Statistical Analysis Plan I8F-MC-GPIN (Version 1.0)

A Study to Evaluate Tirzepatide Concentrations in Breastmilk Following Administration of Single Dose of Tirzepatide by Subcutaneous Injection in Healthy Lactating Females

NCT05978713

Approval Date: 26 JUN 2023

STATISTICAL ANALYSIS PLAN

A Study to Evaluate Tirzepatide Concentrations in Breastmilk Following Administration of Single Dose of Tirzepatide by Subcutaneous Injection in Healthy Lactating Females

Statistical Analysis Plan Status: Final
Statistical Analysis Plan Version: 1.0
Statistical Analysis Plan Date: 22 June 2023

Investigational Medicinal Product: Tirzepatide (LY3298176)

Protocol Reference: I8F-MC-GPIN
Labcorp Drug Development Study: 8505415

Clinical Phase I

1. TABLE OF CONTENTS

2. ABBREVIATIONS

Abbreviations pertain to the Statistical Analysis Plan (SAP) only (not the tables, figures and listings [TFLs]).

%AUC(t_{last}-∞)	Percentage of AUC(0-∞) extrapolated
AE	Adverse event
$Ae_{(t1-t2)}$	Amount of dose excreted in breastmilk within the time interval t1 to t2
AUC	Area under the concentration versus time curve
AUC(0-∞)	Area under the concentration versus time curve from time zero to infinity
AUC(0-t_{last})	Area under the concentration versus time curve from time zero to time t, where t is the last time point with a measurable concentration
BQL	Below the quantifiable lower limit of the assay
CL/F	Apparent total body clearance of drug calculated after extra-vascular administration
C_{last}	Last quantifiable drug concentration
C_{max}	Maximum observed drug concentration
CRF	Case Report Form
CRU	Clinical Research Unit
CSR	Clinical Study Report
CV	Coefficient of variation
DMP	Data Management Plan
ECG	Electrocardiogram
$Fe_{(t1-t2)}$	Fraction of dose excreted in breastmilk within the time interval t1 to t2
ICF	Informed consent form
ICH	International Conference on Harmonisation
LLOQ	Lower limit of quantification
M/P	Milk to plasma ratio
MedDRA	Medical Dictionary for Regulatory Activities
MRE	Magnetic resonance elastography
PG	Plasma Glucose
PK	Pharmacokinetic

SAP	Statistical Analysis Plan
SD	Standard deviation
$t_{1/2}$	Half-life associated with the terminal rate constant (λ_z) in non-compartmental analysis
TFLs	Tables, Figures, and Listings
t_{max}	Time of maximum observed drug concentration
V_z/F	Apparent volume of distribution during the terminal phase after extra-vascular administration
WHO	World Health Organization

3. INTRODUCTION

This SAP has been developed after review of the Clinical Study Protocol (final version dated 17 May 2023).

This SAP describes the planned analysis of the safety, tolerability and pharmacokinetic (PK) data from this study. A detailed description of the planned TFLs to be presented in the clinical study report (CSR) is provided in the accompanying TFL shell document.

The intent of this document is to provide guidance for the statistical and PK analyses of data. In general, the analyses are based on information from the protocol, unless they have been modified by agreement with Eli Lilly and Company. A limited amount of information concerning this study (e.g., objectives, study design) is given to help the reader's interpretation. This SAP must be finalized prior to first participant visit. When the SAP and TFL shells are agreed upon and finalized, they will serve as the template for this study's CSR.

This SAP supersedes the statistical considerations identified in the protocol; where considerations are substantially different, they will be so identified. If additional analyses are required to supplement the planned analyses described in this SAP, they may be performed and will be identified in the CSR. Any substantial deviations from this SAP will be agreed upon with Eli Lilly and Company and identified in the CSR. Any minor deviations from the TFLs may not be documented in the CSR.

This SAP is written with consideration of the recommendations outlined in the International Conference on Harmonisation (ICH) E9 Guideline entitled Guidance for Industry: Statistical Principles for Clinical Trials[1] and the ICH E3 Guideline entitled Guidance for Industry: Structure and Content of Clinical Study Reports[2].

4. STUDY OBJECTIVES AND ENDPOINTS

Objectives	Endpoints
Primary	
To evaluate tirzepatide PK in breastmilk of healthy lactating females	Area under the tirzepatide concentration-time curve in breastmilk from time zero extrapolated to infinity [$AUC_{0-\infty}$]
Secondary	
CCI	

5. STUDY DESIGN

This is a postmarketing, single-site, open-label, single-treatment arm, single-dose, lactation study, assessing PK of tirzepatide in breastmilk and plasma in healthy lactating women.

Approximately 14 healthy lactating females will be enrolled so that about 8 females complete the study.

Participants will receive a single dose of tirzepatide CCI delivered by subcutaneous injection.

The study duration for individual participants, inclusive of screening, is expected to be approximately 8 weeks.

Screening

Screening may occur up to 27 days prior to Day -1. During the screening visit, participants will undergo screening tests as mentioned in the protocol. Participants who are not enrolled within 28 days of screening may be subject to an additional medical assessment, clinical measurements, or both, to confirm their eligibility. In such instances, at a minimum, the following screening tests and procedures should be repeated:

- weight
- vital signs
- safety 12-lead electrocardiogram (ECG)
- clinical laboratory tests, and
- pregnancy test.

Inpatient Treatment Period

Participants will be admitted to the clinical research unit (CRU) on Day -1 to establish baseline measurements.

Participants will receive a single subcutaneous (SC) tirzepatide CCI dose on Day 1 following overnight fasting of approximately 8 hours. Participants will remain in the CRU until Day 5 following the completion of study procedures. The inpatient stay for the participant may be extended at the clinical discretion of the investigator (for example, necessary for the participant's clinical wellbeing and management and monitoring of adverse events (AEs)).

Breastmilk and plasma samples for tirzepatide PK will be collected. Breastmilk volume will be measured across the study duration as indicated in the protocol. Safety will be assessed by physical examination, medical assessments or both, vital signs, ECGs, safety laboratory tests, glucose safety monitoring, and the recording of AEs. Breastfeeding must be discontinued prior to the administration of tirzepatide and for the duration of the study.

Outpatient Follow-up Period

On Days 6, 8, 15 ± 1, and 22 ± 1, participants may either return to the CRU for outpatient visits or have these visits be performed remotely by home health nurses as deemed appropriate by the study investigator. On Day 29 ± 1, the participants will return to the CRU for the final follow-up visit.

6. BLINDING

This is a non-randomized, open-label study.

7. TREATMENT

The study treatment name "CCI tirzepatide SC" will be used in the TFLs.

A footnote stating "Abbreviations: SC = subcutaneous" will be utilized.

8. SAMPLE SIZE JUSTIFICATION

Approximately CCI participants will be enrolled so that about [redacted] participants provide evaluable PK data. Since inter-participant variability of primary PK parameter in breastmilk is unknown, sample size is not chosen to achieve a specific precision in estimation of the primary endpoint.

9. DEFINITION OF ANALYSIS POPULATIONS

The "Enrolled" population will consist of all participants who signed the informed consent form (ICF), regardless of whether they take any doses of Tirzepatide.

The "Safety" population will consist of all participants who received a single SC dose of tirzepatide, whether or not they completed all protocol requirements. Participants will be analyzed according to the intervention they actually received.

The "Pharmacokinetic" population will consist of all participants who received a single SC dose of tirzepatide and have evaluable PK samples.

All protocol deviations that occur during the study will be considered for their severity/impact and will be taken into consideration when participants are assigned to analysis populations.

10. STATISTICAL METHODOLOGY

10.1 General

Data listings will be provided for all data that is databased. Summary statistics and statistical analysis will only be presented for data where detailed in this SAP. For continuous data, summary statistics will include the arithmetic mean, arithmetic standard deviation (SD), median, minimum, maximum, and number of observations; for log-normal data (e.g., the PK parameters: area under the concentration versus time curves (AUCs) and C_{max}) the geometric mean and geometric coefficient of variation (CV%) will also be presented. For categorical data, frequency count and percentages will be presented. Data listings will be provided for all participants up to the point of withdrawal, with any participants excluded from the relevant population highlighted.

Summary statistics and statistical analyses will generally only be performed for participants included in the relevant analysis population. For the calculation of summary statistics and statistical analysis, unrounded data will be used.

For change from baseline summary statistics, each individual change from baseline will be calculated by subtracting the individual participant's baseline value from the value at that time point. The individual participants' change from baseline values will be used to calculate the summary statistics (arithmetic mean, arithmetic SD, median, minimum, maximum, and number of observations) using a SAS procedure such as Proc Univariate.

Data analysis will be performed using SAS® Version 9.4 or greater.

10.2 Demographics and Participant Disposition

Participant disposition will be listed. The demographic variables age, sex, race, ethnicity, country of enrolment, site ID, body weight, height, and body mass index (BMI) will be summarized and listed. All other demographic variables will be listed only.

10.3 Pharmacokinetic Assessment

10.3.1 Pharmacokinetic Analysis

The following PK parameters will be determined where possible from the plasma and breast milk concentrations of tirzepatide (LY3298176) using noncompartmental methods in validated software program Phoenix WinNonlin (Certara, Version 8.1 or higher):

Parameter	Units[a]	Definition
$AUC_{(0\text{-}tlast)}$	h.ng/mL	area under the concentration-time curve from time 0 to the time of the last observable concentration (t_{last})[b]
$AUC_{(0\text{-}\infty)}$	h.ng/mL	area under the concentration-time curve from time 0 to infinity[b]
$\%AUC_{(tlast\text{-}\infty)}$	%	fraction of $AUC_{(0\text{-}\infty)}$ extrapolated
C_{max}	ng/mL	maximum observed concentration
t_{max}	h	time of the maximum observed concentration
t_{last}	h	time of the last quantifiable concentration
$t_{1/2}$	h	half-life associated with the terminal rate constant (λz) in non-compartmental analysis
CL/F	L/h	apparent total clearance (plasma only)
V_z/F	L	apparent volume of distribution during the terminal phase (plasma only)
M/P		Milk to plasma ratio, calculated as $AUC_{0\text{-}\infty,\ breastmilk}$ / $AUC_{0\text{-}\infty,\ plasma}$
$Ae_{(t1\text{-}t2)}$	mg	amount of dose excreted in breastmilk within the time interval t1 to t2
$Fe_{(t1\text{-}t2)}$	%	fraction of dose excreted in breastmilk within the time interval t1 to t2

[a] Units are based on concentration units (provided by the bioanalytical lab or preferred units for presentation of PK parameters) and dose units used in the study.
[b] The AUC will be calculated using a combination of the linear and logarithmic trapezoidal methods (linear-log trapezoidal rule)

Additional PK parameters may be calculated, as appropriate. The software and version used for the final analyses will be specified in the CSR. Any exceptions or special handling of data will be clearly documented within the final CSR.

Formatting of tables, figures and abbreviations will follow the Eli Lilly Global PK/PD/TS Tool: NON-COMPARTMENTAL PHARMACOKINETIC STYLE GUIDE. The version of the tool effective at the time of PK analysis will be followed.

General PK Parameter Rules

- Actual sampling times will be used in the final analyses of individual PK parameters, except for pre-dose sampling times which will be set to zero.

- The actual start times of each breastmilk collection will be used for the calculation of individual breastmilk PK parameters.

- C_{max} and t_{max} will be reported from observed values. If C_{max} occurs at more than one time point, t_{max} will be assigned to the first occurrence of C_{max}.

- AUC parameters will be calculated using a combination of the linear and logarithmic trapezoidal methods (linear-log trapezoidal rule). The linear trapezoidal method will be applied up to t_{max} and then the logarithmic trapezoidal method will be used after t_{max}. The minimum requirement for the calculation of AUC will be the inclusion of at least three consecutive concentrations above the lower limit of quantification (LLOQ), with at least one of these concentrations following C_{max}. $AUC_{(0-\infty)}$ values where the percentage of the total area extrapolated is more than 20% will be flagged. Any $AUC_{(0-\infty)}$ value excluded from summary statistics will be noted in the footnote of the summary table.

- Half-life ($t_{1/2}$) will be calculated, when appropriate, based on the apparent terminal log-linear portion of the concentration-time curve. The start of the terminal elimination phase for each participant will be defined by visual inspection and generally will be the first point at which there is no systematic deviation from the log-linear decline in plasma concentrations. Half-life will only be calculated when a reliable estimate for this parameter can be obtained comprising of at least 3 data points. If $t_{1/2}$ is estimated over a time window of less than 2 half-lives, the values will be flagged in the data listings. Any $t_{1/2}$ value excluded from summary statistics will be documented in the footnote of the summary table.

- A uniform weighting scheme will be used in the regression analysis of the terminal log-linear portion of the concentration-time curve.

- The parameters based on predicted last quantifiable drug concentration (C_{last}) will be reported (except in bioequivalence and bioavailability studies, where only the observed parameters will be reported).

Individual PK Parameter Rules

- Only quantifiable concentrations will be used to calculate PK parameters with the exception of special handling of certain concentrations reported below the lower limit of quantitation (BQL). Concentrations reported as BQL will be set to a value of zero when all of the following conditions are met:
    - The compound is non-endogenous.
    - The samples are from the initial dose period for a participant or from a subsequent dose period following a suitable wash-out period.
    - The time points occur before the first quantifiable concentration.
- All other BQL concentrations that do not meet the above criteria will be set to missing.
- Also, where two or more consecutive concentrations are BQL towards the end of a profile, the profile will be deemed to have terminated and therefore any further quantifiable concentrations will be set to missing for the calculation of the PK parameters unless it is considered to be a true characteristic of the profile of the drug.

Individual Concentration vs. Time Profiles

- Individual concentrations will be plotted utilizing actual sampling times.
- The terminal point selections will be indicated on a semi-logarithmic plot.

Average Concentration vs. Time Profiles

- The average concentration profiles will be graphed using scheduled (nominal) sampling times.
- The average concentration profiles will be graphed using arithmetic average concentrations.
- The pre-dose average concentration will be set to zero. Otherwise, only quantifiable concentrations will be used to calculate average concentrations.
- Concentrations at a sampling time exceeding the sampling time window specified in the protocol, or ± 10%, will be excluded from the average concentration profiles.
- Concentrations excluded from the mean calculation will be documented in the final CSR.
- A concentration average will be plotted for a given sampling time only if 2/3 of the individual data at the time point have quantifiable measurements that are within ± 10% of the nominal time. An average concentration estimated with less than 2/3 but more than 3 data points may be displayed on the mean concentration plot if determined to be appropriate and will be documented within the final CSR.

Treatment of Outliers during PK Analysis

Application of this procedure to all PK analyses is not a requirement. Rather, this procedure provides justification for exclusion of data when scientifically appropriate. This procedure describes the methodology for identifying an individual value as an outlier for potential exclusion but does not require that the value be excluded from analysis. The following methodology will not be used to exclude complete profiles from analysis.

Data within an Individual Profile

A value within an individual profile may be excluded from analysis if any of the following criteria are met:

- For PK profiles during single dosing of non-endogenous compounds, the concentration in a pre-dose sample is quantifiable.
- For any questionable datum that does not satisfy the above criteria, the profile will be evaluated and results reported with and without the suspected datum.

Data between Individual Profiles

1. If $n<6$, then the dataset is too small to conduct a reliable range test. Data will be analyzed with and without the atypical value, and both sets of results will be reported.
2. If $n\geq6$, then an objective outlier test will be used to compare the atypical value to other values included in that calculation:
    a. Transform all values in the calculation to the logarithmic domain.
    b. Find the most extreme value from the arithmetic mean of the log transformed values and exclude that value from the dataset.
    c. Calculate the lower and upper bounds of the range defined by the arithmetic mean ±3*SD of the remaining log-transformed values.
    d. If the extreme value is within the range of arithmetic mean ±3*SD, then it is not an outlier and will be retained in the dataset.
    e. If the extreme value is outside the range of arithmetic mean ±3*SD, then it is an outlier and will be excluded from analysis.

If the remaining dataset contains another atypical datum suspected to be an outlier and $n \geq 6$ following the exclusion, then repeat step 2 above. This evaluation may be repeated as many times as necessary, excluding only one suspected outlier in each iteration, until all data remaining in the dataset fall within the range of arithmetic mean ±3*SD of the log-transformed values.

Reporting of Excluded Values

Individual values excluded as outliers will be documented in the final CSR. Approval of the final CSR will connote approval of the exclusion.

10.3.2 Pharmacokinetic Statistical Methodology

The primary PK parameter for analysis will be $AUC_{(0-\infty)}$ in breast milk. Summary of $AUC_{(0-\infty)}$ and 95% confidence interval for the geometric mean will be provided.

Summaries of CCI [redacted] will be provided.

Additional parameters may be estimated as deemed necessary.

10.4 Safety and Tolerability Assessments

10.4.1 Adverse events

Where changes in severity are recorded in the Case Report Form (CRF), each separate severity of the AE will be reported in the listings, only the most severe will be used in the summary tables. A pre-existing condition is defined as a condition that starts before the participant has provided written informed consent and is ongoing at consent. A non-treatment emergent AE is defined as an AE which starts after informed consent but prior to dosing. A treatment-emergent AE (TEAE) is defined as an AE which occurs postdose or which is present prior to dosing and becomes more severe postdose.

All AEs will be listed. If the frequency of events allows, then the TEAEs will be summarized by severity and relationship to the study drug. The frequency (the number of AEs, the number of participants experiencing an AE and the percentage of participants experiencing an AE) of TEAEs will be summarized by Medical Dictionary for Regulatory Activities (MedDRA) (version is documented in the Data Management Plan [DMP]) system organ class and preferred term. The summary and frequency AE tables will be presented for all causalities and those considered related to the study drug by the investigator. Any serious AEs (SAEs) will be listed.

Discontinuations due to AEs will be listed. AE of special interest (AESI) will be listed, this includes.

- pancreatitis
- major adverse cardiovascular events
- deaths
- hypoglycemia (Level 2 and 3)
- thyroid malignancies and C-cell hyperplasia
- arrhythmias and cardiac conductive disorders
- hypersensitivity events

- severe injection-site reactions (ISRs), and
- severe gastrointestinal (GI) AEs.

10.4.2 Glucose Monitoring and Hypoglycemia

During the study, blood glucose concentrations will be monitored for safety assessments. Glucose data will be listed and summarized by timepoint together with changes from baseline, where baseline is defined as Day 1 predose.

Hypoglycemic events will be appropriately recorded in the CRF. In the case of a hypoglycemic event, the actual blood glucose value, if measured, will be recorded in the CRF, together with any treatments administered. Each category of hypoglycemic events (defined below) will be listed and summarized. Hypoglycemia is defined as follows:

Level 1 Hypoglycemia:

Glucose <70 mg/dL (3.9 mmol/L) and ≥54 mg/dL (3.0 mmol/L): Level 1 hypoglycemia can alert a person to take action such as treatment with fast-acting carbohydrates. Providers should continue to counsel participants to treat hypoglycemia at this glucose alert value.

Level 2 Hypoglycemia:

Glucose <54 mg/dL (3.0 mmol/L): This is also referred to as documented or blood glucose confirmed hypoglycemia with glucose <54 mg/dL (3.0 mmol/L). This glucose threshold is clinically relevant regardless of the presence or absence of symptoms of hypoglycemia.

Level 3 Hypoglycemia:

Severe hypoglycemia (in adults): A severe event characterized by altered mental and/or physical status requiring assistance for treatment of hypoglycemia. For example, participants had altered mental status and could not assist in their own care, were semiconscious or unconscious, or experienced coma with or without seizures; the assistance of another person was needed to actively administer carbohydrate, glucagon, or other resuscitative actions. Glucose measurements may not be available during such an event, but neurological recovery attributable to the restoration of glucose concentration to normal is considered sufficient evidence that the event was induced by a low glucose concentration.

- The determination of a hypoglycemic event as an episode of severe hypoglycemia, as defined above, is made by the investigator based on the medical need of the participant to have required assistance and is not predicated on the report of a participant simply having received assistance.

- If a hypoglycemic event meets the criteria of severe hypoglycemia, the investigator must record the event as serious on the AE CRF and report it to Lilly as an SAE.

Nocturnal Hypoglycemia:

Nocturnal hypoglycemia is a hypoglycemia event (including severe hypoglycemia) that occurs at night and presumably during sleep.

10.4.3 Concomitant medication

Concomitant medication will be coded using the WHO drug dictionary (version is documented in the DMP). Concomitant medication will be listed.

10.4.4 Clinical laboratory parameters

All clinical chemistry and hematology data will be summarized by time point and listed. Urinalysis data will be listed. Additionally, clinical chemistry, hematology, and urinalysis data outside the reference ranges will be listed and flagged on individual participant data listings.

Values recorded as $<x$, $\leq x$, $>x$, or $\geq x$ will be displayed in the listings as recorded. For the calculation of summary statistics, $<x$ and $\leq x$ values will be set to $0.5 \times x$, whereas $>x$ and $\geq x$ values will be set to $1.1 \times x$.

10.4.5 Vital signs

Vital signs data will be summarized by time point together with changes from baseline, where baseline is defined as the Day 1 predose assessment.

Values for individual participants will be listed.

10.4.6 Electrocardiograms

ECGs will be performed for safety monitoring purposes only and will not be presented. Any clinically significant findings from ECGs will be reported as an AE.

10.4.7 Hepatic Monitoring

If a participant experiences elevated laboratory parameters, as detailed in Section 8.2.10.1 of the protocol, additional tests will be performed to confirm the abnormality. Additional safety data may be collected if required, as defined in the protocol. Where applicable, the following will be presented.

The participants' liver disease history and associated person liver disease history data will be listed. Use of acetaminophen during the study, which has potential for 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 summarized, if deemed appropriate, and listed. 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 participant data listings.

10.4.8 Injection-Site Reactions

Injection-site reaction data (erythema, induration, categorical pain, pruritus, and edema) will be listed and summarized in frequency tables.

10.4.9 Other assessments

All other safety assessments not detailed in this section will be listed but not summarized or statistically analyzed.

10.4.10 Safety and Tolerability Statistical Methodology

No inferential statistical analyses are planned.

11. INTERIM ANALYSES

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

12. CHANGES FROM THE PROTOCOL SPECIFIED STATISTICAL ANALYSES

There were no changes from the protocol specified statistical analyses.

13. REFERENCES

1. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, ICH Harmonized Tripartite Guideline, Statistical Principles for Clinical Trials (E9), 5 February 1998.

2. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, ICH Harmonized Tripartite Guideline, Structure and Content of Clinical Study Reports (E3), 30 November 1995.

14. DATA PRESENTATION

14.1 Derived Parameters

Individual derived parameters (e.g., PK parameters) and appropriate summary statistics will be reported to three significant figures. Observed concentration data, e.g., C_{max}, should be reported as received. Observed time data, e.g., t_{max}, should be reported as received. Number of observations and percentage values should be reported as whole numbers. Median values should be treated as an observed parameter and reported to the same number of decimal places as minimum and maximum values.

14.2 Missing Data

Missing data will not be displayed in listings.

14.3 Insufficient Data for Presentation

Some of the TFLs may not have enough participants or data for presentation. If this occurs, the blank TFL shell will be presented with a message printed in the center of the table, such as, "No serious adverse events occurred for this study."

15. APPENDICES

Appendix 1: Document History

Status and Version	Date of Change	Summary/Reason for Changes
Final Version 1.0	NA	NA; the first version.

NA = not applicable

Signature Page for VV-CLIN-121654 v1.0

Approval	PPD 23-Jun-2023 11:30:49 GMT+0000
Approval	PPD 26-Jun-2023 08:28:01 GMT+0000
Approval	PPD 26-Jun-2023 16:00:13 GMT+0000
Approval	PPD 26-Jun-2023 16:57:01 GMT+0000

Signature Page for VV-CLIN-121654 v1.0

Approved on 26 Jun 2023 GMT