Protocol I8F-MC-GPHX (a)

Disposition of [14C]-Tirzepatide Following Subcutaneous Administration in Healthy Male Subjects

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EUDRA CTA: 2019-003664-44

Confidential Information

Tirzepatide (LY3298176)

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Clinical Pharmacology Protocol Electronically Signed and Approved by Lilly on 18-Feb-2020.

Amendment (a) Electronically Signed and Approved by Lilly on approval date provided below.

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1. Protocol Synopsis

Title of Study:
Disposition of [^{14}C]-Tirzepatide Following Subcutaneous Administration in Healthy Male Subjects.

Rationale:
Tirzepatide, a dual agonist of glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) is being developed for the treatment of type 2 diabetes mellitus (T2DM). This study is being conducted to determine the disposition of radioactivity and tirzepatide (LY3298176) in healthy male subjects following a single subcutaneous (SC) injection of approximately 4.1 mg tirzepatide containing approximately 100 µCi [^{14}C]-tirzepatide. The 100 µCi dose of the radiotracer will be administered to each subject to facilitate characterization of physiological disposition and metabolism of tirzepatide.

Objectives/Endpoints:

Objectives	Endpoints
Primary To determine the disposition of radioactivity in healthy male subjects following SC administration of a single dose of approximately 4.1 mg (approximately 100 µCi) [^{14}C]-tirzepatide.	Urinary and fecal excretion of total radioactivity over time expressed as a percentage of the total radioactive dose.
Secondary • To determine the PK of tirzepatide in plasma and total radioactivity in plasma and whole blood. • To assess the mass balance of tirzepatide by quantifying radioactivity recovered in urine, feces, and expired air (if applicable). • To assess the metabolism of tirzepatide in plasma, urine, and feces (if applicable). • To assess the safety and tolerability of a single dose of tirzepatide in healthy male subjects.	• AUC(0-t_{last}), AUC(0-∞), and C_{max} for tirzepatide in plasma and radioactivity in plasma and whole blood. • Total radioactivity recovered in urine, feces, and expired air (if applicable). • Total number of metabolites. • Incidence of AEs.

Abbreviations: AE = adverse event; AUC(0-t_{last}) = area under the concentration-time curve from time 0 to time of the last measurable concentration; AUC(0-∞) = area under the concentration-time curve from time 0 extrapolated to infinity; C_{max} = maximum observed drug concentration; PK = pharmacokinetics; SC = subcutaneous.

Summary of Study Design:
Study I8F-MC-GPHX is an open-label, single-center study to determine the disposition of radioactivity and pharmacokinetics (PK) of tirzepatide in healthy male subjects following a single dose of approximately 4.1 mg tirzepatide containing approximately 100 µCi of [^{14}C]-tirzepatide administered as an SC injection.

Treatment Arms and Planned Duration for an Individual Subject:
Subjects will participate in a screening visit, a single study period, follow-up visit(s), and a follow-up assessment. Subjects will be admitted to the clinical research unit (CRU) on the day prior to dosing (Day -1) and will receive a single subcutaneous dose of [^{14}C]-tirzepatide on Day 1. Subjects will remain resident at the CRU for a minimum of

7 days postdose (Day 8) up to a maximum of 14 days postdose (Day 15). Subjects can be discharged from the CRU at any time after 7 days postdose (Day 8) if both of the following release criteria have been met:

- ≥90% of the administered radioactivity (based on the actual dose) has been recovered.
- <1.0% of the total administered radioactivity is recovered in each combined urine and feces collections from 2 consecutive 24-hour urine and fecal sample collections (where both collections have occurred).

If subjects have not met both release criteria by 14 days postdose (Day 15), they will be required to return to the CRU for up to seven 48-hour residential inpatient follow-up visits. Samples will be collected for PK and the measurement of remaining radioactivity in urine and feces during the inpatient follow-up visits. Subjects will be required to attend all inpatient follow -up visits as scheduled until such time that the second release criterion (<1.0% total radioactivity in excreta) is met or up to a maximum of 63 days postdose (Day 64), whichever occurs first.

A follow-up assessment, conducted by telephone, will be performed 7 ± 2 days after each subject has completed the final study visit or after early discontinuation from the study.

Number of Subjects:
Up to 8 subjects will be enrolled. It is planned that up to 6 subjects will be dosed initially and 2 additional subjects will be dosed if needed, in order that a minimum of 4 subjects complete the study.

Statistical Analysis:
Pharmacokinetic parameter estimates for plasma and whole blood radioactivity, and plasma tirzepatide will be calculated by standard noncompartmental methods of analysis and summarized using descriptive statistics. The whole blood:plasma ratio of total radioactivity will be calculated for each time point. The percent of radiolabeled dose recovered in feces, urine, and expired air (if applicable) will be calculated. Safety assessments will be conducted for all enrolled subjects, whether or not they complete all protocol requirements. These parameters will be listed and summarized using standard descriptive statistics. No formal statistical analyses are planned.

2. Schedule of Activities

Study Schedule Protocol I8F-MC-GPHX

	Screening	Check-in	Study Days		Discharge[a]	Follow-up 48-hour I/P Visits[b]	Follow-up O/P Visit[c]	Follow-up Phone Assessment	Comments
Procedure	Days -28 to -2	Day -1	Day 1	Day 2 up to 14	Up to Day 15 or ED	Days 20 to 22, 27 to 29, 34 to 36, 41 to 43, 48 to 50, 55 to 57 and 62 to 64	Day 29	7 ± 2 days after last visit or ED	
Informed Consent	X								
Subject Admission to CRU		X				Day 20, 27, 34, 41, 48, 55 and 62			Subjects will be resident in the CRU for 48 hours during Follow-up inpatient visits.
Subject Discharge from CRU					X[d]	Day 22, 29, 36, 43, 50, 57 and 64			In case of ED, efforts will be made to perform all discharge procedures.
Follow-up Assessment								X	Follow-up assessment may be conducted by telephone and include recording of AEs and concomitant medication.
[^{14}C]-tirzepatide Subcutaneous Administration			0 hours						Dosing occurs after all predose procedures and predose sample collections are completed.
Medical History	X								
Height	X								
Weight	X					Day 20, 27, 34, 41, 48, 55 and 62	X		
Temperature	X		Predose						
Urine Drug Screen and Alcohol Breath Test	X	X				Day 20, 27, 34, 41, 48, 55 and 62	X		Only the alcohol breath test will be conducted at the follow-up visit(s)
Cotinine Screen	X	X							

	Screening	Check-in	Study Days		Discharge[a]	Follow-up 48-hour I/P Visits[b]	Follow-up O/P Visit[c]	Follow-up Phone Assessment	Comments
Procedure	Days -28 to -2	Day -1	Day 1	Day 2 up to 14	Up to Day 15 or ED	Days 20 to 22, 27 to 29, 34 to 36, 41 to 43, 48 to 50, 55 to 57 and 62 to 64	Day 29	7 ± 2 days after last visit or ED	
Physical Exam/Medical Assessment	X	X			X[d]	Day 20, 27, 34, 41, 48, 55 and 62			After screening, medical assessment only performed to include medical review and targeted examination, as appropriate. At admission to the CRU for any follow-up visits a symptom-directed physical exam will be performed.
Genetic Sample			Predose						Single sample taken prior to dosing on Day 1
Clinical Lab Tests	X	X			X[d]	Day 29 and 57	X		See Appendix 2, Clinical Laboratory Tests, for details.
Vital Signs (supine)	X	X	Predose, 12 hours	24, 48 and 72 hours	X[d]	Day 20, 27, 34, 41, 48, 55 and 62	X		Additional time points may be added, if warranted at the investigator's clinical discretion.
12-Lead ECG	X		Predose,	24 hours	X[d]		X		Single safety ECG will be collected. ECGs must be recorded before collecting any blood samples at each time point. Subjects must be supine for 5 minutes before ECG collection, and remain supine but awake during ECG collection. Additional timepoints may be added, if

	Screening	Check-in	Study Days		Discharge[a]	Follow-up 48-hour I/P Visits[b]	Follow-up O/P Visit[c]	Follow-up Phone Assessment	Comments
Procedure	Days -28 to -2	Day -1	Day 1	Day 2 up to 14	Up to Day 15 or ED	Days 20 to 22, 27 to 29, 34 to 36, 41 to 43, 48 to 50, 55 to 57 and 62 to 64	Day 29	7 ± 2 days after last visit or ED	
									warranted, at the investigator's clinical discretion.
AEs/Concomitant Medications	X	X	X	X	X	X	X	X	Ongoing assessment
Immunogenicity Samples			Predose			Day 29	X		Where applicable, collection times should match with PK sampling time points. In the event of immediate or non-immediate drug hypersensitivity reactions, unscheduled samples will be collected as detailed in Section 9.4.6. Subjects with TE-ADA at follow-up will undergo additional follow-up as detailed in Section 9.7.
Blood Glucose Monitoring[e]			Predose and 12 hours	24, 36, 48 and 72 hours					Performed using a bedside glucose monitor. Additional unscheduled measurements may be taken at the discretion of the investigator where clinically indicated.

	Screening	Check-in	Study Days		Discharge[a]	Follow-up 48-hour I/P Visits[b]	Follow-up O/P Visit[c]	Follow-up Phone Assessment	Comments
Procedure	Days -28 to -2	Day -1	Day 1	Day 2 up to 14	Up to Day 15 or ED	Days 20 to 22, 27 to 29, 34 to 36, 41 to 43, 48 to 50, 55 to 57 and 62 to 64	Day 29	7 ± 2 days after last visit or ED	
PK Samples[e] (tirzepatide and total radioactivity)			Predose and 8 hours	24, 48, 72, 96, 120, 144, 168, 240, and 288 hours	336 hours	Day 22, 29, 36, 43, 50, 57 and 64	X		Sampling times are relative to the time of study treatment administration and are given as targets to be achieved within reasonable limits (±10 minutes for time points from 2 to 24 h; ±1 h for time points later than 24 h). One sample will be collected at each follow-up visit.
Metabolite Profiling Samples			8 hours	24, 48, 72 96, 120, 144, 168, 240, and 288 hours	336 hours				Metabolite profiling samples will be collected separately to PK samples.
Urine Samples[f]			Predose, 0-12 and 12-24 hours	X	X	X (2 x 24-hour collections at each visit)			Samples collected for 24-hour intervals, except as specified on Day 1, until release criteria are met. Urine samples to be collected for the determination of total radioactivity and metabolite profiling (total number and identity) only.

	Screening	Check-in	Study Days		Discharge[a]	Follow-up 48-hour I/P Visits[b]	Follow-up O/P Visit[c]	Follow-up Phone Assessment	Comments
Procedure	Days -28 to -2	Day -1	Day 1	Day 2 up to 14	Up to Day 15 or ED	Days 20 to 22, 27 to 29, 34 to 36, 41 to 43, 48 to 50, 55 to 57 and 62 to 64	Day 29	7 ± 2 days after last visit or ED	
Feces Samples[f]			Predose and 0-24 hours	X	X	X (2 x 24-hour collections at each visit)			Samples collected for 24-hour intervals until release criteria are met. The inability to produce a fecal sample will not be considered a protocol deviation. Feces samples to be collected for the determination of total radioactivity and metabolite profiling (total number and identity) only.
Expired Air Samples			Predose, 2, 4, and 8 hours	X	X				Samples collected for 24-hour intervals, except as specified on Day 1, until release criteria are met.

Abbreviations: AE = adverse event; CRU = clinical research unit; ECG = electrocardiogram; ED = early discontinuation; I/P = inpatient; O/P = outpatient; PK = pharmacokinetics; TE-ADA = treatment-emergent antidrug antibodies.

[a] Subjects may be discharged from the CRU at any time, once the study release criteria have been met, up to a maximum stay of 14 days postdose (Day 15).

[b] If subjects do not meet the release criteria by Day 15 (Section 5.1) they will return for up to seven 48-hour I/P visits until <1.0% of the total administered radioactivity is excreted in 2 consecutive 24-hour urine and fecal sample collections or until a maximum of Day 64.

[c] Immunogenicity and PK samples are required from all subjects on Day 29 (28 days postdose). These samples will be collected during the course of the Day 27 to 29 I/P follow-up visit; any subjects not attending the Day 27 to 29 I/P follow-up visit will alternatively be required to return for an outpatient visit on Day 29 for the collection of these samples.

[d] Only to be performed at the time of discharge or early discontinuation for each subject.

[e] Specified times are approximate, and actual times will be recorded. Actual sampling time should not exceed 1 hour prior to dosing for the predose sample.

[f] Predose urine and feces will be collected as spot/single samples. A urine spot sample will be collected within 3 hours prior to dosing and a single predose fecal sample collected from Day -1 to 0 hours on Day 1.

Note: if multiple procedures take place at the same time point, the following order of the procedure should be use: ECGs, vital signs, PK sample (record of actual PK sampling time is the priority), urine and feces sample for radioactivity, metabolite profiling samples, blood glucose, clinical laboratory sample, immunogenicity and pharmacogenetic sample. Unless otherwise specified, predose procedures and samples may be performed within an hour prior to [^{14}C]-tirzepatide dosing.

3. Introduction

3.1. Study Rationale

Tirzepatide is being developed for the treatment of type 2 diabetes mellitus (T2DM) as an adjunct to diet and exercise. In addition, it is being developed as a therapy for the indications of chronic weight management and nonalcoholic steatohepatitis.

Study I8F-MC-GPHX (GPHX) is being conducted to determine the disposition of radioactivity and pharmacokinetics (PK) of tirzepatide (LY3298176) in healthy male subjects following a single subcutaneous (SC) injection of approximately 4.1 mg tirzepatide containing approximately 100 μCi [^{14}C]-tirzepatide. The 100-μCi dose of the radiotracer will be administered to each subject to facilitate characterization of physiological disposition and metabolism of tirzepatide. The tirzepatide dose is therefore dependent upon the specific activity of the drug substance. At the target drug substance specific activity, the tirzepatide dose will be 4.1 mg. However, based on variability in the drug substance specific activity, the final tirzepatide dose will be approximately 4.1 mg and will be no higher than 5 mg.

3.2. Background

The available preclinical and clinical data indicate that dual-stimulation of glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (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. 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 1 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 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. A few subjects experienced transient elevations in lipase and/or amylase levels, but these episodes were not associated with any relevant clinical outcomes. Once-weekly doses of 1, 5, 10, and 15 mg 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.

Doses above 5 mg of tirzepatide were attained via step-wise dose escalation. Results from the two Phase 2 studies demonstrated that tirzepatide at doses between 5 and 15 mg provided clinically meaningful efficacy in both glucose- and body weight-lowering. Gastrointestinal-related AEs (nausea, diarrhea, vomiting) were the most frequently reported AEs in Phase 2 studies. The majority of the treatment-emergent adverse events (TEAEs) were mild or moderate in severity. There were no other clinically relevant safety observations in the Phase 1 and 2 studies.

Tirzepatide terminal half-life was estimated to be approximately 5 days, with maximum observed drug concentration (C_{max}) occurring 24 to 72 hours postdose. The pharmacokinetics of tirzepatide appears linear across the dose range evaluated (single doses from 0.25 to 8 mg or multiple weekly doses up to 15 mg) previously in clinical studies.

Overall, the safety and tolerability, and PK/pharmacodynamic profiles of tirzepatide support further development of tirzepatide in patients with T2DM. Further details can be found in the Investigator's Brochure (IB).

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, increases in heart rate, and hypoglycemic events (GLP-1 receptor agonist class effect).

No clinically significant safety or tolerability concerns have been identified in subjects to date for a single dose of tirzepatide up to the maximum tolerated dose of 5 mg (dose escalation from 0.25 to 8 mg) or multiple weekly doses when escalated up to 15 mg. Based on this information, the single maximum 5-mg dose to be administered in Study GPHX is reasonably anticipated to be tolerable in this group of healthy subjects.

There is no anticipated therapeutic benefit for the subjects.

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 IB.

4. Objectives and Endpoints

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

Table GPHX.1. Objectives and Endpoints

Objectives	Endpoints
Primary To determine the disposition of radioactivity in healthy male subjects following SC administration of a single dose of approximately 4.1mg (approximately 100 µCi) [^{14}C]-tirzepatide.	Urinary and fecal excretion of total radioactivity over time expressed as a percentage of the total radioactive dose.
Secondary • To determine the PK of tirzepatide in plasma and total radioactivity in plasma and whole blood. • To assess the mass balance of tirzepatide by quantifying radioactivity recovered in urine, feces, and expired air (if applicable). • To assess the metabolism of tirzepatide in plasma, urine, and feces (if applicable). • To assess the safety and tolerability of a single dose of tirzepatide in healthy male subjects.	• $AUC(0\text{-}t_{last})$, $AUC(0\text{-}\infty)$, and C_{max} for tirzepatide in plasma and radioactivity in plasma and whole blood. • Total radioactivity recovered in urine, feces, and expired air (if applicable). • Total number of metabolites. • Incidence of AEs.

Abbreviations: AE = adverse event; $AUC(0\text{-}t_{last})$ = area under the concentration-time curve from time 0 to time of the last measurable concentration; $AUC(0\text{-}\infty)$ = area under the concentration-time curve from time 0 extrapolated to infinity; C_{max} = maximum observed drug concentration; PK = pharmacokinetics; SC = subcutaneous.

5. Study Design

5.1. Overall Design

This is a Phase 1, open-label, single-center study in healthy male subjects following a single dose of approximately 4.1 mg tirzepatide containing approximately 100 μCi of [^{14}C]-tirzepatide administered as an SC injection.

Subjects will participate in a screening visit, a single study period, up to 7 follow-up visits and a follow-up telephone assessment. Subjects will be admitted to the clinical research unit (CRU) on the day prior to dosing (Day -1) and will receive a single SC injection of [^{14}C]-tirzepatide, administered by clinical site staff, on Day 1.

Subjects will remain resident in the CRU for 14 days postdose (Day15), but, may be discharged earlier if both the following release criteria have been met:

- ≥90% of the administered radioactivity (based on the actual dose) has been recovered, AND
- 24-hour urine and fecal samples from 2 consecutive collections (where both collections have occurred) where each combined urine and feces collection has a radioactivity level <1.0% of the total administered radioactivity.

If subjects have not met both release criteria by 14 days postdose (Day 15), they will be required to return to the CRU for up to seven 48-hour residential inpatient follow-up visits. Samples will be collected for PK and the measurement of remaining radioactivity in urine and feces during the inpatient follow-up visits. Subjects will be required to attend all inpatient follow -up visits as scheduled until such time that the second release criterion (<1.0% total radioactivity in excreta) is met or up to a maximum of 63 days postdose (Day 64), whichever occurs first.

An immunogenicity and corresponding PK sample are required from all subjects on Day 29. If subjects have not previously met the release criteria and are returning for the Day 27 to 29 inpatient follow-up visit, then the immunogenicity and PK samples will be collected in the course of this visit. Subjects that have met the release criteria prior to the Day 27 to 29 inpatient visit will be required to attend a single outpatient visit on Day 29 for the collection of PK and immunogenicity samples.

A follow-up assessment will be conducted by telephone and will include recording of AEs and concomitant medication. The follow-up assessment will be performed 7 ± 2 days after each subject has completed the final study visit or following early discontinuation from the study.

Sequential blood samples will be obtained predose and after dose administration to quantify the PK of the total radioactivity in whole blood and plasma, and tirzepatide in plasma. Separate blood samples will also be taken at selected time points for metabolite profiling.

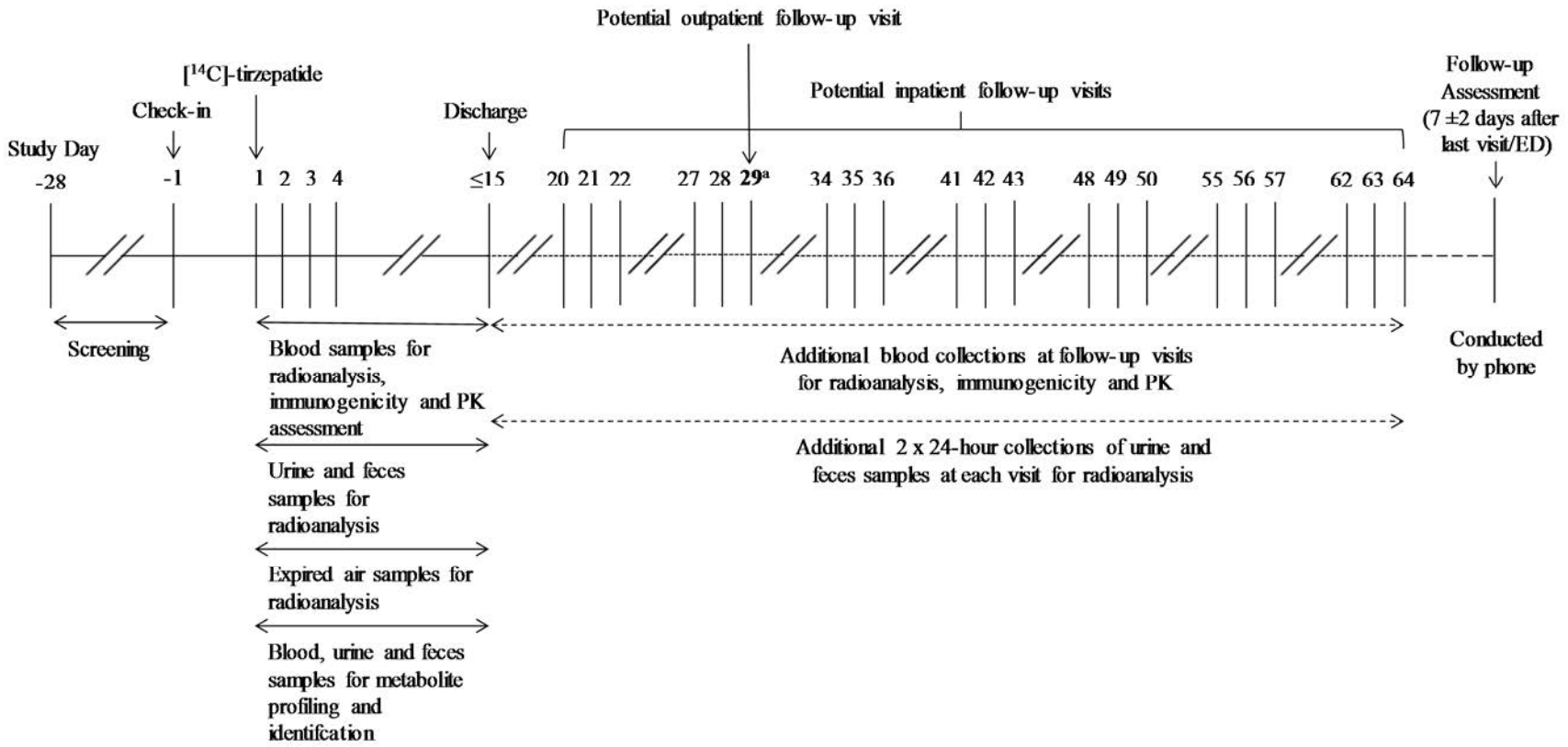
Sequential urine and fecal samples will be obtained to determine the mass balance of tirzepatide by quantification of radioactivity and to identify metabolites. Samples of expired air will also be collected for the analysis of $^{14}CO_2$ at selected time points. If a significant amount of

administered radioactivity is present in expired air samples, these data will be extrapolated to estimate the radioactive dose recovery. The percent of the dose eliminated in excreta will be estimated by measuring the amount of radioactivity in the urine and/or feces for each collection period.

Safety evaluations will include recording of AEs, clinical laboratory tests, glucose monitoring, injection-site and hypersensitivity reactions, vital sign measurements, 12-lead electrocardiograms (ECGs), and physical examinations.

Study governance considerations are described in detail in Appendix 3.

Figure GPHX.1 illustrates the study design.



Abbreviations: ED = early discontinuation; PK = pharmacokinetic

[a] Immunogenicity and PK samples are required from all subjects on Day 29 (28 days postdose). Subjects attending the Day 27 to 29 inpatient visit will have these samples collected during the course of the visit. Subjects not attending the Day 27 to 29 inpatient visit will return to the CRU for a single outpatient visit on Day 29 for the collection of these samples.

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

5.2. Number of Participants

Up to 8 subjects may be enrolled. It is planned that up to 6 subjects will be dosed initially and 2 additional subjects may be dosed if needed, in order that a minimum of 4 subjects complete the study. For purposes of this study, a subject completes the study when all scheduled procedures shown in the Schedule of Activities (Section 2) 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.

5.4. Scientific Rationale for Study Design

This clinical study will be conducted to determine the disposition of radioactivity and PK of tirzepatide in healthy male subjects following a single SC injection of tirzepatide labeled with ^{14}C. The radiolabeled tirzepatide enables identification of circulatory and excretory metabolites and understanding of the clearance pathways.

Conducting the study in healthy subjects mitigates the potential confounding effects of the disease state and of concomitant medications. This study has an open-label design with no control treatment. This type of design is standard and widely used in radiolabeled studies. Subcutaneous administration has been chosen as this is the intended clinical route of tirzepatide administration.

Based on available clinical PK data for tirzepatide and human excretion data from a related GLP-1 analog (Jensen et al., 2017), the duration of clinical confinement will be up to 14 days postdose (Day 15).

5.5. Justification for Dose

Based on data from a quantitative whole-body autoradiography disposition study for tirzepatide in male rats, a whole-body radiation dose in a 70-kg man following administration of a single SC 100-µCi (3.7-MBq) dose of [^{14}C]-tirzepatide was calculated to be 97.7 mrem (0.977 mSv). This effective radiation dose is defined as being within dose limits for members of the public (Category II study; World Health Organization, 1977) with a minor associated risk (risk Category IIa; International Commission on Radiological Protection [ICRP], 1992). Therefore, administration of a single SC 100-µCi (3.7-MBq) dose of [^{14}C]-tirzepatide would not be expected to represent a significant radiation exposure risk in man.

The specific activity of [^{14}C]-tirzepatide is approximately 20-24 µCi/mg. A dose of tirzepatide that can provide a radioactive dose of 100 µCi per subject, is considered sufficient to facilitate characterization of the physiological disposition and metabolism of tirzepatide whilst complying with the “as low as (is) reasonably achievable” (ALARA) principle prescribed by the ICRP. Hence a tirzepatide dose of approximately 4.1 mg has been selected for this study.

Data from Phase 1 study GPGA has shown that a 5-mg dose of tirzepatide was well tolerated by healthy subjects and patients with T2DM, and is also planned as one of the doses to be

investigated in Phase 3 studies. Doses higher than 5 mg were achieved via escalation since a 5 mg dose was considered the MTD when administered as a single dose.

The study will be submitted for approval by the Administration of Radioactive Substances Advisory Committee.

6. Study Population

Eligibility of subjects for the study will be based on the results of screening medical history, physical examination, vital signs, clinical laboratory tests, and ECGs.

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 27 days prior to check-in. Subjects who are not enrolled within 27 days of screening may be subjected to an additional medical assessment and/or clinical measurements 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 and/or check-in:

[1] are overtly healthy males, as determined by medical history and physical examination

[1a] male subjects, regardless of their fertility status, with non-pregnant women of childbearing potential 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 at least 90 days following administration of the investigational product (IP). A full list of permitted highly effective and effective methods of contraception is presented in Appendix 7.

[1b] male subjects 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.

[1c] periodic abstinence, declaration of abstinence just for the duration of a trial, and withdrawal are not acceptable methods of contraception.

[1d] male subjects with pregnant partners should use condoms during intercourse for the duration of the study and for at least 90 days after dosing.

[1e] male subjects should refrain from sperm and blood donation for the duration of the study and for at least 90 days after dosing.

[1f] male subjects who are in exclusively same-sex relationships (as their preferred and usual lifestyle) are not required to use contraception.

[2] are 30 to 55 years old, inclusive, at the time of screening.

[3] have a body mass index (BMI) of 18.0 to 32.0 kg/m^2, inclusive, at screening.

[4] have clinical laboratory test results within normal reference range for the population or investigative site, or results with acceptable deviations that are judged to be not clinically significant by the investigator.

[5] have venous access sufficient to allow for blood sampling as per the protocol.

[6] are willing to receive study treatment by SC injections.

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

[8] are able and willing to give signed informed consent.

6.2. Exclusion Criteria

Subjects will be excluded from study enrollment if they meet any of the following criteria at screening and/or check-in:

[9] are investigative site personnel directly affiliated with this study and their immediate families. Immediate family is defined as a spouse, biological or legal guardian, child, or sibling.

[10] are Lilly or Covance employees.

[11] 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.

[12] have participated, within the last 3 months, in a clinical study involving an IP. If the previous IP has a long half-life, 5 half-lives or 3 months (whichever is longer) should have passed, prior to dosing on Day 1.

[13] have previously completed or withdrawn from this study or any other study investigating tirzepatide, and have previously received the IP.

[14] have had any exposure to tirzepatide, other GLP-1 analogs, or other related compounds within the prior 3 months, or any history ever of allergies to these medications.

[15] have any abnormality in the 12-lead ECG at screening that, in the opinion of the investigator, increases the risks associated with participating in the study.

[16] have an abnormal blood pressure and/or pulse rate as determined by the investigator at screening or check-in.

[17] have significant history of or current cardiovascular, respiratory, hepatic, renal, gastrointestinal, endocrine, hematological, or neurological disorders capable of significantly altering the absorption, metabolism, or elimination of drugs; of constituting a risk when taking the IP; or of interfering with the interpretation of data.

[18] have evidence of significant active neuropsychiatric disease, as determined by the investigator.

[19] have a positive alcohol breath test result or positive urine drug screen (confirmed by repeat) at screening or check-in.

[20] show evidence of human immunodeficiency virus (HIV) infection and/or positive human HIV antibodies.

[21] show evidence of hepatitis C and/or positive hepatitis C antibody.

[22] show evidence of hepatitis B and/or positive hepatitis B surface antigen.

[23] have used or plan to use over-the-counter or prescription medication, and/or herbal supplements (with the exception of vitamin/mineral supplements and/or thyroid replacement therapy) within 14 days prior to dosing and for the duration of the study, including any medications that reduce GI motility, including, but not limited to, anticholinergics, antispasmodics, 5-hydroxytryptamine-3 receptor antagonists, dopamine antagonists, and opiates.

[24] have donated blood of more than 500 mL within the previous 3 months of study screening.

[25] have an average weekly alcohol intake that exceeds 21 units per week, or are unwilling to stop alcohol consumption from 36 hours prior to check-in and while resident in the CRU (1 unit = 1/2 pint or 284 mL of beer; 25 mL of distilled spirits; 3 units = 250 mL of wine).

[26] are unwilling to refrain from consuming caffeine- or xanthine-containing food and drink from 48 hours prior to admission and while resident in the CRU.

[27] are currently or have been smokers, users of tobacco, users of nicotine replacement products, or users of any vaping/e-cigarette devices within the 3 months prior to admission and/or have positive cotinine at screening or check-in.

[28] have had exposure to significant diagnostic, therapeutic, or employment-related radiation within 12 months prior to dosing (e.g., serial x-ray or computed tomography scans, barium meal, current employment in a job requiring radiation exposure monitoring).

[29] have a history of constipation or have had acute constipation within 3 weeks prior to check-in.

[30] have evidence of active renal disease (e.g., diabetic renal disease, polycystic kidney disease) or an estimated creatinine clearance of <80 mL/minute, calculated using the Chronic Kidney Disease-Epidemiology.

[31] have participated in any clinical trial involving a radiolabeled IP within 12 months prior to check-in.

[32] have a history or presence of pancreatitis (history of chronic pancreatitis or idiopathic acute pancreatitis), elevation in serum amylase or lipase (>1.5-fold the upper limit of normal [ULN]).

[33] have a GI disorder (e.g., relevant esophageal reflux or gall bladder disease), or any GI disease which impacts gastric emptying (e.g., gastric bypass surgery, pyloric stenosis [with the exception of appendectomy]) or could be aggravated by GLP-analogs. Subjects with dyslipidemia and subjects who had cholecystolithiasis (with removal of gallstones) and/or cholecystectomy (removal of the gall bladder) in the past, with no further sequelae, may be included in the study, at the discretion of the investigator.

[34] have a history of atopy or clinically significant multiple or severe drug allergies, or severe post-treatment hypersensitivity reactions (including, but not limited to, erythema multiforme major, linear immunoglobulin A dermatosis, toxic epidermal necrolysis, or exfoliative dermatitis).

[35] have a personal or family history of medullary thyroid carcinoma or have multiple endocrine neoplasia syndrome type 2, or a screening calcitonin ≥20 pg/mL at screening.

[36] have Gilbert's syndrome.

[37] have serum aspartate aminotransferase (AST) or alanine aminotransferase (ALT) >2 × ULN or total bilirubin (TBL) >1.5 × ULN at screening.

[38] have a history of malignancy within 5 years prior to screening.

[39] have a triglyceride ≥5 mmol/L (442.5 mg/dL) at screening.

[40] in the opinion of the investigator or sponsor, are unsuitable for inclusion in the study.

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 being administered the SC dose of tirzepatide, and when clinical laboratory test samples are taken (Schedule of Activities; Section 2). A meal will be offered to study subjects at approximately 2 hours postdose. During inpatient stays, subjects will receive a standardized, high-fiber diet at scheduled times that do not conflict with other study-related activities. Prune juice may be administered on an as-needed

basis to aid in normal bowel function and will not be considered a concomitant medication. Water may be consumed freely.

6.3.2. Caffeine, Alcohol, and Tobacco

- Caffeine - Subjects will refrain from consuming caffeine- or xanthine-containing food and drink from 36 hours prior to admission until discharge from the CRU.
- Alcohol - Subjects will not consume alcohol for 36 hours prior to admission until discharge from the CRU and will abide by study restrictions (i.e. no more than 3 units of alcohol per day - see Exclusion Criteria [25] for unit definition) at other times throughout the study.
- Tobacco - Subjects will not be permitted to smoke, use tobacco, or use products containing nicotine within 3 months prior to admission until final discharge from the study.

6.3.3. Activity

No strenuous physical activity will be allowed for 48 hours prior to dosing until discharge from the CRU, and 24 hours prior to each follow-up visit (inpatient and outpatient).

6.4. Screen Failures

Individuals who do not meet the criteria for participation in this study (screen failure) may not be re-screened.

7. Treatment

7.1. Treatment Administered

After an overnight fast of at least 8 hours, IP is to be administered subcutaneously to each subject by injection into the abdomen. The IP will consist of [^{14}C]-tirzepatide to provide approximately 4.1 mg tirzepatide with approximately 100 µCi of radioactivity in each unit dose. The proposed [^{14}C]-tirzepatide IP will be manufactured extemporaneously as sterile solutions for SC injection, as described in the Pharmacy Protocol approved by the sponsor. All doses will be administered by clinical site staff. The study regimen is indicated in Table GPHX.2.

Prior to injection, the investigator or designee will clean the subject's skin and the injection will be administered to the lower abdominal quadrant, approximately 5 cm from the umbilicus (i.e., left or right lower quadrant). Detailed instructions for use will be provided by the sponsor. A limited number of clinical site staff will perform SC administration for consistency reasons.

Each dose syringe will be retained for analysis of residual radioactivity. Any unused assembled unit doses will be retained until completion of the study.

Table GPHX.2. Treatments Administered

Treatment Name	[^{14}C]-tirzepatide
Dosage Formulation	Solution
Unit Dose Strength/Dosage Level	Approx. 4.1 mg tirzepatide (~100 µCi)/1 mL
Route of Administration	SC injection
Delivery Method	Fixed Needle Syringe

Abbreviations: Approx. = approximately; SC = subcutaneous.

The investigator or designee is responsible for:

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

7.1.1. Packaging and Labeling

[^{14}C]-tirzepatide will be supplied by the sponsor or its designee in accordance with current good manufacturing practice, labeled according to the country's regulatory requirements.

7.2. Method of Treatment Assignment

All subjects will receive the same treatment; the study will not be subject to randomization.

7.2.1. Selection and Timing of Dose

The IP will be administered on the morning of Day 1 after a minimum 8-hour fast. The actual time of dose administration will be recorded in the subject's electronic case report form (eCRF).

7.3. Blinding

This study is open-label.

7.4. Dose Modification

Dose modification is not permitted in this study.

7.4.1. Study Release Criteria

Subjects will remain at the CRU until they have met the specified release criteria (Section 5.1) or up to a maximum of 14 days postdose (Day 15). In no case will subjects be retained in CRU longer than the maximum duration of 14 days postdose but may be required to return for up to 7 inpatient follow-up visits each consisting of a 48-hour residential stay in the CRU, if release criteria have not been met by Day 15 (14 days postdose). Subjects will attend inpatient follow-up visit(s) until the second release criterion (i.e., < 1% radioactivity in the excreta for two 24-hour collections) it met or up to a maximum of 63 days postdose (Day 64). A PK and an immunogenicity sample are required from all subjects on Day 29, subjects not attending an inpatient follow-up visit at this time will be required to return for a single outpatient visit on Day 29 for the collection of these samples.

7.5. Preparation/Handling/Storage/Accountability

The [^{14}C]-tirzepatide solution for administration will be prepared in the CRU by qualified pharmacy staff, details of dose preparation will be provided in the Pharmacy Protocol.

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 subjects enrolled in the study may receive IP or study materials, and only authorized site staff may administer the 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, institution, or the head of the medical institution (where applicable) is responsible for study treatment accountability, reconciliation, and record maintenance (such as receipt, reconciliation, and final disposition records).

7.5.1. Safety Procedure in the Event of a Retrospective Positive Sterility Finding from Extemporaneously Prepared Study Treatment

If a positive sterility finding were to arise in the sterile filtered product, the subjects who were dosed from the impacted batch should be immediately contacted and asked to return to the CRU for a full medical examination. This should include a physical examination, including blood pressure, pulse rate, and body temperature. Blood samples should be collected for culture and assayed for inflammatory markers such as C-reactive protein and elevations in white blood cell counts.

If the signs and symptoms indicate a subject is suffering from possible infection(s), they will be clinically managed, treated, and followed up until resolution. Any AEs will be recorded as appropriate.

7.6. Treatment Compliance

The IP will be administered at the clinical site, and documentation of treatment administration will occur at the site.

7.7. Concomitant Therapy

In general, concomitant medication should be avoided; however, acetaminophen (maximum 3 g/24 hours) may be administered at the discretion of the investigator for treatment of headaches etc. If the need for concomitant medication (other than acetaminophen) arises, inclusion or continuation of the subject may be at the discretion of the investigator after consultation with a Lilly CP, CRP or designee. Any medication used during the course of the study must be documented.

7.8. Treatment after the End of the Study

Not applicable.

8. Discontinuation Criteria

8.1. Discontinuation from Study Treatment

Subjects discontinuing from the study prematurely for any reason must complete AE and follow-up procedures per Section 2 of this protocol.

8.1.1. 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 CP/CRP and the investigator to determine if the patient may continue in the study. If both agree it is medically appropriate to continue, the investigator must obtain documented approval from the Lilly CP/CRP to allow the inadvertently enrolled subject to continue in the study.

8.2. Discontinuation from the Study

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 (GCP). The study will be halted, and the risk to other potential subjects evaluated if any of the following criteria are met:
    - if serious adverse reaction (i.e. an SAE considered at least possibly related to [^{14}C]-tirzepatide administration) occur in 1 (or more) subject;
    - severe non-serious adverse reactions (ie severe non-serious AE considered as, at least possibly related to [^{14}C]-tirzepatide administration) in 2 subjects, independent of within or not within the same system organ class.
- Investigator Decision
    - the investigator decides that the subject should be discontinued from the study.
- Subject Decision
    - the subject 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 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 clinical laboratory tests that will be performed for this study.

Appendix 5 provides a summary of the approximate number and volume of invasive samples, for all blood 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 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 must notify the sponsor in writing via a file note to facilitate data reconciliation.

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 informed consent form 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, study device, 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, study device 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.

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 patient or may require intervention to prevent one of the other outcomes listed in the definition above.

Study site personnel must alert the Lilly CP/CRP, 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 subject 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 of a female partner (paternal exposure to IP) does not meet the definition of an AE. 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. Complaint Handling

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

9.3. Treatment of Overdose

For the purposes of this study, an overdose of tirzepatide is considered any dose higher than the planned study dose.

There is no specific antidote for tirzepatide. In the event of an overdose, the subject should receive appropriate supportive care and any AEs should be documented.

Refer to the IB.

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).

9.4.1.1. Amylase and Lipase Measurements

Serum amylase and lipase measurements will be collected as part of the clinical laboratory testing and as specified in the Schedule of Activities (Section 2). Additional measurements may be performed at the investigator's discretion. Further diagnostic assessments will be recommended as per the algorithm (refer to Appendix 6) for the monitoring of pancreatic events whenever lipase and/or amylase is confirmed to be ≥3 × ULN at any visit postdose, even if the subject is asymptomatic.

9.4.2. Glucose Monitoring

For safety purposes, blood glucose measurements will be performed using a bedside glucose monitor as specified in the Schedule of Activities (Section 2). Additional blood glucose monitor measurements may also be taken during the study as deemed necessary by the investigator where clinically indicated.

9.4.2.1. Hypoglycemia

Site personnel will collect information on episodes of hypoglycemia at each study visit according to the Schedule of Activities. Subjects will be trained by site personnel about signs and symptoms of hypoglycemia, how to treat hypoglycemia, and how to collect appropriate

information for each episode of hypoglycemia. Site personnel will enter this information into the eCRF at each visit.

Investigators should use the following definitions and criteria when diagnosing and categorizing an episode considered to be related to hypoglycemia (the plasma glucose [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) (ADA 2019).

Glucose Alert Value (Level 1):

- **Documented symptomatic hypoglycemia** is defined as any time a patient 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 patient feels that he or 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 (BG) 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 eCRF and report it to Lilly as an SAE (see Section 9.2.1 for details regarding SAE reporting).

To avoid duplicate reporting, all consecutive PG 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 by the investigator. The subject should receive additional education, if deemed appropriate.

9.4.3. Vital Signs

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

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

Unscheduled orthostatic vital signs should be assessed, if possible, during any AE of dizziness or posture-induced symptoms. Additional vital signs may be measured, if warranted, at the clinical discretion of the investigator.

9.4.4. Electrocardiograms

For each subject, ECGs should be collected according to the Schedule of Activities (Section 2).

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

For each subject, a single 12-lead digital ECGs will be collected according to the Schedule of Activities. Electrocardiograms must be recorded before collecting any blood samples. Subjects must be supine for approximately 5 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 physician (the investigator 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 AE.

Additional ECGs may be measured, if warranted, at the clinical discretion of the investigator.

9.4.5. 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.

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).

Investigational 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 in a standardized manner for record-keeping purposes; however, the photographs will not be used to evaluate the severity of the injection-site reaction.

9.4.6. Hypersensitivity Reactions

All hypersensitivity reactions will be reported by the investigator as either AEs or, if any serious criterion is met, as SAEs.

In the event of suspected drug hypersensitivity reactions (immediate or non-immediate) in subjects who experience moderate-to-severe injection reactions as assessed by the investigator, unscheduled blood samples will be collected for PK and antidrug antibody analyses at the following time points:

- as close as possible to the onset of the event
- at the resolution of the event
- 30 (±3) days following the event.

Additionally, unscheduled serum samples for immune safety laboratory testing (including, but not limited to β tryptase, total immunoglobulin E, complement and cytokine panel testing) should also be collected at approximately 60 to 120 minutes and 4 to 6 weeks after the onset of the event in these subjects.

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.

9.4.7. Safety Monitoring

The Lilly CP/CRP will monitor safety data throughout the course of the study.

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

- trends in safety data
- laboratory analytes including glucose, amylase, and lipase
- adverse events.

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

9.4.7.1. Hepatic Safety

9.4.7.1.1. Close hepatic monitoring

If a subject who had normal or near normal baseline ALT, AST, alkaline phosphatase (ALP), TBL (i.e., <1.5x ULN), experiences elevated ALT≥3x ULN, AST≥3x ULN, ALP≥2x ULN, or TBL≥2x, laboratory tests (Appendix 4) should be repeated within 48 to 72 hours, including ALT, AST, ALP, TBL, direct bilirubin, gamma-glutamyltransferase, and creatinine phosphokinase to confirm the abnormality and to determine if it is increasing or decreasing.

In subjects enrolled with elevated baseline ALT, AST, ALP or TBL (≥1.5x ULN), the thresholds for close monitoring are ALT≥2x baseline, AST≥2x baseline, ALP≥2x baseline, or TBL≥2x baseline. 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 medical monitor. 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 subject's clinical condition and hepatic biochemical tests. Subsequently, the frequency of monitoring may be lowered to once every 1 to 2 weeks, if the subject'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.

9.4.7.1.2. Comprehensive hepatic evaluation

If a study subject, who had baseline ALT, AST, ALP, TBL<1.5x ULN, experiences elevated ALT≥5x ULN, AST≥5x ULN, ALP ≥3x ULN, TBL ≥2x ULN, or elevated ALT, AST ≥3x ULN with hepatic signs/symptoms (severe fatigue, nausea, vomiting, right upper quadrant abdominal pain, fever, rash, and/or eosinophilia>5%), a comprehensive evaluation should be performed to search for possible causes of liver injury.

In subjects who had elevated baseline ALT, AST, ALP, or TBL (≥1.5x ULN), the thresholds for performing this evaluation are ALT≥3x baseline, AST≥3x baseline, ALP≥2x baseline, TBL≥1.5x baseline, or ALT, AST ≥2x baseline with hepatic signs/symptoms.

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, viral hepatitis A, B, C, E, tests for autoimmune hepatitis, and an abdominal imaging study (for example, ultrasound or computed tomography [CT] scan).

Based on the patient's history and initial results, further testing should be considered, in consultation with the Lilly-designated medical monitor, including tests for hepatitis D virus, cytomegalovirus, Epstein-Barr virus, acetaminophen levels, acetaminophen protein adducts, urine toxicology screen, Wilson's disease, blood alcohol levels, urinary ethylglucuronide, and serum phosphatidylethanol. Based on the circumstances and the investigator's assessment of the subject's clinical condition, the investigator should consider referring the subject for a hepatologist/ gastroenterologist consultation, magnetic resonance cholangio-pancreatography, endospopic retrograde cholangio-pancreatography, cardiac echocardiogram, or a liver biopsy.

9.4.7.1.3. Additional hepatic data collection (hepatic safety eCRF) in subjects who have abnormal liver tests during the study

Additional hepatic safety data collection (hepatic safety eCRF) should be performed in subjects who meet 1 or more of the following 5 conditions:

1. Elevation of serum ALT to ≥5× ULN on 2 or more consecutive blood tests (if baseline ALT<1.5x ULN)
    - In subjects with baseline ALT≥1.5x ULN, the threshold is ALT≥3x baseline on 2 or more consecutive tests
2. Elevated TBL to ≥2× ULN (if baseline TBL <1.5x ULN)
    - In subjects with baseline TBL ≥1.5x ULN, the threshold should be TBL ≥2x baseline
3. Elevation of serum ALP to ≥2× ULN on 2 or more consecutive blood tests (if baseline ALP <1.5x ULN)
    - In subjects with baseline ALP≥1.5x ULN, the threshold is ALP ≥2x baseline on 2 or more consecutive blood tests
4. Hepatic event considered to be an SAE

5. Discontinuation of the IP due to a hepatic event

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

9.5. Pharmacokinetics

9.5.1. Whole Blood and Plasma Samples

At the visits and times specified in the Schedule of Activities (Section 2), venous blood samples (approximate volumes listed in Appendix 5) will be collected to determine the whole blood and plasma concentrations of total radioactivity, the plasma concentrations of tirzepatide, and metabolite profiling (total number and identity) of tirzepatide. A maximum of 3 samples may be collected at additional time points during the study if warranted and agreed upon between both the investigator and sponsor, this excludes any additional samples required for safety monitoring. Instructions for the collection and handling of blood samples will be provided by the sponsor in a Laboratory Manual. The actual date and time (24-hour clock time) of each sampling will be recorded.

Plasma samples for metabolite profiling will be stored frozen pending confirmation from the sponsor of which samples are to be analyzed.

9.5.2. Urine and Feces Sampling

Urine and feces samples will be collected for the determination of total radioactivity and metabolite profiling (total number and identity) only. Urine and feces will be collected before IP administration (control samples); however, the inability to produce a fecal sample will not be considered a protocol deviation. After dosing with IP, cumulative feces and urine samples will be collected in specified containers according to the Schedule of Activities (Section 2) until the specified release criteria have been met.

Urine will be collected at the specified intervals, into 1 or more containers (depending on volume excreted), according to the Schedule of Activities (Section 2) until the specified release criteria have been met. An aliquot of each sample will be analyzed to yield the percentage radioactivity recovered within that interval as well as to determine its metabolic profile.

Feces from each bowel movement will be collected and the time of collection will be noted. Fecal samples will be pooled over each 24-hour collection period (if applicable), see the Schedule of Activities (Section 2), and analyzed to yield the percentage radioactivity recovered over that period as well as to determine its metabolic profile.

9.5.3. Expired Air Samples

A sample of expired breath will be collected for analysis of $^{14}CO_2$ at the times indicated in the Schedule of Activities (Section 2). If a significant amount of the radioactivity is present in breath samples, the raw data will be extrapolated to provide an estimate of the percentage dose eliminated via $^{14}CO_2$. Additional breath samples may be collected at some or all of the times that blood samples are drawn and only analyzed if needed.

9.5.4. Bioanalysis

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

Concentrations of tirzepatide will be assayed using a validated liquid chromatography tandem mass spectrometry method. Metabolite identification will be determined using appropriate techniques such as liquid chromatography (LC)/radiodetection and LC/mass spectroscopy.

Whole blood, plasma, expired air, urine, and fecal concentrations of radioactivity will be determined using liquid scintillation counting techniques. Whole blood and feces will be combusted prior to the liquid scintillation counting.

Bioanalytical samples collected during radiolabeled studies 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 additional metabolism and/or protein binding work.

9.6. Pharmacodynamics

Not applicable.

9.7. Immunogenicity Assessments

At the times specified in the Schedule of Activities (Section 2), venous blood samples will be collected and stored for potential future analysis to determine antibody production against tirzepatide. Antibodies may be further characterized for cross-reactive binding to native GIP and GLP-1 and their ability to neutralize the activity of tirzepatide. To interpret the results of immunogenicity, a venous blood sample will be collected at the same time points to determine the plasma concentrations of tirzepatide. All samples for immunogenicity should be taken predose when applicable and possible. In the event of drug hypersensitivity reactions, unscheduled blood samples will be collected as specified in Section 9.4.7.

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

9.8. Genetics

A blood sample will be collected for pharmacogenetic analysis as specified in the Schedule of Activities, 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 it 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.

9.9. Biomarkers

Not applicable.

9.10. Health Economics

This section is not applicable for this study.

10. Statistical Considerations and Data Analysis

10.1. Sample Size Determination

Up to 8 subjects will be enrolled. It is planned that up to 6 subjects will be dosed initially and 2 additional subjects will be dosed if needed, in order that a minimum of 4 subjects complete the study.

The sample size is customary for [^{14}C]-disposition studies (Penner et al. 2009) and is chosen to provide adequate PK data while limiting the number of subjects exposed to radiopharmaceuticals in non-therapeutic research.

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.

10.2.2. Study Participant Characteristics

The subject's age, sex, weight, height, BMI, race, and other demographic characteristics will be recorded and summarized using descriptive statistics.

10.3. Statistical Analyses

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

Pharmacokinetic analyses will be conducted on data from all subjects who receive the IP and have evaluable PK.

Safety summaries will be conducted for all enrolled subjects, 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 to avoid issues with post-hoc analyses and incomplete disclosures of analyses.

10.3.1. Safety Analyses

10.3.1.1. Clinical Evaluation of Safety

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

The incidence of symptoms for each treatment will be presented by severity and by association with IP as perceived by the investigator. Symptoms 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 summarized include incidence of AEs, safety lab parameters (including BG, amylase, and lipase) and vital signs. The parameters will be listed, and summarized using standard descriptive statistics. Additional analysis will be performed if warranted upon review of the data.

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

10.3.1.3. Injection-Site Reactions

Incidence of erythema, induration, pain, itching, and edema will be listed and summarized.

Additional analyses may be performed, if appropriate.

10.3.2. Pharmacokinetic Analyses

10.3.2.1. Pharmacokinetic Parameter Estimation

Pharmacokinetic parameter estimates for plasma and whole blood total radioactivity, and plasma tirzepatide will be calculated by standard noncompartmental methods of analysis and summarized using standard descriptive statistics.

The primary PK parameters for analysis will be C_{max}, area under the concentration-time curve (AUC) from time 0 to time of the last measurable concentration (AUC[0-t_{last}]) and AUC from time 0 extrapolated to infinity (AUC[0-∞]). Other noncompartmental parameters, such as time to C_{max} (t_{max}), half-life associated with the terminal rate constant in noncompartmental analysis ($t_{1/2}$), apparent clearance (CL/F), and apparent volume of distribution (V/F) may be reported. The whole blood:plasma ratio of total radioactivity will be calculated for each time point.

The percent of radiolabeled dose recovered in feces, urine, and expired air (if applicable) will be calculated.

10.3.2.2. Pharmacokinetic Statistical Inference

No formal statistical analyses are planned.

10.3.3. Interim Analyses

No interim analyses are planned for this study. If an unplanned interim analysis is deemed necessary, the Lilly CP, CRP, 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 patient 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	alanine aminotransferase
assent	Agreement from a child or other individual who is not legally capable of providing consent, but who can understand the circumstances and potential risks involved in participating in a study (required by some institutional review boards [IRBs]/ethical review boards [ERBs]).
AST	aspartate aminotransferase
AUC	area under the concentration-time curve
AUC0-∞	AUC from time 0 extrapolated to infinity
AUC0-t_{last}	AUC from time 0 to time of the last measurable concentration
BG	blood glucose
BMI	body mass index
CIOMS	Council for International Organizations of Medical Sciences
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.

CRU	clinical research unit
CT	computerised tomography
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
GCP	good clinical practice
GI	gastrointestinal
GIP	glucose-dependent insulinotropic polypeptide
GLP-1	glucagon-like peptide-1
IB	Investigator's Brochure
ICH	International Council for Harmonization
ICRP	International Commission on Radiological Protection
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.
open-label	A study in which there are no restrictions on knowledge of treatment allocation, therefore the investigator and the study subjects are aware of the drug therapy received during the study.
PG	plasma glucose
PK	pharmacokinetic(s)
randomize	the process of assigning subjects/patients to an experimental group on a random basis

SAE	serious adverse event
SC	subcutaneous
screen	The act of determining if an individual meets minimum requirements to become part of a pool of potential candidates for participation in a clinical study.
SUSARs	suspected unexpected serious adverse reactions
T2DM	type 2 diabetes mellitus
TBL	total bilirubin
TE-ADA	treatment-emergent antidrug antibodies
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 last measurable concentration
ULN	upper limit of normal

Appendix 2. Clinical Laboratory Tests

Safety Laboratory Tests

Hematology	Clinical Chemistry
Hematocrit	Sodium
Hemoglobin	Potassium
Erythrocyte count (RBC)	Bicarbonate
Mean cell volume	Chloride
Mean cell hemoglobin	Calcium
Mean cell hemoglobin concentration	Glucose (fasting)
Leukocytes (WBC)	Blood urea nitrogen (BUN)
	Total protein
Absolute counts of:	Albumin
Neutrophils	Total bilirubin
Lymphocytes	Alkaline phosphatase (ALP)
Monocytes	Aspartate aminotransferase (AST)
Eosinophils	Alanine aminotransferase (ALT)
Basophils	Creatinine
Platelets	Amylase
	Lipase
	Triglycerides
	Calcitonin[a]
Urinalysis	
Specific gravity	
pH	Urine drug screen[b]
Protein	Cotinine (urine)
Glucose	Ethanol testing (breath test)[b]
Ketones	Hepatitis B surface antigen[a]
Bilirubin	Hepatitis C antibody[a]
Urobilinogen	HIV[a]
Blood	
Nitrite	
Leukocytes (WBC)	
Microscopic examination of sediment[c]	

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

[a] Performed at screening only.

[b] Urine drug screen and ethanol level may be repeated prior to admission to the clinical research unit and at other times indicated in the Schedule of Activities.

[c] Test only if dipstick result is abnormal (i.e., positive for blood, protein, or nitrites) if clinically indicated, per investigator discretion.

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 or legal representative. 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 investigational product.
- 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 subject or the subject's legal representative 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 ERB was properly constituted and convened as required by International Council for Harmonization (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. Lilly or its representatives must approve the ICF before it is used at the investigative site. 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

Selected tests may be obtained in the event of a treatment-emergent hepatic abnormality and may be required in follow-up with subjects in consultation with Lilly or its designee CP/CRP.

Hepatic Monitoring Tests

Hepatic Hematology[a]	**Haptoglobin[a]**
Hemoglobin	
Hematocrit	**Hepatic Coagulation[a]**
RBC	Prothrombin Time
WBC	Prothrombin Time, INR
Neutrophils	
Lymphocytes	**Hepatic Serologies[a,b]**
Monocytes	Hepatitis A antibody, total
Eosinophils	Hepatitis A antibody, IgM
Basophils	Hepatitis B surface antigen
Platelets	Hepatitis B surface antibody
	Hepatitis B core antibody
Hepatic Chemistry[a]	Hepatitis C antibody
Total bilirubin	Hepatitis E antibody, IgG
Conjugated bilirubin	Hepatitis E antibody, IgM
Alkaline phosphatase	
ALT	**Anti-nuclear antibody[a]**
AST	**Alkaline phosphatase isoenzymes[a]**
GGT	**Anti-smooth muscle antibody (or anti-actin antibody)[a]**
CPK	

Abbreviations: ALT = alanine aminotransferase; AST = aspartate aminotransferase; CPK = creatinine phosphokinase; GGT = gamma-glutamyl transferase; Ig = immunoglobulin; INR = international normalized ratio; RBC = red blood cells; WBC = white blood cells.

[a] Assayed by Lilly-designated or local laboratory.

[b] Reflex/confirmation dependent on regulatory requirements and/or testing availability.

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-GPHX Sampling Summary

Purpose	Blood Volume per Sample (mL)	Number of Blood Samples	Total Volume (mL)
Screening tests (clinical laboratory test and serology)[a]	11	1	11
Clinical laboratory tests[a]	7.5	4	30
Pharmacokinetics (tirzepatide and total radioactivity)	8	19 (+3)	176
Metabolite profiling	20	11	220
Immunogenicity (storage only)	10	2	20
Pharmacogenetics (stored sample)	10	1	10
Total			467
Total for clinical purposes rounded up to nearest 10 mL			470

[a] Additional samples may be drawn if needed for safety purposes.

If extra blood samples are required, the maximum blood volume withdrawn per subject during the study will not exceed that donated during a standard blood donation (approximately 500 mL).

Appendix 6. Pancreatic Monitoring

Glucagon-like peptide-1 agonists have been associated with a possible risk of acute pancreatitis. In 2006, the United States prescribing information for exenatide was revised to include the event of pancreatitis. In 2007, the United States prescribing information for this medication was amended to include pancreatitis under "Precautions." Epidemiologic studies have indicated that there is an increased incidence and prevalence of pancreatitis in persons with T2DM.

To enhance understanding of the natural variability of pancreatic enzymes in the T2DM population and, in order to assess for any potential effects of tirzepatide on the exocrine pancreas, amylase and lipase values will be monitored in all current and future clinical trials with tirzepatide.

Additional monitoring will be requested for amylase and/or lipase values ≥3× ULN at any visit, even in asymptomatic subjects (see figure below). Lipase and amylase values may also be obtained at any time during the clinical trials for any subject suspected of having symptoms suggestive of pancreatitis (such as severe GI signs and/or symptoms), at the investigator's discretion.

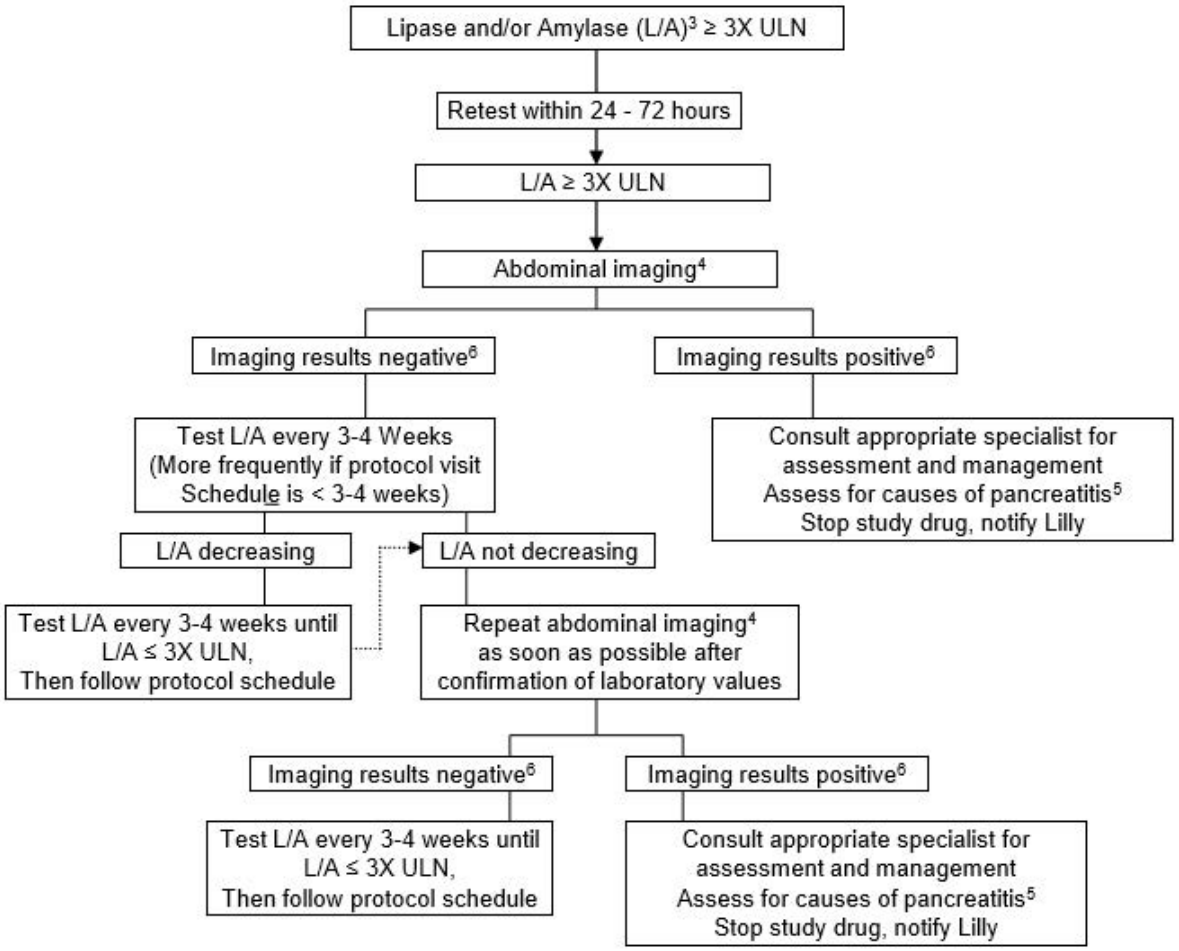
Acute pancreatitis is an AE defined as an acute inflammatory process of the pancreas that may also involve peripancreatic tissues and/or remote organ systems. The diagnosis of acute pancreatitis requires 2 of the following 3 features:

- abdominal pain characteristic of acute pancreatitis
- serum amylase and/or lipase ≥3× ULN
- characteristic findings of acute pancreatitis on CT scan or magnetic resonance imaging

Most subjects with acute pancreatitis experience abdominal pain that is located generally in the epigastrium, and radiates to the back in approximately one-half of the cases. The pain is often associated with nausea and vomiting. However, experience with GLP-1 agonists has demonstrated that some subjects asymptomatic for classic pancreatitis may demonstrate significant elevations of lipase and/or amylase. For subjects considered by investigators to be asymptomatic for pancreatitis, but whose value(s) for lipase and/or amylase are ≥3× ULN, an algorithm is in place to follow these subjects safely and to quickly reach (or not reach) a diagnosis of pancreatitis.

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 investigational product.

Appendix 7. Classification of Contraceptive Methods

Highly Effective (Less Than 1% Failure Rate) Methods of Contraception:

- Combined oral contraceptive pill and mini pill
- NuvaRing
- Implantable contraceptives
- Injectable contraceptives (such as Depo-Provera®)
- Intrauterine device (such as Mirena® and ParaGard®)
- Contraceptive patch – females ONLY <198 pounds or 90 kg
- Total abstinence or in a same-sex relationship (if this is their preferred and usual lifestyle). Note: periodic abstinence (for example, calendar, ovulation, symptothermal, postovulation methods), declaration of abstinence just for the duration of a trial, and withdrawal are not acceptable methods of contraception
- Vasectomy – for males in clinical trials

Effective Methods of Contraception (Must Use Combination of 2 Methods):

- Male condom with spermicide*
- Female condom with spermicide*
- Diaphragm with spermicide
- Cervical sponge
- Cervical cap with spermicide

* the use of male and female condoms as a double-barrier method is not considered acceptable.

Appendix 8. Protocol Amendment I8F-MC-GPHX (a) Summary Disposition of [^{14}C]-Tirzepatide Following Subcutaneous Administration in Healthy Male Subjects

Overview

Protocol I8F-MC-GPHX 'Disposition of [^{14}C]-Tirzepatide Following Subcutaneous Administration in Healthy Male Subjects' 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 not considered to be substantial.

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

- Clarified and expanded on further details in Section 8 on discontinuation criteria in response to the recommendations provide by the Medicines and Healthcare Products Regulatory Agency of the United Kingdom.
- 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.

8.2 Discontinuation from the Study

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 (GCP). The study will be halted, and the risk to other potential subjects evaluated if any of the following criteria are met:
    - if serious adverse reaction (i.e. an SAE considered at least possibly related to [^{14}C]-tirzepatide administration) occur in 1 (or more) subject;
    - severe non-serious adverse reactions (ie severe non-serious AE considered as, at least possibly related to [^{14}C]-tirzepatide administration) in 2 subjects, independent of within or not within the same system organ class.
- Investigator Decision
    - the investigator decides that the subject should be discontinued from the study.
- Subject Decision
    - the subject requests to be withdrawn from the study.

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