished from those reported as new or increased in severity during the study. Each symptom will be classified by the most suitable term from the medical regulatory dictionary.

The number of IP-related SAEs will be reported.

10.3.1.2. Statistical Evaluation of Safety

Safety parameters that will be assessed include safety laboratory parameters, vital signs, [redacted] TEAEs (including TEAEs of special interest), and SAEs. The parameters will be listed and summarized using standard descriptive statistics. Additional analysis will be performed if warranted upon review of the data. All AEs related to study or protocol procedure will be listed, and if the frequency of events allows, will be also summarized using descriptive methodology.

Hypoglycemic events, as defined in Section 9.2.2.1, will be summarized and listed.

Physical examinations and ECGs will be performed for safety monitoring purposes and will not be presented. If warranted, additional analyses will be performed upon review of the data.

10.3.2. Pharmacokinetic Analyses

10.3.2.1. Pharmacokinetic Parameter Estimation

Pharmacokinetic parameter estimates for tirzepatide will be calculated by standard noncompartmental methods of analysis. The primary parameters for analysis will be C_{max}, $AUC_{0-\infty}$, $AUC0\text{-}t_{last}$, and t_{max}. Other noncompartmental parameters, such as half-life, apparent clearance, and apparent volume of distribution may be reported.

10.3.2.2. Pharmacokinetic Statistical Inference

Planned PK parameters will be summarized with descriptive statistics.

10.3.3. Pharmacodynamic Analyses

10.3.3.1. Pharmacodynamic Parameter Estimation

Acetaminophen PK parameters will be estimated to assess the effect of tirzepatide on gastric emptying delay. The PK parameter estimates for acetaminophen will be calculated by standard noncompartmental methods of analysis. The primary parameters for analysis will be the C_{max}, the $AUC0\text{-}t_{last}$, and the t_{max} of acetaminophen. Other parameters, such as half-life, apparent clearance, and apparent volume of distribution, may be reported.

A comparison of acetaminophen parameters on Day -1 (prior to study drug) and following first and sixth dose of study drug will be summarized.

10.3.3.2. Pharmacodynamic Statistical Inference

Pharmacokinetic parameters will be evaluated to estimate the impact of tirzepatide on gastric emptying and on the PK of acetaminophen. Log-transformed C_{max} and AUC will be evaluated in a linear mixed-effects model, with day (-1, 2, 37) as a fixed effect and subject as a random

effect. The differences in C_{max} and AUC between acetaminophen + tirzepatide (Test; Days 2 and 37) and acetaminophen alone (Reference; Day -1) will be back-transformed to present the ratios of geometric least squares means and the corresponding 90% CI. Other parameters may be analyzed in this way as needed.

The t_{max} will be analyzed using a Wilcoxon signed rank test. Estimates of the median difference based on the observed medians and 90% CIs from the Wilcoxon test will be calculated.

Planned PK parameters will also be summarized with descriptive statistics.

Pharmacodynamic parameters that will be assessed include body weight, appetite VAS scores and HbA1c. The parameters will be listed and summarized using standard descriptive statistics. Additional analysis will be performed if warranted upon review of the data.

10.3.4. Evaluation of Immunogenicity

The frequency and percentage of subjects with preexisting ADA and with TE ADA+ to tirzepatide will be tabulated. Treatment-emergent ADAs are defined as those with a titer 2-fold (1 dilution) greater than the minimum required dilution if no ADAs were detected at baseline (treatment-induced ADA) or those with a 4-fold (2 dilutions) increase in titer compared to baseline if ADAs were detected at baseline (treatment-boosted ADA). The minimum required dilution of the ADA assay is 1:10. For the TE ADA+ subjects the distribution of maximum titers will be described. If cross-reactivity with native GLP-1 and GIP or neutralizing antibodies against native GLP-1 and GIP assays are performed, the frequency of each will be reported.

10.3.5. Interim Analyses

No interim analyses are planned for this study. If an unplanned interim analysis is deemed necessary, the Lilly clinical pharmacologist, CRP/investigator, or designee will consult with the appropriate medical director or designee to determine if it is necessary to amend the protocol.

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12. Appendices

Appendix 1. Abbreviations and Definitions

Term	Definition
ADA	antidrug antibodies
AE	adverse event: Any untoward medical occurrence in a subject or clinical investigation subject administered a pharmaceutical product that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.
ALP	alkaline phosphatase
ALT	alkaline phosphatase
AST	aspartate aminotransferase
AUC	area under the concentration versus time curve
$AUC_{0-\infty}$	area under the concentration versus time curve from time zero to infinity
$AUC_{0-tlast}$	area under the concentration versus time curve from time zero to time t, where t is the last time point with a measurable concentration
BMI	body mass index
CI	confidence interval
C_{max}	maximum observed drug concentration
complaint	A complaint is any written, electronic, or oral communication that alleges deficiencies related to the identity, quality, purity, durability, reliability, safety or effectiveness, or performance of a drug or drug delivery system.
compliance	Adherence to all the study-related requirements, good clinical practice (GCP) requirements, and the applicable regulatory requirements.
confirmation	A process used to confirm that laboratory test results meet the quality requirements defined by the laboratory generating the data and that Lilly is confident that results are accurate. Confirmation will either occur immediately after initial testing or will require that samples be held to be retested at some defined time point, depending on the steps required to obtain confirmed results.
CP	Clinical Pharmacologist
CRP	Clinical Research Physician: Individual responsible for the medical conduct of the study. Responsibilities of the CRP may be performed by a physician, clinical research scientist, global safety physician or other medical officer.

C-SSRS	Columbia-Suicide Severity Rating Scale
CV	coefficient of variation
DDI	drug-drug interaction
ECG	electrocardiogram
eCRF	electronic case report form
enroll	The act of assigning a subject to a treatment. Subjects who are enrolled in the study are those who have been assigned to a treatment.
enter	Subjects entered into a study are those who sign the informed consent form directly or through their legally acceptable representatives.
ERB	ethical review board
FSH	follicle-stimulating hormone
GCP	good clinical practice
GE	gastric emptying
GI	gastrointestinal
GIP	glucose-dependent insulinotropic peptide
GLP-1	glucagon-like peptide 1
HCV	hepatitis C virus
HIV	human immunodeficiency virus
IB	Investigator's Brochure
ICF	informed consent form
ICH	International Council for Harmonization
informed consent	A process by which a subject voluntarily confirms his or her willingness to participate in a particular study, after having been informed of all aspects of the study that are relevant to the subject's decision to participate. Informed consent is documented by means of a written, signed and dated informed consent form.
Investigational product (IP)	A pharmaceutical form of an active ingredient or placebo being tested or used as a reference in a clinical study, including products already on the market when used or assembled (formulated or packaged) in a way different from the authorized form, or marketed products used for an unauthorized indication, or marketed products used to gain further information about the authorized form.
investigator	A person responsible for the conduct of the clinical study at a study site. If a study is conducted by a team of individuals at a study site, the investigator is the responsible leader of the team and may be called the principal investigator.

MAD	multiple ascending dose
MEN 2	multiple endocrine neoplasia syndrome type 2
MTC	medullary thyroid carcinoma
open-label	A study in which there are no restrictions on knowledge of treatment allocation, therefore the investigator and the study participant are aware of the drug therapy received during the study.
PD	pharmacodynamics
PFS	pre-filled syringe
PHQ-9	Patient Health Questionnaire-9
PK	pharmacokinetics
PK/PD	pharmacokinetic/pharmacodynamic
PG	plasma glucose
QW	once-weekly
RNA	ribonucleic acid
SAD	single ascending dose
SAE	serious adverse event
SC	subcutaneous
screen	The act of determining if an individual meets the minimum requirements to become part of a pool of potential candidates for participation in a clinical study.
SHSF	Self-Harm Supplementary Form
SMPG	self-monitoring of plasma glucose
SUSARs	suspected unexpected serious adverse reactions
TBL	total bilirubin
TE-ADA	treatment-emergent antidrug antibody
TEAE	treatment-emergent adverse event: Any untoward medical occurrence that emerges during a defined treatment period, having been absent pretreatment, or worsens relative to the pretreatment state, and does not necessarily have to have a causal relationship with this treatment
t_{max}	time of maximum observed drug concentration
T2DM	type 2 diabetes mellitus

ULN	upper limit of normal
VAS	visual analog scale
WOCBP	woman of childbearing potential

Appendix 2. Clinical Laboratory Tests

Safety Laboratory Tests

Hematology[a]	Clinical Chemistry (fasting)[a]
Hematocrit	Sodium
Hemoglobin	Potassium
Erythrocyte count (RBC)	Bicarbonate
Mean cell volume	Chloride
Mean cell hemoglobin	Calcium
Mean cell hemoglobin concentration	Glucose
Leukocytes (WBC)	Blood urea nitrogen
	Total protein
Absolute counts of:	Albumin
Neutrophils	Total bilirubin
Lymphocytes	Alkaline phosphatase
Monocytes	Aspartate aminotransferase
Eosinophils	Alanine aminotransferase
Basophils	Creatinine
Platelets	Amylase
	Lipase
Hemoglobin A1c[a,b]	Uric acid
	Phosphorous
Urinalysis[a]	Calcitonin[c]
	Follicle-stimulating hormone[c]
Specific gravity	Hepatitis B surface antigen[c,d]
pH	Hepatitis C antibody, hepatitis C RNA[c,d]
Protein	HIV[c,d]
Glucose	Pregnancy test[e]
Ketones	
Bilirubin	Lipid Panel (Fasting)[a,b]
Urobilinogen	Triglycerides
Blood	Total Cholesterol
Nitrite	LDL
Microscopic examination of sediment[f]	HDL

Abbreviations: HIV = human immunodeficiency virus; RBC = red blood cells; WBC = white blood cells.

[a] Performed by local laboratory. Results will be validated by the laboratory at the time of initial testing.

[b] Performed at Screening, Day -2, Day 29, and FU/ED.

[c] Performed by local laboratory at screening only.

[d] Tests may be waived if they have been performed within 6 months before screening with reports available for review.

[e] Serum pregnancy test will be performed at screening and urine pregnancy tests at subsequent visits.

[f] Test only if dipstick result is abnormal (i.e., positive for blood, protein, or nitrites).

Appendix 3. Study Governance, Regulatory and Ethical Considerations

Informed Consent

The investigator is responsible for:

- ensuring that the subject understands the nature of the study, the potential risks and benefits of participating in the study, and that their participation is voluntary.
- ensuring that informed consent is given by each subject. This includes obtaining the appropriate signatures and dates on the ICF prior to the performance of any protocol procedures and prior to the administration of IP.
- answering any questions the subject may have throughout the study and sharing in a timely manner any new information that may be relevant to the subject's willingness to continue his or her participation in the study.
- providing a copy of the ICF to the participant and retaining a copy on file.

Recruitment

Lilly or its designee is responsible for the central recruitment strategy for subjects. Individual investigators may have additional local requirements or processes. Study specific recruitment material should be approved by Lilly.

Ethical Review

The investigator must give assurance that the ethical review board (ERB) was properly constituted and convened as required by ICH guidelines and other applicable laws and regulations.

Documentation of ERB approval of the protocol and the ICF must be provided to Lilly before the study may begin at the investigative site(s). Lilly or its representatives must approve the ICF before it is used at the investigative site(s). All ICFs must be compliant with the ICH guideline on GCP.

The study site's ERB(s) should be provided with the following:

- the current IB and updates during the course of the study
- ICF
- relevant curricula vitae

Regulatory Considerations

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

1) consensus ethics principles derived from international ethics guidelines, including the Declaration of Helsinki and Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines
2) applicable ICH GCP Guidelines
3) applicable laws and regulations

Some of the obligations of the sponsor will be assigned to a third party organization.

Protocol Signatures

The sponsor's responsible medical officer will approve the protocol, confirming that, to the best of his or her knowledge, the protocol accurately describes the planned design and conduct of the study.

After reading the protocol, the principal investigator will sign the protocol signature page and send a copy of the signed page to a Lilly representative.

Final Report Signature

The investigator or designee will sign the clinical study report for this study, indicating agreement with the analyses, results, and conclusions of the report.

The sponsor's responsible medical officer and statistician will sign/approve the final clinical study report for this study, confirming that, to the best of his or her knowledge, the report accurately describes the conduct and results of the study.

Data Quality Assurance

To ensure accurate, complete, and reliable data, Lilly or its representatives will do the following:

- provide instructional material to the study sites, as appropriate.
- provide training to instruct the investigators and study coordinators. This training will give instruction on the protocol, the completion of the eCRFs, and study procedures.
- make periodic visits to the study site.
- be available for consultation and stay in contact with the study site personnel by mail, telephone, and/or fax.
- review and evaluate eCRF data and/or use standard computer edits to detect errors in data collection.
- conduct a quality review of the database.

In addition, Lilly or its representatives will periodically check a sample of the subject data recorded against source documents at the study site. The study may be audited by Lilly and/or regulatory agencies at any time. Investigators will be given notice before an audit occurs.

The investigator will keep records of all original source data. This might include laboratory tests, medical records, and clinical notes. If requested, the investigator will provide the sponsor, applicable regulatory agencies, and applicable ERBs with direct access to the original source documents.

Data Collection Tools/Source Data

An electronic data capture system will be used in this study. The site must define and retain all source records and must maintain a record of any data where source data are directly entered into the data capture system.

Data Protection

Data systems used for the study will have controls and requirements in accordance with local data protection law.

The purpose and use of subject personal information collected will be provided in a written document to the subject by the Sponsor.

Study and Site Closure

Discontinuation of Study Sites

Study site participation may be discontinued if Lilly or its designee, the investigator, or the ERB of the study site judges it necessary for medical, safety, regulatory, or other reasons consistent with applicable laws, regulations, and GCP.

Discontinuation of the Study

The study will be discontinued if Lilly or its designee judges it necessary for medical, safety, regulatory, or other reasons consistent with applicable laws, regulations, and GCP.

Appendix 4. Hepatic Monitoring Tests for Treatment-Emergent Abnormality

Evaluation of subjects with treatment-emergent abnormal hepatic biochemical tests during a clinical trial. Extent and type of work-up may vary by subject's history, severity of liver injury, underlying disease, and geography. Laboratory assays should preferably be performed locally for faster turnaround.

Recommended Evaluation	Competing Causes of Abnormal Liver Tests
1st Line Testing	
ALT, AST, ALP, TBL, direct bilirubin, GGT, CK	Routine follow up
Thorough history of symptoms, co-existing medical conditions, concomitant medications, dietary and nutritional supplements, excessive exercise or muscle injury, alcohol consumption, illicit substances.	Systemic infection/ sepsis; ischemic/ congestive hepatic injury; gallstone disease; alcoholic liver disease; muscle injury/ rhabdomyolysis; acetaminophen toxicity; DILI due to another drug, herbal or dietary supplement.
Serum CK	Muscle injury/rhabdomyolysis[a]
Anti-HAV (IgM)	Acute HAV infection
HBsAg Anti-HBc IgG, IgM	Acute hepatitis B; exacerbation of chronic hepatitis B
Anti-HCV HCV RNA (PCR)[b]	Acute hepatitis C[c]; exacerbation of chronic hepatitis C
Anti-HEV (IgG, IgM), HEV RNA[d]	Acute hepatitis E
ANA, ASMA, quantitative immunoglobulins (IgG, IgM, IgA)	Autoimmune hepatitis[e]
Hepatobiliary imaging (ultrasonography, CT scan, MRI, MRCP)[f]	Biliary obstruction; pancreatitis; gallstones; portal-vein/ hepatic vein thrombosis; hepatic metastasis
2nd Line Testing	
PT-INR	For subjects with elevated TBL or suspected liver failure
Serological tests for EBV, CMV, HSV May need to obtain acute and convalescent serological tests	Hepatic injury caused by CMV, EBV, HSV
EBV-DNA, CMV- DNA, HSV-DNA by PCR. Liver biopsy needed to confirm HSV	Hepatic injury caused by CMV, EBV, HSV
Additional Tests[g]	
Bone specific ALP (ALP fractionation)	Differentiate bone from liver origin
LKM-1 antibody	Autoimmune hepatitis
Urinary ethylglucuronide[h], serum phosphatidylethanol[i]	Alcoholic liver disease
Serum acetaminophen level; acetaminophen protein adducts	Acetaminophen toxicity
Review of blood pressure, pulse, electrocardiogram, echocardiogram, cardiology consult	Ischemic or congestive hepatic injury

Urine toxicology screen	Hepatotoxicity due to cocaine, opiates and other illicit substances
Anti-HDV	Hepatitis D
Blood or urine cultures	Systemic infection, sepsis
Serum ceruloplasmin, serum copper Slit lamp eyes examination for Kayser-Fleischer rings, genetic testing	Wilson's disease

Abbreviations: ALP = alkaline phosphatase; ALT = alanine aminotransferase; ANA = antinuclear antibody; anti- = antibody; ASMA = anti-smooth muscle antibody; AST = aspartate aminotransferase; CK = creatinine kinase; CMV = cytomegalovirus; CT = computed tomography; DILI = drug-induced liver injury; EBV = Epstein Bar Virus; GGT = gamma-glutamyl transferase; HAV = hepatitis A virus; HBc = hepatitis B core; HBsAg = hepatitis B surface antigen; HCV = hepatitis C virus; HDV = hepatitis D virus; HEV = hepatitis E virus; HSV = herpes simplex virus; Ig = immunoglobulin; INR = international normalized ratio; LKM-1 = liver kidney microsomal type 1; MRCP = magnetic resonance cholangiopancreatography; MRI = magnetic resonance imaging; PCR = polymerase chain reaction; PT-INR = prothrombin time-international normalized ratio; RNA = ribonucleic acid; TBL = total bilirubin.

[a] Serum AST typically (although not always) is higher than ALT

[b] If anti-HCV positive, HCV RNA is required to confirm HCV infection

[c] Acute hepatitis C may be anti-HCV negative but HCV RNA positive

[d] If anti-HEV IgM positive, consider confirmation with HEV RNA by nested PCR

[e] A liver biopsy is needed to confirm a diagnosis of autoimmune hepatitis

[f] If cholestatic injury, MRCP may be recommended

[g] Based on medical history and clinical judgment

[h] Alcohol consumption in past 3 to 5 days

[i] Alcohol consumption in past 3 weeks

Appendix 5. Blood Sampling Summary

This table summarizes the approximate number of venipunctures and blood volumes for all blood sampling (screening, safety laboratories, and bioanalytical assays) during the study.

Protocol I8F-MC-GPHU Sampling Summary

Purpose	Blood Volume per Sample (mL)	Number of Blood Samples	Total Volume (mL)
Screening tests[a,b]	25	1	25
Safety laboratory tests[a,b]	12.5	5	62.5
Hemoglobin A1c[b]	4	4	16
Tirzepatide pharmacokinetics[c]	3	19 (+3)	66
Immunogenicity[c]	10	4 (+3)	70
Acetaminophen pharmacodynamics[c]	3	35 (+3)	114
6-point plasma glucose[c]	2	6 × 3 occasions	36
Plasma glucose[c]	2	9	18
Pharmacogenetics[c]	10	1	10
Total			417.5
Total for clinical purposes			420

a Additional samples may be drawn if needed for safety purposes. Safety laboratory tests will be inclusive of lipid panels on days when they are performed (refer to Appendix 2)

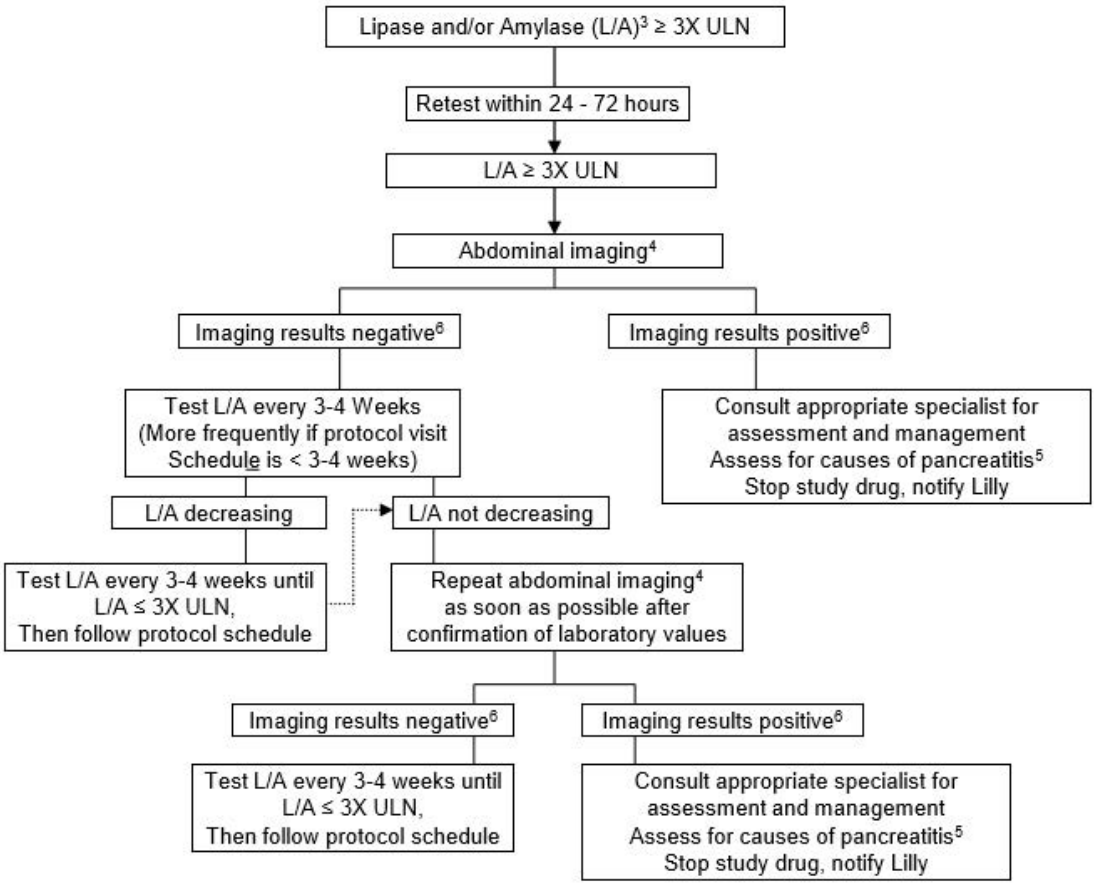
b Performed on site or in a local laboratory.

c Performed at a central or referral laboratory approved by the sponsor, including for storage

Appendix 6. Pancreatic Monitoring

Pancreatic Enzymes: Safety Monitoring Algorithm for Subjects/Patients without Symptoms of Pancreatitis[1,2]

Follow this algorithm when the value(s) for serum lipase and/or amylase are ≥ 3X ULN.



1. Symptomatic – related primarily to abdominal pain consistent with pancreatitis; however, severe nausea, vomiting and other symptoms may be considered by the investigator as symptomatic as well.

2. If, at any time, in the opinion of the investigator, patient/subject has symptoms of acute pancreatitis irrespective of L/A results:
   (a) Consult appropriate specialist for assessment and management
   (b) Assess for causes of pancreatitis
   (c) Stop study drug
   (d) Notify Lilly

3. L/A = Lipase and/or amylase. Either or both enzymes can be measured and either or both can be used to meet the algorithm criteria.

4. Abdominal imaging is most valuable when performed at the time of elevated enzyme values. If in the opinion of the radiologist or investigator, it is safe for the patient/subject to receive contrast, an enhanced abdominal CT is preferred. MRI is also an acceptable imaging modality.

5. As minimum, test hepatic analytes, triglycerides, and calcium, and record all concomitant medications

6. Imaging results positive or negative for signs of acute pancreatitis

Abbreviations: CT = computed tomography; MRI = magnetic resonance imaging; ULN = upper limit of normal.

Subjects diagnosed with pancreatitis will be discontinued from the study. Investigators will be responsible for following, through an appropriate healthcare option, these pancreatitis AEs until the events resolve or are explained. Adverse events that meet the diagnostic criteria of acute pancreatitis will be captured as SAEs. For all other pancreatic AEs (such as idiopathic or asymptomatic pancreatic enzyme abnormalities), the investigator will be responsible for determining the seriousness of the event (AE or SAE) and the relatedness of the event to study drug.

Appendix 7. Protocol Amendment I8F-MC-GPHU (a) Summary
The Impact of Tirzepatide on Gastric Emptying (GE) in Overweight/Obese Non-diabetic Subjects and in Overweight/Obese Subjects with Type 2 Diabetes Mellitus

Overview

Protocol I8F-MC-GPHU 'The Impact of Tirzepatide on Gastric Emptying (GE) in Overweight/Obese Non-diabetic Subjects and in Overweight/Obese Subjects with Type 2 Diabetes Mellitus' has been amended. The new protocol is indicated by Amendment (a) and will be used to conduct the study in place of any preceding version of the protocol. This amendment is considered to be non-substantial since it does not alter the subject safety and the primary and secondary objectives of the study.

The overall changes and rationale for the changes made to this protocol are as follows:

- Lipid panel collection time has been removed from the schedule of activities (Section 2) to avoid information duplication with Appendix 2.
- Footnote attributions for hemoglobin A1c and lipid panel of Appendix 2 have been corrected.
- Blood sampling volume listed in Appendix 6 has been corrected to reflect updates in actual laboratory sampling requirement.
- Minor editorial changes and formatting corrections were made but are not necessarily documented in the revision below.

Revised Protocol Sections

Note:	All deletions have been identified by ~~strikethroughs~~. All additions have been identified by the use of underscore.

2. Schedule of Activities

Study Schedule Protocol I8F-MC-GPHU Screening and Baseline

	Screening	Baseline			Comments
Procedure	D-28 to -4	D-3	D-2	D-1	
…	…	…	…	…	…
Clinical Laboratory Tests	X		X		See Appendix 2 for details. Baseline may be collected anytime from D-3 to D-1 as long as results are reviewed prior to first tirzepatide dosing on D1. Performed by a local laboratory.

CCI

…	…	…	…	…	….
~~Lipid Panel~~	~~X~~				

Study Schedule Protocol I8F-MC-GPHU Treatment and Follow-up

	Treatment and Follow Up														Comments
Procedure	D1	D2	D3	D4	D8 ±1	D15 ±1	D22 ±1	D29 ±1	D35	D36	D37	D38	D43 ±2	FU/ ED[a]	
…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…

CCI

Clinical Laboratory Tests						P		P					X	X	Refer to screening and baseline comments
…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…

4. Objectives and Endpoints

Table GPHU.3. Objectives and Endpoints

Objectives	Endpoints
Exploratory	
CCI	
To evaluate the PD effects of tirzepatide on appetite and weight	Body weight, waist circumference, appetite VAS scores

CCI

Appendix 2 Clinical Laboratory Tests

Safety Laboratory Tests

Hematology[a]	Clinical Chemistry (fasting)[a]
Hematocrit	Sodium
Hemoglobin	Potassium
Erythrocyte count (RBC)	Bicarbonate
Mean cell volume	Chloride
Mean cell hemoglobin	Calcium
Mean cell hemoglobin concentration	Glucose
Leukocytes (WBC)	Blood urea nitrogen
	Total protein
Absolute counts of:	Albumin
Neutrophils	Total bilirubin
Lymphocytes	Alkaline phosphatase
Monocytes	Aspartate aminotransferase
Eosinophils	Alanine aminotransferase
Basophils	Creatinine
Platelets	Amylase
	Lipase
Hemoglobin A1c[a,b]	Uric acid
	Phosphorous
Urinalysis[a]	Calcitonin[c]
	Follicle-stimulating hormone[c]
Specific gravity	Hepatitis B surface antigen[c,d]
pH	Hepatitis C antibody, hepatitis C RNA[c,d]
Protein	HIV[c,d]
Glucose	Pregnancy test[e]
Ketones	
Bilirubin	Lipid Panel (Fasting)[a,e,b]
Urobilinogen	Triglycerides
Blood	Total Cholesterol
Nitrite	LDL
Microscopic examination of sediment[f]	HDL

Abbreviations: HIV = human immunodeficiency virus; RBC = red blood cells; WBC = white blood cells.

[a] Performed by local laboratory. Results will be validated by the laboratory at the time of initial testing.

[b] Performed at Screening, Day -2, Day 29, and FU/ED.

[c] Performed by local laboratory at screening only.

[d] Tests may be waived if they have been performed within 6 months before screening with reports available for review.

[e] Serum pregnancy test will be performed at screening and urine pregnancy tests at subsequent visits.

[f] Test only if dipstick result is abnormal (i.e., positive for blood, protein, or nitrites).

Appendix 5 Blood Sampling Summary

This table summarizes the approximate number of venipunctures and blood volumes for all blood sampling (screening, safety laboratories, and bioanalytical assays) during the study.

Protocol I8F-MC-GPHU Sampling Summary

Purpose	Blood Volume per Sample (mL)	Number of Blood Samples	Total Volume (mL)
Screening tests[a,b]	~~21~~25	1	~~21~~25
Safety laboratory tests[a,b]	~~17~~12.5	5	~~85~~62.5
Hemoglobin A1c[b]	4	4	16
~~Calcitonin[b]~~	~~2~~	~~1~~	~~2~~
Tirzepatide pharmacokinetics[c]	3	19 (+3)	66
Immunogenicity[c]	10	~~7~~4 (+3)	~~100~~70
Acetaminophen pharmacodynamics[c]	~~2~~3	35 (+3)	~~76~~114
6-point plasma glucose[c]	2	6 × 3 occasions	36
Plasma glucose[c]	2	9	18
Pharmacogenetics[c]	10	1	10
Total			~~424~~417.5
Total for clinical purposes			~~423.5~~420

a Additional samples may be drawn if needed for safety purposes. ~~Triglycerides and total cholesterol will be excluded on study days where a lipid panel pharmacodynamics sample is collected.~~ Safety laboratory tests will be inclusive of lipid panels on days when they are performed (refer to Appendix 2)

b Performed on site or in a local laboratory.

c Performed at a central or referral laboratory approved by the sponsor, including for storage

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