Statistical Analysis Plan Version 3 I8F-MC-GPGT

The Effect of Tirzepatide on α and β Cell Function and Insulin Sensitivity in Patients with Type 2 Diabetes Mellitus

NCT03951753

Approval Date: 27-May-2021

1. Statistical Analysis Plan
I8F-MC-GPGT: The Effect of Tirzepatide on α and β Cell Function and Insulin Sensitivity in Patients with Type 2 Diabetes Mellitus

LY3298176 for Type 2 Diabetes Mellitus

Phase 1, multicenter, randomized, sponsor, investigator, and patient blind, parallel arm study in patients with Type 2 Diabetes to compare tirzepatide 15-mg dose to placebo and semaglutide 1-mg dose (active control).

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Protocol I8F-MC-GPGT
Phase 1

Statistical Analysis Plan electronically signed and approved by Lilly on date provided below.

Approval Date: 27-May-2021 GMT

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3. Revision History

Statistical analysis plan (SAP) Version 1 was approved prior to the first data transfer.

The SAP Version 2 was approved before the database lock. The overall changes made in SAP Version 2 are as follows:

- add derivation and analyses details for pharmacodynamic (PD) parameters
- update and clarify PD parameters and its description
- clarify population for analyses
- specify adverse events of special interest (AESI) in Appendix 1
- add definitions of treatment exposure and compliance
- editorial changes and formatting corrections

This is SAP Version 3. The third version is approved before the primary database lock. The overall changes made in this SAP are as follows:

- update and clarify population for analyses
- clarify treatment compliance calculation taking into account early termination
- clarify and add details in secondary and exploratory PD analyses
- update details in summary tables and listings for safety analyses
- add algorithm details on lab measurement when below or above the quantification limit
- editorial changes and formatting corrections

4. Study Objectives

Table GPGT.4.1 shows the objectives and endpoints of the study.

Table GPGT.4.1. Objectives and Endpoints

Objectives	Endpoints
Primary To compare the effect of tirzepatide 15 mg dose and placebo on the total cDI after 28 weeks of treatment, including the dose escalation phase, in T2DM patients on metformin.	• The change from baseline in total cDI (clamp disposition index)
Secondary To compare the effects of tirzepatide 15 mg relative to placebo (except for the primary efficacy measure) and semaglutide 1 mg after 28 weeks of treatment on:	
• Insulin secretion and insulin sensitivity combined outcome	• The change from baseline in total cDI (for tirzepatide versus semaglutide comparison only)
• Glucose control	• The change from baseline in fasting and postmeal glucose (total and incremental $AUC_{0\text{-}240min}$) during sMMTT • The change from baseline in hemoglobin A1c
• Insulin secretion	• The change from baseline in the first phase incremental $ISR_{0\text{-}8min}$ (i.e., first phase insulin secretion rate) from hyperglycemic clamp • The change from baseline in second phase total $ISR_{20\text{-}120min}$ (i.e., second phase insulin secretion rate) from hyperglycemic clamp • The change from baseline in total $ISR_{0\text{-}120min}$ (i.e., total insulin secretion rate) from hyperglycemic clamp • The change from baseline in insulin response to arginine (incremental $insulin_{arginine0\text{-}10min}$ and incremental $insulin_{arginine0\text{-}30min}$) from hyperglycemic clamp • The change from baseline in β cell GS (i.e., glucose sensitivity) from hyperglycemic clamp • The change from baseline in β cell GS from sMMTT • The change from baseline in ISRg (ISR at fixed glucose concentration) from sMMTT
• Insulin sensitivity	• The change from baseline in hyperinsulinemic euglycemic clamp M-value
• Glucagon secretion	• The change from baseline in glucagon concentration at fasting and postmeal during sMMTT (total and incremental $AUC_{0\text{-}240min}$)
• Appetite and food intake	• The change from baseline in food intake during ad libitum meal served buffet style

Objectives and Endpoints

Objectives	Endpoints
Exploratory To compare the effects of tirzepatide 15 mg relative to placebo and semaglutide 1 mg after 28 weeks of treatment on:	
• Glucose metabolism and turnover in adipose tissue	• The change from baseline in glucose concentration and blood flow in adipose tissue during hyperglycemic clamp (microdialysis$_{0\text{-}120min}$) • The change from baseline in glucose concentration and blood flow in adipose tissue during sMMTT (microdialysis$_{0\text{-}240min}$)
• Insulin secretion	• The change from baseline in basal ISR from hyperglycemic clamp • The change from baseline in basal insulin concentration (basal insulin$_{[-10\text{-}0min]}$) from hyperglycemic clamp • The change from baseline in the first phase insulin response (incremental insulin AUC$_{0\text{-}10min}$) from hyperglycemic clamp • The change from baseline in the second phase insulin response (total insulin AUC$_{20\text{-}120min}$) from hyperglycemic clamp • The change from baseline in steady-state ISR (total ISR$_{80\text{-}120min}$) from hyperglycemic clamp • The change from baseline in total insulin response (total insulin AUC$_{0\text{-}120min}$) from hyperglycemic clamp • The change from baseline in basal ISR (basal ISR at 0 min) prior to sMMTT • The change from baseline in total ISR (total ISR AUC$_{0\text{-}240min}$) from sMMTT • The change from baseline in ISRgb (ISRg [ISR at fixed glucose concentration] adjusted for basal potentiation) from sMMTT • The change from baseline in RS (rate sensitivity) from sMMTT • The change from baseline in PFR (potentiation ratio) from sMMTT • The change from baseline in ICLb (basal insulin clearance) prior to sMMTT • The change from baseline in ICLm (insulin clearance during sMMTT) • The change from baseline in ratio of GS from sMMTT and from hyperglycemic clamp • The change from baseline in fasting proinsulin to insulin ratio

	• The change from baseline in II_{30min} (insulinogenic index at 30 minutes) during the sMMTT • The change from baseline in fasting and postmeal insulin (total and incremental $AUC_{0-240min}$) during the sMMTT • The change from baseline in fasting and postmeal C-peptide (total and incremental $AUC_{0-240min}$) during the sMMTT
Exploratory (continued) • Insulin sensitivity	• The change from baseline in hyperinsulinemic euglycemic M/I value (M-value divided by total insulin concentration) • The change from baseline in HOMA2-IR • The change from baseline in postprandial insulin sensitivity indices (Matsuda, OGIS, Stumvoll indices) from sMMTT
• Glucagon secretion and PP secretion	• The change from baseline in total glucagon $AUC_{0-120min}$ during hyperglycemic clamp • The change from baseline in incremental glucagon concentration after arginine stimulation (incremental $glucagon_{arginine0-30min}$) from hyperglycemic clamp • The change from baseline in glucagon/insulin ratio at fasting and during sMMTT (ratio of total glucagon $AUC_{0-240min}$ and total insulin $AUC_{0-240min}$) • The change from baseline in PP concentration at fasting and postmeal (total and incremental $AUC_{0-240min}$) during sMMTT
• Appetite (VAS) and food intake	• The change in fasting appetite (VAS) • The change in appetite (VAS) score during sMMTT
• Lipid metabolism and turnover in adipose tissue	• The change from baseline in blood samples (triglycerides, β-hydroxybutyrate, pyruvate, lactate, FFA, and glycerol) and adipose tissue (pyruvate, lactate, and glycerol [$microdialysis_{0-120min}$]) lipid parameters and blood flow during hyperglycemic clamp • The change from baseline in blood samples (triglycerides, β-hydroxybutyrate, pyruvate, lactate, FFA, glycerol, ApoB-48, ApoB-100, ApoC-III, LPL) and adipose tissue (pyruvate, lactate, and glycerol [$microdialysis_{0-240min}$]) lipid parameters and blood flow at fasting and during sMMTT • The change from baseline in fasting concentration of leptin, adiponectin, IGFBP 1 and 2

Objectives and Endpoints

Objectives	Endpoints
Exploratory (continued) • Body composition	• The change from baseline in lean body mass (actual value and %) • The change from baseline in body fat mass (actual value and %) • The change from baseline in body weight • The change from baseline in waist circumference
• Energy expenditure and substrate utilization	• The change from baseline in resting metabolic rate (indirect calorimetry) • The change from baseline in respiratory quotient, diet-induced thermogenesis, carbohydrate and fat oxidation rates before (-120 and -30 minutes) and 60, 120, 180, and 240 minutes after the start of sMMTT (indirect calorimetry)
• Safety and tolerability	• Adverse events • Safety laboratory parameters • Incidence of hypoglycemia

Abbreviations: ApoB-48 = apolipoprotein B-48; ApoB-100 = apolipoprotein B-100; ApoC-III = apolipoprotein C-III; AUC = area under the concentration versus time curve; $AUC_{0\text{-}10min}$ = first phase insulin response; $AUC_{0\text{-}120min}$ = AUC from time zero to 120 minutes after start of the meal; $AUC_{0\text{-}240min}$ = AUC from time zero to 240 minutes after start of the meal; $AUC_{20\text{-}120min}$ = second phase insulin response; $AUC_{arginine0\text{-}10min}$ = AUC in response to arginine from time zero to 10 minutes; $AUC_{arginine0\text{-}30min}$ = AUC in response to arginine from time zero to 30 minutes; basal $insulin_{\_10\text{-}0min}$ = average insulin concentration between -10 minutes and 0 minutes; cDI = clamp disposition index; FFA = free fatty acid; GS = glucose sensitivity; HOMA2 = Homeostatic Model Assessment of Insulin Resistance; HOMA2-IR = insulin resistance as measured by the HOMA2 method; ICLb = basal insulin clearance; ICLm = insulin clearance during sMMTT; IGFBP = insulin-like growth factor binding protein; II_{30min}= insulinogenic index at 30 minutes; ISI = insulin sensitivity index; ISR = insulin secretion rate; $ISR_{\_10\text{-}0min}$ = basal insulin secretion rate; $ISR_{0\text{-}8min}$ = first phase insulin secretion rate; $ISR_{0\text{-}120min}$ = total insulin secretion rate from hyperglycemic clamp; $ISR_{20\text{-}120min}$ = second phase insulin secretion rate; $ISR_{0\text{-}240min}$ = total insulin secretion rate during sMMTT; $ISR_{80\text{-}120min}$ = steady-state insulin secretion rate; ISRg = ISR at fixed glucose concentration; ISRgb = ISR at fixed glucose concentration adjusted for basal potentiation; LPL = lipoprotein lipase; $microdialysis_{0\text{-}120min}$ = microdialysis from zero to 120 minutes; $microdialysis_{0\text{-}240min}$ = microdialysis from zero to 240 minutes; OGIS = Oral Glucose Insulin Sensitivity Index; PFR = potentiation ratio; PP = pancreatic polypeptide; RS = rate sensitivity; sMMTT = standardized mixed-meal tolerance test; T2DM = type 2 diabetes mellitus; VAS = visual analog scale.

5. Study Design

5.1. Study Design and Treatment

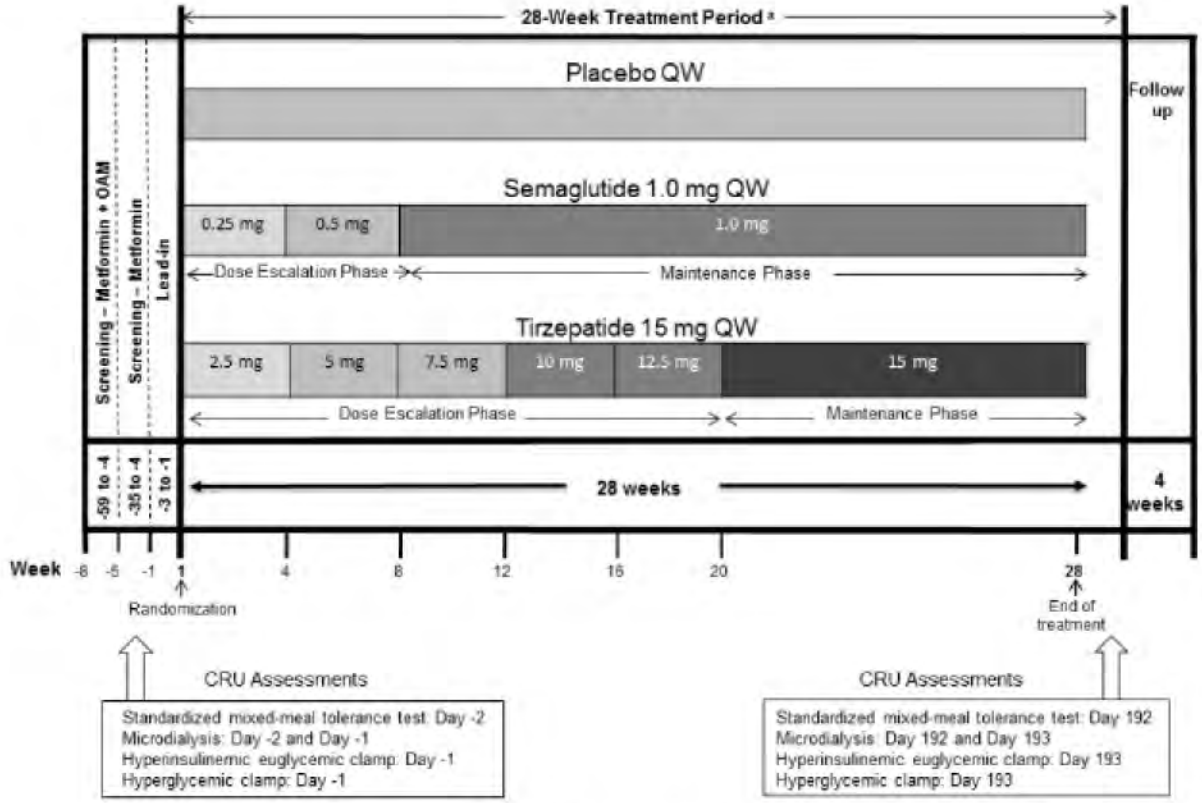
This is a Phase 1, multicenter, randomized, sponsor-, investigator-, and patient-blind, parallel-arm study in patients with type 2 diabetes mellitus (T2DM) to compare the effect of tirzepatide 15-mg dose and placebo on total clamp disposition index (cDI) after 28 weeks of treatment, including the dose-escalation phase. The study will also compare the effects of tirzepatide 15 mg relative to placebo and semaglutide (active control) on additional parameters of pancreatic α and β cell function, insulin sensitivity, glucose and lipid metabolism, and energy balance (food intake and energy expenditure). Ozempic® (semaglutide) will be used as an active control for PD of GLP-1 pharmacology to investigate potential differences in the mechanisms of action between a dual GIPRA/GLP-1RA and a selective GLP-1RA.

The study will consist of 3 periods: an approximately 5- to 8-week screening/lead-in period, a 28-week treatment period, and a 4-week safety follow-up period. Patients will be randomized in a 3:3:2 ratio to once-weekly (QW) tirzepatide 15 mg, semaglutide 1 mg, or placebo. Active treatments will be initially escalated with lower doses to reduce the risk of gastrointestinal (GI) adverse events (AEs). Prior to randomization and approximately 1 to 3 days after the last dose of study drug, the patients will be admitted to the clinical research unit (CRU) to perform planned baseline and end-of-treatment inpatient study procedures, respectively. After the completion of the treatment period, a follow-up visit will be performed 4 weeks later to assess patient safety.

Figure GPGT.5.1 illustrates the study design.

5.2. Determination of Sample Size

Approximately 117 patients are planned to be randomized so that at least 99 patients complete the clamp procedures, assuming 15% discontinuation rate. The 117 patients will be randomized in a 3:3:2 ratio to QW tirzepatide 15 mg, semaglutide 1 mg, or placebo. To power the study, simulated data from pharmacokinetic/pharmacodynamics (PK/PD) models were used. The log-scale variability of cDI is estimated to be 0.586 from the model and previous trials and the log-scale treatment difference is estimated to be 0.913 (tirzepatide - placebo) and 0.417 (tirzepatide - semaglutide). This provides over 90% power to show greater cDI for tirzepatide relative to placebo and 85% power for the comparison of tirzepatide versus semaglutide.



Abbreviations: CRU = clinical research unit; OAM = oral antihyperglycemia medication; QW = once weekly.

[a] Patients who are being treated with other OAMs, in addition to metformin, should discontinue these other OAMs after their eligibility is established, and should complete a 4-week washout period. Once the 4-week washout period is completed, patients can be enrolled in the study. Screening for patients who need to undergo washout of an additional OAM may last from Day -59 to Day -4, inclusive. This period includes the required 4-week washout. Screening procedures may be performed before washout from Day -59 to Day -31.

Figure GPGT.5.1. Illustration of study design for I8F-MC-GPGT.

6. A Priori Statistical Methods

6.1. Analysis Populations

For the purpose of analysis, Table GPGT.6.1 defines 3 analysis sets.

Table GPGT.6.1. Analysis Populations/Data Sets

Population	Definition
All randomized population	All patients who are randomly assigned to a treatment arm.
Safety population	All randomized patients who are exposed to at least 1 dose of the IP (tirzepatide, semaglutide, or placebo), regardless of whether they completed all protocol requirements.
PD analysis set	Data from all randomized patients who received at least 1 dose of the IP and have evaluable PD data*.

Abbreviations: ICF = informed consent form; IP = investigational product; PD = pharmacodynamic.

* Pharmacodynamic data may not be evaluable, for example due to missing data and incorrect execution of planned study procedures (which may produce protocol deviations). Protocol deviations that (1) wrong insulin concentration was prepared for infusion or breach in insulin infusion for hyperinsulinemic euglycemic clamp procedure (2) incorrect amount of glucose bolus infused at pre-hyperglycemic clamp are considered for their severity/impact and taken into consideration whether subjects should be excluded from PD analysis set for hyperinsulinemic euglycemic clamp or hyperglycemic clamp data related analyses due to unevaluable data.

6.2. General Considerations

Statistical analysis of this study will be the responsibility of Eli Lilly and Company (Lilly) or its designee. Any change to the statistical methods described in the protocol will require a protocol amendment only if it changes a principal feature of the protocol. Any other change to the statistical analyses and the justification for the change will be documented in the SAP or clinical study report. Some analyses and summaries described in this analysis plan may not be conducted if not warranted by data. Additional analyses of data may be conducted as deemed appropriate without further changes made to the protocol or SAP.

Unless otherwise specified, safety analyses will be conducted on safety population (Table GPGT.6.1) and PD analyses will be conducted on PD analysis set (Table GPGT.6.1). Data will be censored once patients discontinue their randomized treatment or initiate additional rescue treatment, in which case they will also be discontinued from the study.

Unless otherwise specified, baseline is defined as the last scheduled non-missing measurement collected during Visit 1 (screening) and Visit 2 (including lead-in) before first dose.

Unless otherwise noted, all tests of treatment effects will be conducted at a 2-sided alpha level of 0.05, and the confidence interval (CI) will be calculated at 95%, 2-sided.

6.3. Patient Characteristics

The following patient baseline demographic and clinical characteristics will be summarized for the safety population by study treatment (including overall):

- age (years)
- age group (<65, ≥65 years)
- sex (male, female)
- race
- ethnicity (Hispanic or Latino, Not Hispanic or Latino)
- body weight (kg)
- height (cm)
- body mass index (BMI) (kg/m^2)
- BMI groups (<30, ≥30 to <35, ≥35 kg/m^2) and (<27, ≥27 kg/m^2)
- duration of diabetes (years)
- hemoglobin A1c (HbA1c) (% and mmol/mol)
- substance use (alcohol, caffeine, tobacco, nicotine replacement therapy) (never, current or former)

6.4. Patient Disposition

A listing of patient disposition for the randomized population will be provided. Patient disposition will be summarized by treatment.

A listing of patients who discontinue from the study for any reason for the randomized population will be provided, and the extent of their participation in the study will be reported. If a reason for their discontinuation from study is known, it will be reported. The listing will also include age, sex, and race.

6.5. Concomitant Therapy

Concomitant medication will be listed, and summarized by treatment and World Health Organization Drug Dictionary Preferred Term for the safety population.

A listing of rescue medication for hyperglycemia will be provided.

6.6. Important Protocol Deviations

Important protocol deviations (IPDs) are defined as deviations from the study protocol that may significantly compromise the data integrity and/or patients' safety. The details of identification of IPDs is provided in a separate document (i.e., the trial issue management plan). A listing/table of IPDs will be provided as deemed appropriate.

6.7. Treatment Exposure and Compliance

6.7.1. Treatment Exposure

The duration of exposure to study treatment (tirzepatide, semaglutide, or placebo) is defined as:

Date of last dose of study treatment - date of first dose of study treatment +7 days

The duration of exposure to study treatment will be summarized by treatment using the safety population. The number and percentage of patients with an extent of exposure within the following exposure ranges for the 28-week treatment period will be summarized by treatment:

- 0 weeks, >0 to ≤1 week, >1 to ≤2 weeks, >2 to ≤3 weeks, >3 to ≤4 weeks, ……, >26 to ≤27 weeks, and >27 to ≤28 weeks…

The study drug exposure (actual dose taken) will be summarized for each week by treatment.

6.7.2. Treatment Compliance

Treatment compliance will be assessed using the safety population. Treatment compliance for each patient will be listed, including the percent compliance for each 4-week interval and the percent compliance for 28-week treatment period taking into account early termination. Treatment compliance will be summarized by treatment.

The percent compliance will be calculated as:

$$(Number\ of\ injections\ administered\ [regardless\ of\ actual\ dose\ in\ mg\ administered] \div total\ number\ of\ injections\ expected\ to\ be\ administered) \times 100$$

The percent compliance for each 4-week interval will be calculated. Overall treatment compliance is defined as patients taking ≥75% of their scheduled doses for each 4-week interval during the 28-week treatment period taking into account early termination (specifically excluding the period after early termination).

The total percent compliance for the entire 28-week treatment period will also be calculated taking into account early termination.

When assessing treatment compliance, the missed doses and interrupted doses will be taken into consideration as described in protocol (Section 7.2.1 Selection and Timing of Doses and Section 8.1.2 Temporary Interruption of Study Drug).

6.8. Pharmacokinetic Analyses

6.8.1. Pharmacokinetic Parameter Estimation

Sparse PK samples will be collected across the 28-week treatment duration. Tirzepatide concentrations will be determined to support an understanding of tirzepatide exposure over the treatment duration to compare with expected tirzepatide PK.

6.8.2. Pharmacokinetic Statistical Inference

No summaries and statistical analyses of PK parameters are planned.

6.9. Pharmacodynamic Analyses

6.9.1. Pharmacodynamic Parameter Estimation

In this study, PD measures are used to assess mechanisms of action of study treatments on insulin secretion, insulin sensitivity, glucose control, glucagon and pancreatic polypeptide (PP) secretion, appetite and food intake, glucose and lipid metabolism and turnover in adipose tissue, body composition, energy expenditure, and substrate utilization. For this study, PD measures are also considered as efficacy measures. The planned assessments will be performed at baseline (lead in) and postbaseline (including at the end of the treatment period at 28 weeks) (see Table GPGT.6.2 for details).

Table GPGT.6.2. A Summary of Pharmacodynamic Assessment-Related Study Procedures

Day -3 (Lead in) Day 191 (Week 28)	Day -2 (Lead in) Day 192 (Week 28)	Day -1 (Lead in) Day 193 (Week 28)
• Body composition • Appetite VAS at fasting • Ad libitum food intake test (test meal, lunch)	• During sMMTT (mixed meal) o Appetite VAS o Indirect calorimetry o Microdialysis	• Hyperinsulinemic euglycemic clamp (morning) • During hyperglycemic clamp (afternoon) o Microdialysis

Abbreviations: HbA1c = hemoglobin A1c; sMMTT = standardized mixed-meal tolerance test; VAS = visual analog scale.

* Appetite VAS at fasting is administered additionally on Day 29 (Week 5), Day 57 (Week 8), Day 85 (Week 13), Day 113 (Week 17), Day 141 (Week 21), Day 169 (Week 25), and safety follow-up visit. Ad libitum food intake (test meal) is provided at noon and will be conducted additionally on Day 50 (Week 8) and Day 106 (Week 16). HbA1c is collected on Day -3 (lead in), Day 85 (Week 13), Day 141 (Week 21), and Day 191 (Week 28).

Pharmacodynamic measurements collection

Hyperinsulinemic euglycemic clamp (Day -1, Day 193)

Note: Hyperinsulinemic euglycemic clamp technique is used to assess insulin sensitivity (DeFronzo et al. 1979). It is aimed to maintain glucose level close to the predefined target 5.5 mmol/L (100 mg/dL) during a constant insulin infusion rate (IIR) of 80 mU/min/m^2, by means of variable glucose infusion rate (GIR), to reach the steady-state conditions. The varying GIR reflects the PD (or glucodynamic) effects of the insulin. The steady-state is defined as stable GIR under stable IIR over a period of time (aimed at 150 to 180 minutes).

Blood sampling

- Blood glucose: intensive measurement (almost every minute from ClampArt)
- Insulin (serum), C-peptide (serum), and glucagon (plasma)
  - at 10 time points: -20, -10, 0, 60, 90, 120, 150, 160, 170, 180 minutes

Note: Super GL Glucose Analyzer is used for safety glucose monitoring and for verification of ClampArt blood glucose measurements to secure there is no need for recalibration/adjustment. Before each clamp, the study site calibrates ClampArt versus Super GL.

Hyperglycemic clamp (Day -1, Day 193)

Note: Hyperglycemic clamp is used to assess α cell function and β cell function. It is a validated method in which the GIR is needed to maintain glucose level at predetermined target 12 mmol/L (216 mg/dL). It is used to determine islet β cell glucose sensitivity and insulin secretion capacity at various stages of the glucose exposure and after arginine stimulation under hyperglycemic conditions (Shah et al. 2016; Hannon et al. 2018). At nominal time 0 minute, hyperglycemic clamp starts. At 120 minutes, 5-g arginine bolus will be administered by intravenous (IV) injection over 30 seconds. At 150 minutes, hyperglycemic clamp ends.

Blood sampling during hyperglycemic clamp

- Blood glucose: intensive measurement (almost every minute from ClampArt)
- Glucagon (plasma), insulin (serum), and C-peptide (serum):
  - at 23 time points: -20, -10, 0, 2, 4, 6, 8, 10, 20, 30, 60, 90, 100, 110, 120 (immediately prior to IV injection of arginine), 122, 123, 124, 125, 130, 135, 140, and 150 minutes
- β-hydroxybutyrate (serum), pyruvate (plasma), lactate (lactic acid; plasma), free fatty acid (FFA) (serum), glycerol (plasma), and triglycerides (serum):
  - at 6 time points, every 30 minutes: 0, 30, 60, 90, 120, 150 minutes

Note: Super GL Glucose Analyzer is used for safety glucose monitoring and for verification of ClampArt blood glucose measurements to secure there is no need for recalibration/adjustment. Before each clamp, the study site calibrates ClampArt versus Super GL. Pre hyperglycemic clamp blood glucose at -20, -10, and 0 minutes are measured by Super GL Glucose Analyzer.

Microdialysis (adipose tissue) during hyperglycemic clamp

Note: Microdialysis is a minimally-invasive procedure to measure concentration of substances in the extracellular fluid of a tissue (Felländer et al. 1996). It is considered as a standard technique to investigate adipose tissue physiology and the effects of interventions on subcutaneous abdominal lipolysis (Arner et al. 1988; Coppack et al. 1996).

Dialysate

- Glucose, pyruvate, lactate (lactic acid), glycerol, and ethanol:
  - at 6 time points, every 30 minutes: 0 (pre clamp), 30, 60, 90, 120, 150 minutes

During sMMTT (Day -2, Day 192)

Note: The key objectives of the standardized mixed-meal tolerance test (sMMTT) are to assess α and β cell function, insulin sensitivity, nutrient utilization and metabolic flexibility under physiological conditions. A fixed nutrient ratio solid mixed meal will be served and consumed by the patient, preferably within 15 minutes (meal starts at nominal time 0 minute and stops at

15 minutes). The standardized meal will be individualized for each patient with a fixed nutrients ratio. The sMMTT will account for 35% of the total energy intake at the respective day.

Blood sampling during sMMTT

- Blood glucose (Super GL Glucose Analyzer):
  - at 11 time points: -10, -1, 15, 30, 60 (immediately before start of indirect calorimetry), 90, 120, 150, 180, 210, 240 minutes
- Insulin (serum) and C-peptide (serum):
  - at 9 time points: -10, -1, 15, 30, 60, 90, 120, 180, 240 minutes
- Glucagon (plasma), PP (plasma), β-hydroxy-butyrate (serum), pyruvate (plasma), lactate (lactic acid; plasma), FFA (serum), and glycerol (plasma):
  - at 6 time points: -1, 60, 90, 120, 180, 240 minutes
- Lipid panel (serum):
  - including triglycerides, total cholesterol, low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C), high-density lipoprotein cholesterol (HDL-C), apolipoprotein B-48 (ApoB-48; serum), apolipoprotein B-100 (ApoB-100; serum), apolipoprotein C-III (ApoC-III; serum), and lipoprotein lipase (LPL; serum):
    - at 6 time points: -1, 60, 90, 120, 180, 240 minutes
- Leptin (serum), adiponectin, insulin-like growth factor binding protein (IGFBP) 1 and 2 (serum) and IGFBP 2 (serum), proinsulin (plasma) sample:
  - at 0 minute (pre meal)

Microdialysis (adipose tissue) during sMMTT

Dialysate

- Glucose, pyruvate, lactate (lactic acid), glycerol:
  - at 7 time points: -60, -30, -1, 60 (immediately before start of indirect calorimetry), 120, 180, 240 minutes
- Ethanol:
  - at 4 time points, every 60 minutes: 60 (immediately before start of indirect calorimetry), 120, 180, 240 minutes

Perfusate solution

- Ethanol: 1 sample

Indirect calorimetry during sMMTT

- Energy expenditure (EE), respiratory quotient (RQ), carbohydrate oxidation rate, and fat oxidation rate are collected
  - at 6 time points: -120, -30, 60, 120, 180, 240 minutes
  - The calorimetry device records 1 measurement every 4 to 5 seconds, and each assessment last 20 minutes. The mean for each 20-minute interval will be calculated and will represent the measurement at above 6 time points for further area under the concentration versus time curve (AUC) calculation.

- The mean of measurements at -120 minutes and -30 minutes will be used as measurements at 0 minutes for AUC calculation.

Note: The EE [kcal/day], RQ [ratio], carbohydrate, and fat oxidation rates [g/min] will be provided to statisticians/programmers for analyses. The study site used the formulas below for calculation:

- EE [kcal/day] = (3.9 × VO2+ 1.1 × VCO2) × 1440min
- RQ [ratio] = VCO2 / VO2

where kcal = kilocalorie, VCO2 = volume of carbon dioxide produced [L/min] and VO2 = volume of oxygen consumed [L/min].

Appetite VAS during sMMTT

- Visual analog scale (VAS; at 5 time points): -1, 60, 120, 180, 240 minutes

Other PD measurements

Body composition

- Body mass, lean body mass (fat-free body mass), and body fat mass from plethysmography
  - on Day -3 (lead in) and Day 191 (Week 28)
- body weight (scale) and waist circumference
  - on screening, Day -3 (lead in), Day 29 (Week 5), Day 57 (Week 9), Day 85 (Week 13), Day 113 (Week 17), Day 141 (Week 21), Day 169 (Week 25), Day 191 (Week 28), and safety follow-up visit

Appetite VAS at fasting

In addition to appetite VAS during sMMTT (Day -2 and Day 192 at different time points), appetite VAS at fasting are collected on:

- Day -3 (lead in), Day 29 (Week 5), Day 57 (Week 9), Day 85 (Week 13), Day 113 (Week 17), Day 141 (Week 21), Day 169 (Week 25), Day 191 (Week 28), and follow-up visit

Food intake (ad libitum food intake, test meal)

Buffet meal will be provided at noon on

- Day -3 (lead in), Day 50 (Week 8), Day 106 (Week 16), and Day 191 (Week 28)

HbA1c (fasting)

- Screening, Day -3 (lead in), Day 85 (Week 13), Day 141 (Week 21), and Day 191 (Week 28) (all predose except Day 191 since Day 190 is the last dosing day).

Insulin, C-peptide

- Day -2 (fasting [-10, -1 min] and during sMMTT), Day -1 (hyperglycemic clamp), Day 85 (Week 13; fasting), Day 141 (Week 21; fasting), Day 192 (Week 28, fasting [-10, -1 minutes] and during sMMTT), Day 193 (Week 28, hyperglycemic clamp)

Glucagon

- Day -2 (fasting [-1 minutes] and during sMMTT), Day -1 (hyperglycemic clamp), Day 85 (Week 13; fasting), Day 141 (Week 21; fasting), Day 192 (Week 28, fasting [-1 minutes] and during sMMTT), Day 193 (Week 28, hyperglycemic clamp)

Pancreatic polypeptide

- Day -2 (fasting [-1 minutes] and during sMMTT), Day 85 (Week 13), Day 141 (Week 21), Day 192 (Week 28, fasting [-1 minutes] and during sMMTT)

Proinsulin, leptin, adiponectin, IGFBP 1 and IGFBP 2

- Day -2 (0 minutes of sMMTT), Day 85 (Week 13; fasting), Day 141 (Week 21; fasting), Day 192 (Week 28, 0 minutes of sMMTT)

Lipid panel

- including triglycerides (serum), total cholesterol, LDL-C,VLDL-C, HDL-C, ApoB-48, ApoB-100, ApoC-III, LPL on
    - Day -2 (full lipid panel; fasting [-1 minutes] and during sMMTT), Day -1 (hyperglycemic clamp, only triglycerides), Day 85 (Week 13; fasting), Day 141 (Week 21; fasting), Day 192 (Week 28; full lipid panel; fasting [-1 minutes] and during sMMTT), Day 193 (Week 28; hyperglycemic clamp, only triglycerides)

AUC calculation

For ISR, the deconvolution software calculates ISR during hyperglycemic clamp as a piecewise-constant function of time, and hence these ISR AUC measures are computed with rectangular integration (instead of trapezoidal rule). All other AUC measures (i.e., except ISR AUC during the hyperglycemic clamp) will be calculated using the trapezoidal rule. For example, if $\{x_k\}$ is a partition of the desired time interval [a,b], where measurements at each x_k is denoted as y_k and $a = x_0 < x_1 < \cdots < x_{N-1} < x_N = b$.

- Total AUC (rectangular rule) is defined as

$$AUC_{[a,b]} = \sum_{k=1}^{N} y_{k-1}(x_k - x_{k-1})$$

- Total AUC (trapezoidal rule) is defined as

$$AUC_{[a,b]} = \sum_{k=1}^{N} \frac{(y_k + y_{k-1})(x_k - x_{k-1})}{2}$$

- Incremental AUC measures use the time zero value relative to the AUC measured unless otherwise specified, i.e.

$$incremental\ AUC_{[a,b]} = AUC_{[a,b]} - y_0 \times (b - a)$$

Note: Unless otherwise specified, in case of no planned collection or missing measurement at 0 minute,

- Blood samples:
  - For pre-sMMTT measurements, the first available measurement in the order of -1, -10 minutes (if applicable) will be used as the value for 0 minute to calculate AUC.
  - For pre-clamp measurements, the first available measurement in the order of 0, -10, -20 minutes (if applicable) will be used as the value for 0 minute to calculate AUC (except preclamp glucose AUC, basal insulin AUC, and insulin secretion rate related parameters).
    - Preclamp glucose AUC, basal insulin AUC, and basal insulin secretion rate from the hyperglycemic clamp were calculated using available hyperglycemic preclamp C-peptide, glucose, and insulin data.
- Adipose tissue samples:
  - For pre-sMMTT measurements, the first available measurement in the order of at -1, -30, -60 minute (if applicable) will be used as the value for 0 minute to calculate AUC.
  - For pre-hyperglycemic clamp measurements, adipose tissues samples are only collected at 0 minute (pre clamp).
- Indirect calorimetry measurements:
  - For pre-sMMTT indirect calorimetry measurements, the mean of measurements at -120 and -30 minutes will be used as measurements at 0 minutes to calculate AUC.

6.9.2. Pharmacodynamic Statistical Inference

Unless specified otherwise, pairwise treatment comparison will be performed for 3 treatment groups (tirzepatide, semaglutide, and placebo) on all endpoints.

6.9.2.1. Primary Pharmacodynamic Analysis

cDI from hyperinsulinemic euglycemic and hyperglycemic clamp

The primary endpoint in the study is the change from baseline (Day -1) to Week 28 (Day 193) in total cDI for comparison of tirzepatide 15 mg versus placebo.

Total cDI, which is derived using insulin secretion and insulin sensitivity, will be calculated from total insulin secretion rate during the 120-minute hyperglycemic clamp determined from C-peptide concentrations (total ISR $AUC_{0\text{-}120min}$) divided by glucose area under the concentration versus time curve from 0 to +120 minutes (total AUC from time 0 to 120 minutes after start of the hyperglycemic clamp [total glucose $AUC_{0\text{-}120min}$]), and multiply by insulin sensitivity expressed as M-value from hyperinsulinemic euglycemic clamp, respectively (van Cauter et al. 1992; Sjaarda et al. 2012).

$$cDI = [(\text{total ISR } AUC_{0\text{-}120min}) / (\text{total glucose } AUC_{0\text{-}120min})] \times \text{M-value}$$

Steps to compute cDI:

1. ISR profile will be obtained from hyperglycemic clamp with Mari's implementation of C-peptide deconvolution according to the van Cauter's model (van Cauter et al. 1992; Mari 1998).
2. Compute total ISR $AUC_{0\text{-}120min}$
3. Compute total glucose $AUC_{0\text{-}120min}$ from hyperglycemic clamp blood glucose data
4. Refer to Section 6.9.2.2 for computation details of M-value from hyperinsulinemic euglycemic clamp
5. Compute cDI
    - cDI [$pmol \cdot m^{-2} \cdot L \cdot min^{-2} \cdot kg^{-1}$] = [(total ISR $AUC_{0\text{-}120min}$ [$pmol/m^2$]) / (total glucose $AUC_{0\text{-}120min}$ [min*mmol/L])] × M-value [mmol/min/kg]

The primary endpoint (change from baseline in cDI) will be calculated by first log transforming the data (if cDI values are positive) then computing the change from baseline. The primary analysis will be analyzed using an analysis of covariance (ANCOVA) model to compare tirzepatide versus placebo. The independent variables will include treatment as a factor, and log-transformed baseline cDI.

Inferential statistics include least squares (LS) means and standard error cDI for of each treatment (tirzepatide, semaglutide, and placebo), and the estimated treatment difference and corresponding 2-sided 95% CI (transformed back to the original scale).

Tirzepatide will be claimed to have a statistically significantly greater change in cDI than placebo if the lower limit of the 2-sided CI of (tirzepatide - placebo) on the log scale (or transformed back to original scale) is greater than 0.

If cDI contains negative values, ANCOVA analyses on original scale data and sensitivity analyses using nonparametric tests (Wilcoxon signed-rank test for change from baseline measures and analysis of variance (ANOVA) model on rank-transformed change from baseline measures) will be conducted instead, if deemed appropriate.

6.9.2.2. Secondary Pharmacodynamic Analysis

The following measurements are secondary endpoints in the study to compare the effect of tirzepatide 15 mg versus placebo (except cDI), and tirzepatide 15 mg versus semaglutide 1 mg after 28 weeks of treatment.

cDI from hyperinsulinemic euglycemic clamp and hyperglycemic clamp

Secondary PD measure to compare the effect to tirzepatide 15 mg versus semaglutide 1 mg on insulin secretion and insulin sensitivity combined outcome:

- change from baseline (Day -1) to Week 28 (Day 193) in total cDI measure, where the calculation of total cDI is described above for the primary measure (Section 6.9.2.1).

The secondary PD parameter cDI to compare tirzepatide 15 mg versus semaglutide 1 mg will be analyzed in a manner similar to the primary PD analysis using ANCOVA in log scale. Tirzepatide will be claimed to have a statistically significantly greater change in cDI than semaglutide if the lower limit of the 2-sided CI of (tirzepatide - semaglutide) on the log scale (or transformed back to original scale) is greater than 0.

Glucose control

Secondary PD measures to compare the effect of tirzepatide 15 mg versus placebo and tirzepatide 15 mg versus semaglutide 1 mg on glucose control:

- change from baseline (Day -2) to Week 28 (Day 192) in fasting and postmeal blood glucose during sMMTT
  - fasting glucose
  - total glucose $AUC_{0\text{-}240min}$
  - incremental glucose $AUC_{0\text{-}240min}$

The fasting glucose, total and incremental glucose $AUC_{0\text{-}240min}$ from sMMTT will be analyzed using ANCOVA on the original scale of data, if deemed appropriate. The independent variables will include treatment as a factor and baseline measure.

Note: The fasting glucose is the mean of -10 min and -1 min measurements of sMMTT. For sMMTT total and incremental glucose $AUC_{0\text{-}240min}$ calculations, the -1 minute timepoint was changed to 0 minute prior to calculating AUCs.

- change from baseline through Week 28 (Day 191) in HbA1c
  - HbA1c (% and mmol/mol)

For HbA1c, it will be analyzed using a mixed-model repeated measure (MMRM) method with restricted maximum likelihood (REML) estimation since it is scheduled to be measured 3 times postbaseline (Day 85 [Week 13], Day 141 [Week 21], and Day 191 [Week 28]). The model will include the treatment, visit, and treatment-by-visit interaction as fixed effects, baseline HbA1c as a covariate, and patient as a random effect. Analyses will be conducted on the original scale of data. An unstructured covariance matrix will be used to model the relationship of within-patient errors. If this model fails to converge, the following variance covariance structures will be tested in order until convergence is achieved:

- heterogeneous Toeplitz
- heterogeneous first order autoregressive
- heterogeneous compound symmetry
- Toeplitz
- first order autoregressive, and
- compound symmetry.

The first covariance structure that converges will be used.

Insulin secretion

Secondary PD measures to compare the effect of tirzepatide 15 mg versus placebo and tirzepatide 15 mg versus semaglutide 1 mg on insulin secretion (β cell function):

From hyperglycemic clamp (Day -1 and Day 193)**:**

- change from baseline to Week 28 in incremental $ISR_{0\text{-}8min}$ from hyperglycemic clamp (first phase insulin secretion rate)
    - ISR profile will be obtained from hyperglycemic clamp with Mari's implementation of C-peptide deconvolution according to the van Cauter's model (van Cauter et al. 1992; Mari 1998).

      incremental $ISR_{0\text{-}8min}$ [pmol/min/m^2] = total ISR $AUC_{0\text{-}8min}$ / 8 min - basal ISR

      where basal ISR from hyperglycemic clamp is defined in Section 6.9.2.3.
- change from baseline to Week 28 in total $ISR_{20\text{-}120min}$ from hyperglycemic clamp (second phase insulin secretion rate)
    - ISR profile will be obtained from hyperglycemic clamp with Mari's implementation of C-peptide deconvolution according to the van Cauter's model (van Cauter et al. 1992; Mari 1998). Then total ISR $AUC_{20\text{-}120min}$ will be derived.

      total $ISR_{20\text{-}120min}$ [pmol/min/m^2] = total ISR $AUC_{20\text{-}120min}$ /100 min
- change from baseline to Week 28 in total $ISR_{0\text{-}120min}$ from hyperglycemic clamp (total insulin secretion rate)
    - ISR profile will be obtained from hyperglycemic clamp with Mari's implementation of C-peptide deconvolution according to the van Cauter's model (van Cauter et al. 1992; Mari 1998). Then total ISR $AUC_{0\text{-}120min}$ will be derived.

      total $ISR_{0\text{-}120min}$ [pmol/min/m^2] = total ISR $AUC_{0\text{-}120min}$ /120 min
- change from baseline to Week 28 in insulin response to arginine, incremental $insulin_{arginine0\text{-}10min}$ and incremental $insulin_{arginine0\text{-}30min}$ from hyperglycemic clamp, defined as incremental insulin AUC/time interval, between +120 to +130 minute and +120 to +150 minute of clamp period, respectively.
    - Incremental $insulin_{arginine0\text{-}10min}$ [pmol/L] = incremental insulin $AUC_{120\text{-}130min}$/10 min
    - Incremental $insulin_{arginine0\text{-}30min}$ [pmol/L] = incremental insulin $AUC_{120\text{-}150min}$/30 min

- change from baseline to Week 28 in β cell glucose sensitivity (GS) from hyperglycemic clamp
    - β cell GS [pmol/min/m^2/(mmol/L)] = ($ISR_{80\text{-}120min}$ – basal ISR [pmol/min/m^2]) / (total glucose $AUC_{80\text{-}120min}$/40 min [mmol/L] – total glucose $AUC_{\text{-}10\text{-}0min}$/10 min [mmol/L])
        - total $ISR_{80\text{-}120min}$ = total ISR $AUC_{80\text{-}120min}$/40 min
        - basal ISR = (ISR(-inf) + ISR(-10))/2
        - (total glucose $AUC_{80\text{-}120min}$ / 40 min – total glucose $AUC_{\text{-}10\text{-}0min}$ / 10 min) is glucose increment from basal to final period

The secondary PD parameters on insulin secretion, total $ISR_{20\text{-}120min}$, total $ISR_{0\text{-}120min}$ from hyperglycemic clamp will be analyzed in a manner similar to the primary PD analysis using ANCOVA on log-transformed data.

The above incremental $ISR_{0\text{-}8min}$, incremental $insulin_{arginine0\text{-}10min}$, incremental $insulin_{arginine0\text{-}30min}$, β cell GS will be analyzed using ANCOVA on original scale data. Sensitivity analyses using nonparametric tests (Wilcoxon signed-rank test for change from baseline measures and ANOVA model on rank-transformed change from baseline measures) will be conducted, if deemed appropriate.

From sMMTT (Day -2 and Day 192)

Note: the sMMTT glucose and C-peptide profiles will be analyzed using the model of Mari et al. (Mari et al. 2002a; Mari et al. 2002b; Mari and Ferrannini 2008; Mari et al. 2016).

- change from baseline to Week 28 in β cell GS from sMMTT, defined as the slope of the dose-response for insulin secretion versus glucose during the sMMTT
    - β cell GS [pmol/min/m^2/(mmol/L)]
- change from baseline to Week 28 in ISRg* (ISR at fixed glucose concentration) from sMMTT, defined as insulin secretion corresponding to a fixed glucose level representative of the basal value in the study population, and calculated from the dose-response.
    - ISRg [pmol/min/m^2]

    * will be determined by averaging the pre-meal glucose values at baseline among all subjects and all treatments.

The above secondary PD parameters, β cell GS and ISRg on insulin secretion from sMMTT will be analyzed using ANCOVA on original scale data. Sensitivity analyses using nonparametric tests (Wilcoxon signed-rank test for change from baseline measures and ANOVA model on rank-transformed change from baseline measures) will be conducted, if deemed appropriate.

Insulin sensitivity M-value from hyperinsulinemic euglycemic clamp (Day -1 and Day 193)

Secondary PD measures to compare the effect of tirzepatide 15 mg versus placebo and tirzepatide 15 mg versus semaglutide 1 mg on insulin sensitivity:

- change from baseline to Week 28 in M-value from hyperinsulinemic euglycemic clamp, is calculated from GIR over the last 30 minutes of the clamp, corresponding to steady state (+150 to +180 minutes) minus a correction factor for non-constant glucose level during the clamp study (DeFronzo et al. 1979).
    - M-value [mg/min/kg] = ($GIR_{150\text{-}180min}$ normalized by body weight [mg/min/kg]) – $SC_{150\text{-}180min}$ [mg/min/kg].

      where, SC (space correction) is the correction for the non-constant glucose level

$$SC_{150-180\,min} = \frac{(G_{180min}[mg/dL] - G_{150min}[mg/dL]) \times 10[dL/L] \times 0.19[L/kg]}{30\,[min]}$$
$$= 0.063\,[dL/min/kg] \times (G_{180min}[mg/dL] - G_{150min}[mg/dL])$$

      where G_{150min} and G_{180min} are the blood glucose concentrations (mg/dL) at +150 and +180 minutes of the hyperinsulinemic euglycemic clamp.

Conventional Units to International System of Units (SI) conversion:

M-value (µmol/min/kg) = 5.551 × M-value (mg/min/kg)

(in addition: M-value (mmol/min/kg) = M-value (mg/min/kg) / 180.14772)

The secondary PD parameter M-value will be analyzed using ANCOVA on the original scale of data.

Additional analyses on alternative M-value that is calculated by

M-value= ($GIR_{150\text{-}180min}$ normalized by fat-free body mass) – $SC_{150\text{-}180min}$

may be conducted using a similar model. The $SC_{150\text{-}180min}$ will be calculated with the glucose distribution volume per kg fat-free mass of 0.27 L/kg (instead of 0.19 L/kg [body weight]).

Glucagon secretion from sMMTT

Secondary PD measures to compare the effect of tirzepatide 15 mg versus placebo and tirzepatide 15 mg versus semaglutide 1 mg on glucagon secretion:

- change from baseline (Day -2) to Week 28 (Day 192) in glucagon concentration at fasting and postmeal during sMMTT
    - fasting glucagon (-1 min of sMMTT)
    - total glucagon $AUC_{0\text{-}240min}$
    - incremental glucagon $AUC_{0\text{-}240min}$

The above secondary PD parameters on glucagon secretion from sMMTT will be analyzed using ANCOVA on the original scale of data.

Appetite and food intake

Secondary PD measures to compare the effect of tirzepatide 15 mg versus placebo and tirzepatide 15 mg versus semaglutide 1 mg on appetite and food intake:

- change from baseline (Day -3) through Week 28 (Day 191) in food intake during ad libitum meal served buffet style measured as total energy intake (kcal)

Additional analyses may be conducted on total food intake in grams, energy intake and percent of energy of macronutrient (carbohydrate, protein, and fat).

Note: The amount of grams of fat, carbohydrates and protein consumed will be documented in electronic Case Report Form (eCRF). There are 4 kcal in a gram of carbohydrate or protein, and 9 kcal in a gram of fat will be used to calculate energy intake related variables.

The energy intake will be analyzed in a manner similar to the secondary PD analysis on HbA1c using MMRM since ad libitum food intake test is scheduled 3 times postbaseline (Day 50 [Week 8], Day 106 [Week 16], and Day 191 [Week 28]). Analyses will be done on the original scale of data.

Inferential statistics include LS means and standard error of each treatment (tirzepatide, semaglutide, and placebo), and the estimated treatment difference and corresponding 2-sided 95% CI (on or transformed back to the original scale).

6.9.2.3. Exploratory Pharmacodynamic Analysis

The following measurements are exploratory endpoints to compare the effect of tirzepatide 15 mg versus placebo, and tirzepatide 15 mg versus semaglutide 1 mg after 28 weeks of treatment.

Glucose metabolism and turnover in adipose tissue from hyperglycemic clamp

Exploratory PD measures to compare the effect of tirzepatide 15 mg versus placebo and tirzepatide 15 mg versus semaglutide 1 mg on glucose metabolism and turnover in adipose tissue:

From hyperglycemic clamp (Day -1 and Day 193)

- change from baseline to Week 28 in glucose concentration in adipose tissue microdialysis from 0 to 120 minutes during hyperglycemic clamp
    - Glucose microdialysis$_{0\text{-}120\text{min}}$ (total AUC)
- change from baseline to Week 28 in blood flow in adipose tissue microdialysis from 0 to 150 minutes during hyperglycemic clamp
    - The ethanol outflow/inflow ratio (ratio of ethanol concentration in the dialysate [6 samples on Day -1 and on Day 193] and perfusate [1 sample on Day -2 and on Day 192]) will be measured as an indicator of adipose tissue blood flow at 6 time points: 0, 30, 60, 90, 120, 150 minutes.

The exploratory PD parameters of Glucose microdialysis$_{0\text{-}120min}$ and adipose tissue blood flow (at each of 6 time points and AUC) from hyperglycemic clamp may be analyzed using ANCOVA on original scale of data, if deemed appropriate. Additional analyses on ethanol concentration (at each of 6 time points and AUC) measures may be conducted, if deemed appropriate.

From sMMTT (Day -2 and Day 192)

- change from baseline to Week 28 in glucose concentration in adipose tissue microdialysis from 0 to 240 minutes during sMMTT
    - Glucose microdialysis$_{0\text{-}240min}$ (total AUC)
- change from baseline to Week 28 in blood flow in adipose tissue microdialysis from 60 to 240 minutes during sMMTT
    - The ethanol outflow/inflow ratio (ratio of ethanol concentration in the dialysate [4 samples on Day -2 and on Day 192] and perfusate [1 sample on Day -2 and on Day 192]) will be measured as an indicator of adipose tissue blood flow at 4 time points: 60, 120, 180, 240 minutes.

The exploratory PD parameters of Glucose microdialysis$_{0\text{-}240min}$ and adipose tissue blood flow (at each of 4 time points and AUC) from sMMTT may be analyzed using ANCOVA on original scale of data, if deemed appropriate. Additional analyses on ethanol concentration (at each of 4 time points and AUC) measures may be conducted, if deemed appropriate.

Insulin secretion

Exploratory PD measures to compare the effect of tirzepatide 15 mg versus placebo and tirzepatide 15 mg versus semaglutide 1 mg on insulin secretion (β cell function):

From hyperglycemic clamp

Change from baseline (Day -1) to Week 28 (Day 193) in

- basal ISR (basal insulin secretion rate) prior to hyperglycemic clamp
    - ISR profile will be obtained from hyperglycemic clamp with Mari's implementation of C-peptide deconvolution according to the van Cauter's model (van Cauter et al. 1992; Mari 1998). Then basal ISR will be derived.

      basal ISR [pmol/min/m^2] = (ISR(-inf) + ISR(-10))/2
- basal insulin$_{-10\text{-}0min}$ (basal insulin concentration) prior to hyperglycemic clamp
    - basal insulin$_{-10\text{-}0min}$ [pmol/L] = total insulin AUC$_{-10\text{-}0min}$/10 min
- incremental insulin AUC$_{0\text{-}10min}$ (first phase insulin response) from hyperglycemic clamp
    - incremental insulin AUC$_{0\text{-}10min}$
- total insulin AUC$_{20\text{-}120min}$ (second phase insulin response) from hyperglycemic clamp
    - total insulin AUC$_{20\text{-}120min}$

- total $ISR_{80\text{-}120min}$ (steady-state insulin secretion rate) from hyperglycemic clamp, defined as average total insulin secretion (total ISR AUC/time interval) during the +80 minutes to +120 minutes hyperglycemic clamp period.
    - ISR profile will be obtained from hyperglycemic clamp with Mari's implementation of C-peptide deconvolution according to the van Cauter's model (van Cauter et al. 1992; Mari 1998). Then total ISR $AUC_{80\text{-}120min}$ will be derived.

      total $ISR_{80\text{-}120min}$ [pmol/min/m^2] = total ISR $AUC_{80\text{-}120min}$/40 min
- total insulin $AUC_{0\text{-}120min}$ (total insulin response) from hyperglycemic clamp
    - total insulin $AUC_{0\text{-}120min}$

The above exploratory PD parameters on insulin secretion from hyperglycemic clamp will be analyzed using ANCOVA. For incremental insulin $AUC_{0\text{-}10min}$, it will be analyzed on original scale data, and sensitivity analyses using nonparametric tests (Wilcoxon signed-rank test for change from baseline measures and ANOVA model on rank-transformed change from baseline measures) may be conducted, if deemed appropriate. For the other parameters (e.g., total insulin $AUC_{20\text{-}120min}$, total insulin $AUC_{0\text{-}120min}$) will be analyzed on the log-transformed data, if deemed appropriate.

From sMMTT

Note: the sMMTT glucose and C-peptide profiles will be analyzed using the model of Mari et al. (Mari et al. 2002a; Mari et al. 2002b; Mari and Ferrannini 2008; Mari et al. 2016).

Change from baseline (Day -2) to Week 28 (Day 192) in

- basal ISR at 0 minute from sMMTT
    - basal ISR at 0 minute [pmol/min/m^2]
- total ISR $AUC_{0\text{-}240min}$ (total insulin secretion rate) from sMMTT, defined as total AUC of insulin secretion rate during the entire sMMTT 240-minute period.
    - total ISR $AUC_{0\text{-}240min}$ [nmol/m^2] (integral of total insulin secretion)
- ISRgb (ISRg [ISR at fixed glucose concentration] adjusted for basal potentiation) from sMMTT, defined as ISRg multiplied by the basal potentiation factor value.
    - ISRgb [pmol/min/m^2] = ISRg × basal potentiation factor value
- rate sensitivity (RS) from sMMTT.
    - RS [pmol/m^2/(mmol/L)]
- potentiation ratio (PFR) from sMMTT, defined as relative enhancement of ISR, as predicted by the dose-response from basal to +120 minute (PFR_{120}), +180 minute (PFR_{180}), and +240 minute (PFR_{240}) time points
    - $PFR_{120\ min}$

- $PFR_{180\ min}$
- $PFR_{240\ min}$

- ICLb (basal insulin clearance) prior to sMMTT, defined as the ratio of insulin secretion to insulin concentration at fasting prior to sMMTT.
  - ICLb [$L/min/m^2$] = basal ISR at 0 min / basal insulin
- ICLm (insulin clearance) during sMMTT, defined as the ratio of insulin secretion to insulin concentration AUCs during the entire sMMTT (0-240 min).
  - ICLm [$L/min/m^2$] = total ISR $AUC_{0\text{-}240min}$ / total insulin $AUC_{0\text{-}240min}$
- ratio of GS from sMMTT and from hyperglycemic clamp (estimate of the incretin effect).
  - GS ratio = GS from sMMTT / GS from hyperglycemic clamp

The above exploratory PD parameters on insulin secretion during sMMTT will be analyzed using ANCOVA. For total ISR $AUC_{0\text{-}240min}$, analyses of using log-transformed data will be conducted. For RS, it will be analyzed on original scale data, and sensitivity analyses using nonparametric tests (Wilcoxon signed-rank test for change from baseline measures and ANOVA model on rank-transformed change from baseline measures) may be conducted, if deemed appropriate.

- fasting proinsulin to insulin ratio
  - Day -2 and Day 192: proinsulin at 0 minutes [pre meal] / insulin at -1 minute [1 minute pre meal]
  - Day 85 and Day 141: fasting proinsulin / fasting insulin
- fasting proinsulin to C-peptide ratio
  - Day -2 and Day 192: proinsulin at 0 minutes [pre meal] / C-peptide at -1 minute [1 minute pre meal]
  - Day 85 and Day 141: fasting proinsulin / fasting C-peptide

The above exploratory PD parameters on fasting proinsulin to insulin, C-peptide ratio will be analyzed in a manner similar to the secondary PD analysis on HbA1c using MMRM since it is collected 3 times postbaseline (Day 50 [Week 8], Day 106 [Week 16], and Day 191 [Week 28]). Analyses will be conducted on the original scale or log-transformed data, as deemed appropriate.

- insulinogenic index at 30 minutes (Π_{30min}), defined as the ratio of increments in insulin and blood glucose concentrations from time point 0 to +30 minutes during the sMMTT.
  - $\Pi_{30min} = (Insulin_{+30min} - Insulin_{0min}) / (Glucose_{+30min} - Glucose_{0min})$

  * If there is no collection for insulin and glucose concentration at 0 minute, values at -1 minute will be used instead for calculation; if missing measurement at -1 minute, measurement at -10 minutes will be used.

- fasting and postmeal insulin during the sMMTT
    - fasting insulin
    - total insulin $AUC_{0\text{-}240min}$
    - incremental insulin $AUC_{0\text{-}240min}$
- fasting and postmeal C-peptide during the sMMTT
    - fasting C-peptide
    - total C-peptide $AUC_{0\text{-}240min}$
    - incremental C-peptide $AUC_{0\text{-}240min}$

The above exploratory PD parameters on insulin secretion during sMMTT will be analyzed using ANCOVA. Analyses will be conducted on the original scale or log-transformed data, as deemed appropriate.

Note: Fasting insulin and C-peptide are the mean of -10 min and -1 min measurements of sMMTT.

Insulin sensitivity

From hyperinsulinemic euglycemic clamp (Day -1 and Day 193)

Exploratory efficacy measures to compare the effect of tirzepatide 15 mg versus placebo and tirzepatide 15 mg versus semaglutide 1 mg on insulin sensitivity:

- change from baseline to Week 28 in hyperinsulinemic euglycemic M/I value, defined as the M-value divided by total insulin over the same time period (+150 to +180 minutes).
    - M/I = M-value / $insulin_{150\text{-}180min}$
        - M-value is a secondary PD measure
        - $Insulin_{150\text{-}180min}$ = total insulin $AUC_{150\text{-}180min}$ / 30 min

The exploratory PD parameter M/I will be analyzed using ANCOVA. Analyses of using original scale data will be conducted, if deemed appropriate. M-value (normalized by body weight or fat free mass) will be used, as deemed appropriate.

From sMMTT (Day -2 and Day 192)

- change from baseline to Week 28 in:
    - **HOMA2-IR** (insulin resistance as measured by the HOMA2 method using fasting plasma glucose [mmol/L] and fasting serum insulin [pmol/L] or fasting serum C-peptide [nmol/L] for calculation) (Hill et al. 2013)
        - Fasting measurement will be the mean of non-missing measurements at -10 and -1 minutes of sMMTT
        - Plasma glucose = 1.11 × blood glucose

- Serum insulin is collected during sMMTT and used for calculation of HOMA2-IR and indices below

- **Matsuda Index** (postprandial insulin sensitivity from sMMTT) (Schlichtkrull et al. 1965; Matsuda and DeFronzo 1999; Service and O'Brien 2001)

$$ISI - Matsuda = \frac{10{,}000}{\sqrt{PG_0 * I_0 * \frac{total\ plasma\ glucose\ AUC_{0-240min}}{240\ min} * \frac{total\ insulin\ AUC_{0-240min}}{240\ min}}}$$

where PG_0 and I_0 are the plasma glucose concentration (mg/dL) and serum insulin concentration (μU/mL) at 0 minute (fasting).

Note: If there is no collection for insulin and glucose concentration at 0 minute, values at -1 minute will be used instead for calculation; if missing measurement at -1 minute, measurement at -10 minutes will be used.

- **OGIS Index** (Oral Glucose Insulin Sensitivity Index; $mL \cdot min^{-1} \cdot m^2$) (Mari et al. 2001)

$$OGIS = \frac{1}{2} \times \left(B + \sqrt{B^2 + 4p_5 p_6 (G_{120min} - G_{CLAMP}) Cl_{OGTT}}\right)$$

Where

$$B = [p_5(G_{120min} - G_{CLAMP}) + 1] \times Cl_{OGTT}$$

$$Cl_{OGTT} = p4 \frac{\frac{p_1 D_0 - V(G_{180min} - G_{120min})/60min}{G_{120min}} + \frac{p_3}{G_0}}{I_{120min} - I_0 + p_2}$$

Where $p_1 = 289, p_2 = 270, p_3 = 14{,}000, p_4 = 440, p_5 = 0.000637, p_6 = 117$

D_0 is an oral glucose dose (expressed in grams per square meter)

(D_0 = carbohydrate intake [gram] during sMMTT / BSA [m^2], where Gehan and George body surface area formula is BSA=0.1640443958298 × weight[kg]$^{0.515}$ × (height[cm]/100)$^{0.422}$ [Bailey and Briars 1996])

V=10,000 mL/m^2 (the total glucose distribution volume)

G_{CLAMP} = 90 mg/dL

G_0 = plasma glucose concentration (mg/dL) at 0 minute (fasting).

I_0 = serum insulin concentration (μU/mL) at 0 minute (fasting).

G_{120min} = plasma glucose concentration (mg/dL) at 120 min

I_{120min} = serum insulin concentration (μU/mL) at 120 min

G_{180min} = plasma glucose concentration (mg/dL) at 180 min

Note: If there is no collection for insulin and glucose concentration at 0 minute, values at -1 minute will be used instead for calculation; if missing measurement at -1 minute, measurement at -10 minutes will be used.

- **Stumvoll Index** (Stumvoll et al. 2000; Stumvoll et al. 2001)

$$Stumvoll\ ISI = 0.222 - 0.00333\ BMI - 0.0000779\ I_{120min} - 0.000422\ Age$$

Where I_{120min} = serum insulin concentration (pmol/L) at 120 min

The exploratory PD parameters insulin sensitivity indices from sMMTT will be analyzed using ANCOVA. Analyses will be conducted on the original scale or log-transformed data, as deemed appropriate.

Glucagon and pancreatic polypeptide (PP) secretion

Exploratory PD measures to compare the effect of tirzepatide 15 mg versus placebo and tirzepatide 15 mg versus semaglutide 1 mg on glucagon and PP secretion:

From hyperglycemic clamp (Day -1 and Day 193)

- change from baseline to Week 28 in total glucagon $AUC_{0\text{-}120min}$ during hyperglycemic clamp
  - total glucagon $AUC_{0\text{-}120min}$
- change from baseline to Week 28 in incremental $glucagon_{arginine0\text{-}30min}$, defined as incremental AUC/time interval, between +120 and +150 minutes of hyperglycemic clamp
  - incremental $glucagon_{arginine0\text{-}30min}$ = incremental glucagon $AUC_{120\text{-}150min}$/30 min

From sMMTT (Day -2 and Day 192)

- change from baseline to Week 28 in glucagon/insulin ratio at fasting and during sMMTT, which is defined as ratio of total glucagon $AUC_{0\text{-}240min}$ and total insulin $AUC_{0\text{-}240min}$
  - glucagon/insulin ratio = total glucagon $AUC_{0\text{-}240min}$ / total insulin $AUC_{0\text{-}240min}$
- change from baseline to Week 28 in PP concentration at fasting and postmeal during sMMTT
  - fasting PP (-1 min of sMMTT)
  - total PP $AUC_{0\text{-}240min}$
  - incremental PP $AUC_{0\text{-}240min}$

The above exploratory PD parameters on glucagon and PP secretion during hyperglycemic clamp and sMMTT will be analyzed using ANCOVA. Analyses will be conducted on original scale or log-transformed data, as deemed appropriate.

Appetite VAS score

Exploratory PD measures to compare the effect of tirzepatide 15 mg versus placebo and tirzepatide 15 mg versus semaglutide 1 mg on appetite and food intake:

- change from baseline (Day -3) through Week 28 (Day 191) in fasting appetite VAS
- change from baseline (Day -2) to Week 28 (Day 192) in appetite VAS score during sMMTT
    - at 5 time points: -1, 60, 120, 180, 240 minutes

Note: The aim of the appetite VAS is to determine the effects of study treatments on appetite sensations and desire for specific foods:

- 4 individual ratings: hunger, fullness, satiety, prospective food consumption
- 4 individual ratings: desire for 4 specific foods (sweet, salty, savory, and fatty)

The VAS scales will be analyzed as continuous variables on the 0 to 100 scale for individual components:

- For the above first 4 individual ratings, 0 = Not at all, 100 = Extremely
- For the last 4 individual ratings on desire for specific foods, 0 = Yes, very much, 100 = No, not at all

The higher overall appetite score indicates less appetite, and the lower score indicates more appetite. The 8 individual VAS scores will be documented in eCRF. Overall appetite score is calculated as the average of the 4 individual scores (Flint et al. 2000; Flint et al. 2013; van Can et al. 2014):

$$\text{overall appetite score} = (\text{satiety} + \text{fullness} + [100 - \text{prospective food consumption}] + [100 - \text{hunger}]) / 4$$

The exploratory PD parameters on fasting appetite VAS will be analyzed in a manner similar to the secondary PD analysis on HbA1c using MMRM since it is scheduled 7 times postbaseline (Day 29 [Week 5], Day 57 [Week 9], Day 85 [Week 13], Day 113 [Week 17], Day 141 [Week 21], Day 169 [Week 25], Day 191 [Week 28]). Analyses will be done on the original scale of data.

The exploratory PD parameters on appetite VAS during sMMTT will be analyzed in a manner similar to the primary PD analysis using ANCOVA at each of the 5 time points (i.e., -1, 60, 120, 180, 240 minutes), respectively. The analyses will be done on the original scale of data.

Analyses will be conducted on the overall appetite score. Additional analyses on individual ratings (satiety, fullness, hunger, prospective food consumption, and desire for 4 specific foods [sweet, salty, savory, and fatty]) of fasting appetite VAS and appetite VAS during sMMTT will be conducted, if deemed appropriate.

Lipid metabolism and turnover in adipose tissue

Exploratory PD measures to assess the effect of the effect of tirzepatide 15 mg versus placebo and tirzepatide 15 mg versus semaglutide 1 mg on lipid metabolism and turnover in adipose tissue:

During hyperglycemic clamp (Day -1 and Day 193)

- change from baseline to Week 28 in blood samples during hyperglycemic clamp:
  - total $AUC_{0\text{-}120min}$ of triglycerides, β-hydroxybutyrate, pyruvate, lactate (lactic acid), FFA, and glycerol
- change from baseline to Week 28 in adipose tissue (microdialysis; dialysate) during hyperglycemic clamp:
  - $microdialysis_{0\text{-}120min}$ (total AUC) of pyruvate, lactate (lactic acid), and glycerol

The above exploratory PD parameters on lipid metabolism and turnover in adipose tissue during hyperglycemic clamp will be analyzed using ANCOVA. Analyses will be conducted on original scale or log-transformed data, as deemed appropriate.

During sMMTT (Day -2 and Day 192)

- change from baseline to Week 28 in blood samples at fasting and during sMMTT
  - fasting triglycerides, β-hydroxybutyrate, pyruvate, lactate (lactic acid), FFA, glycerol, ApoB-48, ApoB-100, ApoC-III, and LPL
  - total $AUC_{0\text{-}240min}$ of triglycerides, β-hydroxybutyrate, pyruvate, lactate (lactic acid), FFA, glycerol, ApoB-48, ApoB-100, ApoC-III, and LPL
- change from baseline to Week 28 in adipose tissue (microdialysis; dialysate) at fasting and during sMMTT
  - fasting pyruvate, lactate (lactic acid), and glycerol
  - $microdialysis_{0\text{-}240min}$ (total AUC) of pyruvate, lactate (lactic acid), and glycerol

The above exploratory PD parameters on lipid metabolism and turnover in adipose tissue during sMMTT may be analyzed using ANCOVA. Analyses of will be conducted on original scale or log-transformed data, as deemed appropriate.

- change from baseline to Week 28 in fasting concentration of
  - leptin, adiponectin, IGFBP 1, IGFBP 2

The above exploratory PD parameters on fasting leptin, adiponectin, IGFBP 1 and 2 will be analyzed in a manner similar to the secondary PD analysis on HbA1c using MMRM since they are scheduled to be measured 3 times postbaseline (Day 85 [Week 13], Day 141 [Week 21], and Day 192 [Week 28]). On Day -2 and Day 192, measurements at 0 minutes of sMMTT will be

used for fasting state. Analyses of using original scale data will be conducted, if deemed appropriate.

Body composition

Exploratory PD measures to compare the effect of tirzepatide 15 mg versus placebo and tirzepatide 15 mg versus semaglutide 1 mg on body composition, assessed with air displacement plethysmography, and body weight and waist circumference:

- change from baseline (Day -3) to Week 28 (Day 191) in lean body mass (fat-free mass) (kg and %)
- change from baseline (Day -3) to Week 28 (Day 191) in body fat mass (kg and %)
- change from baseline through Week 28 in body weight
- change from baseline through Week 28 in waist circumference

The exploratory PD parameters on lean body mass and body fat mass measured from plethysmography will be analyzed on original scale of data using ANCOVA.

For body weight (scale) and waist circumference, they will be analyzed in a manner similar to the secondary PD analysis on HbA1c using MMRM with 8 postbaseline measurements (Day 29 [Week 5], Day 57 [Week 9], Day 85 [Week 13], Day 113 [Week 17], Day 141 [Week 21], Day 169 [Week 25], Day 191 [Week 28]). Analyses will be conducted on the original scale of data.

Indirect calorimetry: energy expenditure and substrate utilization during sMMTT
(Day -2 and Day 192)

Exploratory PD measures to compare the effect of tirzepatide 15 mg versus placebo and tirzepatide 15 mg versus semaglutide 1 mg on energy expenditure and substrate utilization assessed with indirect calorimetry using ventilated hood system:

- change from baseline to Week 28 in RMR (this is fasting energy expenditure) (kcal/day)
    - RMR/body mass, RMR/body fat-free mass

Note: The mean of measurements at -120 minutes and -30 minutes will be used as measurements for fasting state.

- change from baseline to Week 28 in RQ (ratio)
    - total $AUC_{0\text{-}240min}$ for RQ
- change from baseline to Week 28 in diet-induced thermogenesis
    - incremental $AUC_{0\text{-}240min}$ for EE/body mass, and RMR/body fat-free mass
- change from baseline to Week 28 in carbohydrate oxidation rate (g/min)
    - total $AUC_{0\text{-}240min}$ for carbohydrate oxidation rate

- change from baseline to Week 28 in fat oxidation rate (g/min)
    - total $AUC_{0\text{-}240min}$ for fat oxidation rate

The above exploratory PD parameters on RMR, diet-induced thermogenesis, RQ, carbohydrate and fat oxidation rates assessed with indirect calorimetry will be analyzed using ANCOVA on the original scale of data. For indirect calorimetry parameters, an adjustment for body composition (e.g. body weight, fat-free mass, fat mass) or energy balance as covariate(s) may be applied in the model, if deemed appropriate.

Inferential statistics include LS means and standard error of each treatment (tirzepatide, semaglutide, and placebo), and the estimated treatment difference and corresponding 2-sided 95% CI (on or transformed back to the original scale).

6.10. Safety Analyses

Safety measures include, but not limited to, AEs, treatment-emergent adverse events (TEAEs), serious adverse events (SAEs), AESIs, vital signs, and safety laboratory measures.

Unless specified otherwise, safety analyses will be performed on the safety population and presented by treatment group.

Unless specified otherwise, safety listings will display values/events during all study periods. Listings of AEs, death, SAE may include (but not limited to): subject identification (ID) number, age, sex, race, treatment arm, dose, Medical Dictionary for Regulatory Activities (MedDRA) System Organ Class (SOC), Preferred Term (PT), and time of onset from the first dose of study drug, duration of the AE, seriousness, severity, relatedness to study drug, action taken, and outcome, as appropriate. Additional safety listings will be provided for safety parameters other than AEs in related sections below.

For safety measurements, summary statistics will be presented by treatment. A summary will be provided for AEs with frequency ≥10 patients with such event.

6.10.1. Adverse Events

A listing of adverse events for the safety population will be provided, which includes MedDRA PT.

6.10.2. Treatment-Emergent Adverse Events

A TEAE is defined as an AE which first occurs post first dose of study drug or which is present prior to first dose of study drug and becomes more severe post first dose. The maximum severity for each AE during the baseline period including ongoing medical history will be used as baseline severity.

Treatment-emergent adverse events will be summarized by treatment, severity, and relationship with study drug.

6.10.3. Serious Adverse Events

A listing of patients with SAEs (including death) will be provided.

6.10.4. Adverse Events Leading to Discontinuation

A listing of patients with AEs leading to discontinuation from study will be provided.

6.10.5. Special Safety Topics

A listing of patients with all AESIs defined in Section 6.10.5 will be provided.

6.10.5.1. Hypoglycemia

A listing of documented clinically significant hypoglycemia (Level 2) and severe hypoglycemia (Level 3) events will be provided. A listing of patients with hypoglycemic events will be provided. The category of hypoglycemic events (see Table GPGT.6.3 for details) will be presented. A summary will be provided by treatment. The incidence of hypoglycemia will be reported.

Severe/serious hypoglycemia is considered an AESI in this trial.

Table GPGT.6.3. Definitions of Hypoglycemic Event Categories

	Symptoms and/or Signs of Hypoglycemia	Plasma Glucose Level
Documented glucose alert value (Level 1)	Yes/No/Unknown	≤70 mg/dL (3.9 mmol/L)
Documented symptomatic hypoglycemia	Yes	
Documented asymptomatic hypoglycemia	No	
Documented unspecified hypoglycemia	Unknown	
Documented clinically significant hypoglycemia (Level 2)	Yes/No/Unknown	<54 mg/dL (3.0 mmol/L)
Clinically significant documented symptomatic hypoglycemia	Yes	
Clinically significant documented asymptomatic hypoglycemia	No	
Clinically significant documented unspecified hypoglycemia	Unknown	

Severe hypoglycemia (Level 3): defined as an episode with severe cognitive impairment requiring the assistance of another person to actively administer carbohydrate, glucagon, or other resuscitative actions. Severe hypoglycemia will be reported as an SAE.

Nocturnal hypoglycemia: defined as any hypoglycemic event that occurs between bedtime and waking.

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

6.10.5.2. Severe, Persistent Hyperglycemia

Data on episodes of severe, persistent hyperglycemia will be reported by the investigator during the study. Events of interest related to hyperglycemia are those that require rescue therapy, per the criteria in Protocol Section 9.2.2.2.

A listing of rescue medication for hyperglycemia will be provided (also noted in Section 6.5).

6.10.5.3. Pancreatitis

A listing of patients with pancreatitis (including investigator-reported and adjudicated) will be provided. Adjudication assessment results will be reported in the listing.

Treatment-emergent adjudication-confirmed pancreatitis will be considered as an AESI.

6.10.5.4. Thyroid Malignancies and C-Cell Hyperplasia

A listing of patients with thyroid malignancies and C-cell hyperplasia (search criteria in Appendix 1) will be provided.

Thyroid malignancies and C-cell hyperplasia will be considered as AESIs.

6.10.5.5. Major Adverse Cardiovascular Events (MACE)

A listing of patients with MACE (including investigator-reported and adjudicated) will be provided. Adjudication assessment results will be reported in the listing.

Only positively adjudicated MACE will be considered as an AESI.

6.10.5.6. Supraventricular Arrhythmias and Cardiac Conduction Disorders

A listing of patients with supraventricular arrhythmias and cardiac conduction disorders (search criteria in Appendix 1) will be provided.

Severe/serious treatment-emergent supraventricular arrhythmias, arrhythmias and cardiac conduction disorders will be considered as AESI. The cardiovascular events will include clinically relevant rhythm and conduction disorders.

6.10.5.7. Hypersensitivity Events

A listing of patients with hypersensitivity reactions (search criteria in Appendix 1) will be provided.

Only the serious/severe cases of hypersensitivity will be considered as AESIs.

6.10.5.8. Injection Site Reactions

Injection-site assessments for local tolerability will be conducted, when reported as an AE from a patient, or a clinical observation from an investigator.

A listing of patients with reported injection-site reactions (edema, erythema, induration, itching, and pain) will be provided. Detailed search criteria can be found in Appendix 1.

Only the severe/serious injection site reactions (e.g., abscess, cellulitis, erythema, hematomas/hemorrhage, exfoliation/necrosis, pain, subcutaneous nodules, swelling, indurating, inflammation) will be considered as AESIs.

6.10.5.9. Diabetic Retinopathy Complications

A listing of patients with diabetic retinopathy complications (search criteria in Appendix 1) will be provided.

The cases with repeat fundoscopy during the course of the trial, based on clinical suspicion of worsening retinopathy, that have either findings of de novo retinopathy or progression of retinopathy, and severe/serious adverse events from the PTs for diabetic retinopathy complications (search criteria in Appendix 1), will be classified as an AESI.

6.10.5.10. Hepatobiliary Disorders

A listing of patients with events of biliary colic, cholecystitis, or other suspected events related to gallbladder disease will be provided. Detailed search criteria can be found in Appendix 1.

Severe/serious hepatobiliary disorders will be considered as AESIs.

6.10.5.10.1. Hepatic Monitoring

The subjects' liver disease history and associated person liver disease history data will be listed. Concomitant medications acetaminophen/paracetamol, which have potential to cause hepatotoxicity, will be listed. Results from any hepatic monitoring procedures, such as a magnetic resonance elastography (MRE) scan, and biopsy assessments will be listed, if performed.

Hepatic risk factor assessment data will be listed. Liver-related signs and symptoms data will be listed, and it will be summarized by treatment if ≥10 patients with such data. Alcohol and recreational drug use data will also be listed.

All hepatic chemistry, hematology, coagulation, and serology data will be listed. Values outside the reference ranges will be flagged on the individual subject data listings.

6.10.5.11. Severe/Serious Gastrointestinal Adverse Events

A listing of patients with severe/serious gastrointestinal AEs, such as nausea, vomiting, and diarrhea will be provided.

Only the PTs in the gastrointestinal MedDRA SOC with serious/severe cases will be considered as AESIs.

6.10.5.12. Acute Renal Events

A listing of patients with acute renal events (search criteria in Appendix 1) will be provided.

Severe/serious acute renal events will be considered as AESIs.

6.10.5.13. Metabolic Acidosis, Including Diabetic Ketoacidosis

A listing of patients with metabolic acidosis (search criteria in Appendix 1) will be provided.

Severe/serious Metabolic Acidosis, including Diabetic Ketoacidosis will be captured as AESIs.

6.10.5.14. Amputation/Peripheral Revascularization

A listing of patients with amputation and peripheral revascularization (search criteria in Appendix 1) will be provided.

Amputation/Peripheral revascularization will be considered as AESIs.

6.10.5.15. Major Depressive Disorder/Suicidal Ideation

A listing of patients with major depressive disorder/suicidal ideation (search criteria in Appendix 1) will be provided.

The severe/serious major depressive disorder/suicidal ideation or behavior will be captured as AESIs.

6.10.6. Vital Signs

Summaries of vital signs will be provided by treatment for the baseline (Day -3), postbaseline values on Day 29 (Week 5), Day 57 (Week 9), Day 85 (Week 13), Day 113 (Week 17), Day 141 (Week 21), Day 169 (Week 25), Day 191 (Week 28), and safety follow-up visit, and change from baseline values. Plots of mean vital signs and mean changes from baseline over time will be provided by treatment.

The treatment-emergent abnormal vital signs will be listed. The criteria for identifying patients with treatment-emergent vital sign abnormalities are stated in Table GPGT.6.4.

Table GPGT.6.4. Categorical Criteria for Treatment-Emergent Abnormal Blood Pressure and Pulse Measurements

Parameter	Low	High
Systolic BP (mmHg) (Supine or sitting – forearm at heart level)	≤90 and decrease from baseline ≥20	≥140 and increase from baseline ≥20
Diastolic BP (mmHg) (Supine or sitting – forearm at heart level)	≤50 and decrease from baseline ≥10	≥90 and increase from baseline ≥10
Pulse (bpm) (Supine or sitting)	<50 and decrease from baseline ≥15	>100 and increase from baseline ≥15

Abbreviations: BP = blood pressure; bpm = beats per minute.

6.10.7. Electrocardiogram

Electrocardiograms (ECGs) will be performed for safety monitoring purposes only and will not be presented. Any clinically significant findings from ECGs will be recorded as AEs.

6.10.8. Safety Laboratory Parameters

All laboratory data will be reported in the SI Units and conventional units. Clinical chemistry, hematology, and endocrine (calcitonin) data and their changes from baseline will be summarized at each planned visit by treatment using descriptive statistics.

Additionally, clinical chemistry, hematology, urinalysis, and endocrine (calcitonin) data outside the reference ranges will be listed.

If any safety lab measurements are (1) below the quantification limit (e.g. < QL), ½ × QL may be used for the calculation of summary statistics; (2) above the quantification limit (e.g. > QL), 1.1 × QL may be used for the calculation of summary statistics, if deemed appropriate.

6.11. Evaluation of Immunogenicity

Baseline immunogenicity sample is collected on Day 1 pre-dose.

For the TE-ADA+ patients, the distribution of maximum titers may be described.

The frequency and percentage of patients with preexisting antidrug antibodies (ADA) and with treatment-emergent ADA+ (TE-ADA+) to tirzepatide may be tabulated.

If cross-reactivity to native GLP-1 and GIP or neutralizing antibodies against native GLP-1 and GIP assays are performed, the frequency and percentage of each may be tabulated.

A listing will be provided of immunogenicity assessments. This includes the tirzepatide concentration from a simultaneous pharmacokinetic sample and the clinical interpretation result (ADA Present, ADA Not Present, ADA Inconclusive, Missing). A listing of TEAE for patients with TE ADA or Injection Site Reaction or Potential Hypersensitivity may be provided.

Cases of TE-ADA that are associated with AEs of either severe/serious hypersensitivity or severe/serious injection site reaction will be classified as AESI.

6.12. Interim Analyses

No interim analyses are planned for this study. Blinded data may be reviewed periodically on an ongoing basis. If an unplanned interim analysis is deemed necessary, the appropriate Lilly clinical pharmacologist, clinical research physician/investigator, or designee, will consult with the appropriate medical director or designee to determine whether it is necessary to amend the protocol.

7. Unblinding Plan

The blinding/unblinding plan is not part of this SAP. Approved blinding/unblinding plan is stored in eTMF.

8. Changes from the Protocol Specified Statistical Analyses

Changes from the protocol specified statistical analyses are as follows:

- Protocol Section 1 states "Safety analyses will be conducted for all enrolled patients whether or not they completed all protocol requirements". In this SAP, it is clarified that safety population includes all patients, who are exposed to at least 1 dose of the investigational product (tirzepatide, semaglutide, or placebo), regardless of whether they completed all protocol requirements.
- Protocol Section 10.3.1.2 states "For change from baseline values, a mixed-model repeated-measure (MMRM) method with treatment, visit, and treatment-by-visit interaction as fixed effects, patient as random effect, and baseline as covariate will be used. An unstructured variance-covariance matrix will be used to model within-patient effects." In this SAP, it is clarified that vital signs will only be summarized, and no MMRM analyses will be conducted.
- Protocol Section 10.3.3.2 states "Tirzepatide will be claimed to have a statistically significant greater change in cDI than placebo if lower limit of the 2-sided CI of tirzepatide – placebo on the log scale is greater than 0…". It is further specified as "Tirzepatide will be claimed to have a statistically significantly greater change in cDI than placebo if the lower limit of the 2-sided CI of (tirzepatide - placebo) on the log scale (or transformed back to original scale) is greater than 0."
- Protocol Section 4, under Secondary Objective "Insulin Secretion," it is stated in Endpoints column that "The change from baseline in insulin response to arginine (incremental $AUC_{arginine0\text{-}10min}$ and incremental $AUC_{arginine0\text{-}30\ min}$" from hyperglycemic clamp), in this SAP, it is changed to "The change from baseline in insulin response to arginine (incremental $insulin_{arginine0\text{-}10min}$ and incremental $insulin_{arginine0\text{-}30min}$) from hyperglycemic clamp" to be consistent with protocol Section 9.1.2.
- Protocol Section 4, under Exploratory Objective "Insulin Secretion," it is stated in Endpoints column that "The change in basal ISR (total $ISR_{-10\text{-}0min}$) prior to sMMTT." It is updated to "The change in basal ISR (basal ISR at 0 minute) prior to sMMTT."
- Protocol Section 4, under Exploratory Objective "Glucagon secretion and PP secretion," it is stated in Endpoints column that "The change from baseline in incremental glucagon concentration after arginine stimulation (incremental $AUC_{arginine0\text{-}30min}$)." In this SAP, it is clarified to be "The change from baseline in incremental glucagon concentration after arginine stimulation (incremental $glucagon_{arginine0\text{-}30min}$)" to be consistent with protocol Section 9.1.3.
- Protocol Section 4, under Exploratory Objective "Safety and tolerability," it is stated in Endpoints column that "Incidence and rate of hypoglycemia." It is updated to "Incidence of hypoglycemia" since it is considered sufficient for this study.
- Protocol Section 4, under Exploratory Objective "Insulin secretion", it is stated in the Endpoints column that "The change from baseline in basal ISR ($ISR_{[-10\text{-}0min]}$) from hyperglycemic clamp." It is updated to "The change from baseline in basal ISR from hyperglycemic clamp."

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10. Appendices

Appendix 1. Search Criteria For Special Safety Topics

The search criteria for AEs of special safety topics and AESIs are stored in CCI [redacted]

CCI

Approver: PPD

Approval Date & Time: 27-May-2021 20:18:23 GMT

Signature meaning: Approved