Statistical Analysis Plan: J2U-MC-YBAA (Version 2)

A Safety, Tolerability, Pharmacokinetic, and Pharmacodynamic Study of Single-and Multiple-Ascending Doses of LY3522348 in Healthy Participants

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STATISTICAL ANALYSIS PLAN

A Safety, Tolerability, Pharmacokinetic, and Pharmacodynamic Study of Single-and Multiple-Ascending Doses of LY3522348 in Healthy Participants

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Clinical Phase I

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2. ABBREVIATIONS

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

%AUC(t_{last}-∞)	Percentage of AUC due to extrapolation from the last quantifiable concentration to infinity
AE	Adverse event
A_e(0-24)	Amount of drug excreted unchanged between time zero and 24 hours post-dose
AIC	Akaike information criterion
AUC	Area under the concentration versus time curve
AUC(0-6)	Area under the concentration versus time curve from time zero to 6 h.
AUC(0-24)	Area under the concentration versus time curve from time zero to 24 hours
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
AUC(6-12)	Area under the concentration versus time curve from time 6 h to 12h.
AUC(12-24)	Area under the concentration versus time curve from time 12 h to 24 h.
AUC_τ	Area under the concentration versus time curve during one dosing interval
BQL	Below the quantifiable lower limit of the assay
CI	Confidence interval
CL/F	Apparent total body clearance of drug calculated after extra-vascular administration
CL_r	Renal clearance
C_{max}	Maximum observed drug concentration
CRU	Clinical Research Unit
CSR	Clinical Study Report
ECG	Electrocardiogram
F_e(0-24)	Fraction of dose excreted unchanged between time zero and 24 hours post-dose
FTT	Fructose tolerance test

HR	Heart rate
ICH	International Conference on Harmonisation
LI	Linearity index
LLOQ	Lower limit of quantification
LS	Least-squares
MAD	Multiple ascending dose
MedDRA	Medical Dictionary for Regulatory Activities
MR	Metabolic ratio
NOAEL	No-observed-adverse-effect level
PD	Pharmacodynamic
PK	Pharmacokinetic
PR	Pulse rate
QD	Once daily
QTcF	QT interval corrected using Fridericia's formula
R_A	Accumulation ratio
RR	Respiratory rate
SAD	Single ascending dose
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SD	Standard deviation
TEAE	Treatment-emergent adverse event
TFLs	Tables, Figures, and Listings
$t_{1/2}$	Half-life associated with the terminal rate constant (λ_z) in non-compartmental analysis
t_{max}	Time of maximum observed drug concentration
ULN	Upper limit of normal
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 14 July 2020), Protocol Amendment (a) (final version dated 08 September 2020), Protocol Amendment (b) (final version 04 March 2021), Protocol Amendment (c) (final version dated 11 August 2021), and SAP Version 1 (final version dated 28 October 2020).

This SAP describes the planned analysis of the safety, tolerability, pharmacokinetic (PK), and pharmacodynamic (PD) 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, PK and PD 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. 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

4.1 Primary Objective

The primary objective of the study is:

- To investigate the safety and tolerability of LY3522348 following single and multiple oral doses

The primary endpoints for the study are:

- Incidence of treatment-emergent adverse events (TEAEs) and serious adverse events (SAEs)
- Clinically significant changes in vital signs, safety laboratory parameters, and electrocardiograms (ECGs)

4.2 Secondary Objective

The secondary objective of the study is:

- To determine the PK of LY3522348 following single and multiple doses

The secondary endpoints of the study are:

- Area under the concentration versus time curve (AUC) from time zero to 24 hours (AUC[0-24]), AUC from time zero to infinity (AUC[0-∞]), maximum observed drug concentration (C_{max}), and time of maximum observed drug concentration (t_{max})

4.3 Exploratory Objectives

The exploratory objectives for the study are:

- To determine the PD of LY3522348 following single and multiple doses
- To investigate the effect of multiple doses of LY3522348 on the PK of midazolam

The exploratory endpoints for the study are:

- AUC(0-24) of fructose concentration over time following fructose tolerance test (FTT)
- For midazolam and its metabolite, 1'-hydroxymidazolam
    - AUC(0-24)
    - C_{max}
    - the ratio of 1'-hydroxymidazolam:midazolam

5. STUDY DESIGN

Study YBAA is a Phase 1, single site, randomized, investigator- and participant-blind, placebo-controlled, 2-part study in healthy participants. Part A is a single ascending dose (SAD) study and Part B is a multiple ascending dose (MAD) study with a drug-drug interaction component.

5.1 Part A

Single-ascending oral dose of LY3522348 or placebo will be administered in up to 6 cohorts. It is planned that each cohort will consist of 8 participants, 6 will receive LY3522348 and 2 will receive placebo. Cohort 6 is an optional cohort that may be assessed based on the available safety and PK data from the previous cohorts.

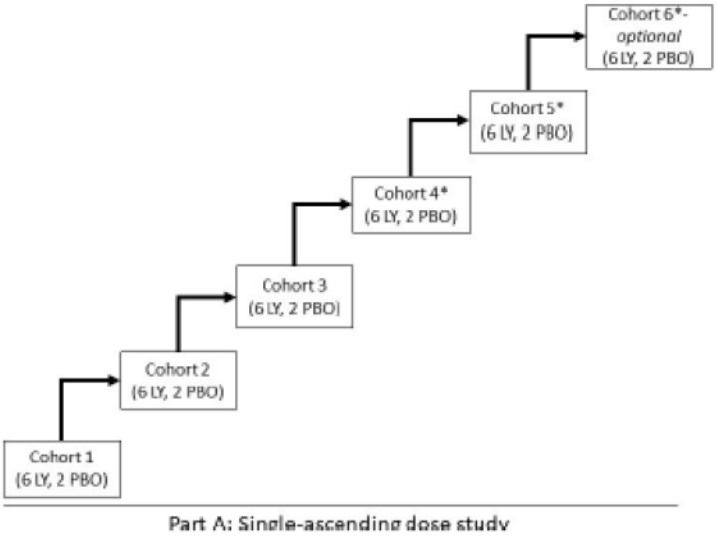
A sentinel dosing strategy will be utilized for Cohorts 4, 5, and 6 anticipated to have predicted mean exposure(s) between 8.1 µg hr/mL and 25.5 µg hr/mL. Two participants (1 LY3522348 and 1 placebo) in each cohort will receive the study drug (sentinel dose) on the same day. After at least 48 hours of safety monitoring post-sentinel dosing, the remaining participants in the

cohort will be dosed based on the available safety data from the 2 sentinel participants previously dosed.

A 7-day inpatient stay to allow intensive monitoring will be included for Cohorts 1 to 6.

A follow-up visit will occur approximately 14 days after the dose of study intervention.

The planned starting dose in Part A is 5 mg of LY3522348. Dose escalation will be based on safety and tolerability results from all previous cohorts and PK/PD information when available. Doses in the SAD study will escalate until the mean predicted AUC reaches approximately 25.5 µg·h/mL, which is 10-fold lower than the Day 1 exposure observed in the male rats at 250 mg/kg (this dose resulted in morbidity in rats) approximately 7.5-fold lower than the exposure observed at the rat no-observed-adverse-effect level (NOAEL). Dose escalation in the SAD study will not proceed if the predicted mean exposure, AUC(0-∞), in the next cohort is higher than 25.5 µg hr/mL. A dose of 400 mg is predicted to result in this exposure. Dose escalation to next cohort will be no more than 3.3-fold (approximately half-log). The current planned doses are 5, 15, 50, 150, and 400 mg, but may be adjusted to reach the desired exposure.



Abbreviations: * = Sentinel dosing; LY = LY3522348; PBO = placebo.

Figure 1: Scheme of study J2U-MC-YBAA for Part A

5.2 Part B

Part B will be initiated after assessing safety and tolerability through Cohort 4 and PK and PD data through Cohort 3 in Part A.

Multiple-ascending oral doses of either LY3522348 or placebo will be administered once daily (QD) for 14 days in Cohorts 1 and 2 and for 15 days in Cohorts 3 and 4. Each cohort will consist of 8 participants, 6 will receive LY3522348 and 2 will receive placebo.

In Cohorts 3 and 4, all participants will receive midazolam on Day -1 and study intervention co-administered with midazolam on Day 15.

A sentinel dosing strategy will be utilized for Cohorts 3 and 4, which are anticipated to result in the exposure(s) between 8.1 µg hr/mL and 19.1 µg hr/mL. Two participants (1 LY3522348 and 1 placebo) in each cohort will receive the study drug (sentinel dose) on the same day. After at least 48 hours of safety monitoring post sentinel dosing, the remaining participants in the cohort will be dosed based on the available safety data.

Participants will be discharged on Day 20 for Cohorts 1 and 2 and on Day 21 for Cohorts 3 and 4.

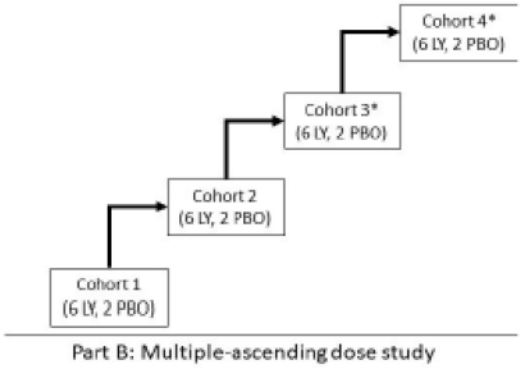
A 21-day inpatient stay to allow intensive monitoring will be included for Cohorts 3 and 4.

A follow-up visit will occur approximately 28 days after the first dose of study intervention.

The current proposed starting dose in Part B is 25 mg of LY3522348, which is predicted to result in plasma concentrations less than the half maximal inhibitory concentration. This dose may be modified based on safety and tolerability results and available PK/PD information in the SAD

study. Doses will be escalated until the mean predicted AUC reaches approximately 19.1 µg·hr/mL, which is 1/10th of the NOAEL exposure in rats in the 1-month toxicity study. Dose escalation to next cohort will be based on safety and tolerability results from all previous cohorts and PK/PD information when available and the dose increase will be no more than 3.3-fold (approximately half-log). The current presumed doses are approximately 25, 75, 150, and 300 mg but may be adjusted to reach the desired exposure.

A general schema for Part B can be seen in Figure 2.



Abbreviations: * = Sentinel dosing; LY = LY3522348; PBO = placebo.

Figure 2: Scheme of study J2U-MC-YBAA for Part B

6. TREATMENTS

The following is a list of the study treatment names that will be used in the TFLs.

Part	Cohort	Study Treatment	Treatment order in TFL
A	All	Placebo	1
	1	5 mg LY3522348	2
	2	15 mg LY3522348	3
	3	50 mg LY3522348	4
	4	150 mg LY3522348	5
	5	400 mg LY3522348	6
	6	TBD mg LY3522348	7
B	1 and 2	Placebo QD	1

3 and 4	Placebo QD + Midazolam (Days -1 and 15)	2
1	Up to 25 mg LY3522348 QD	3
2	Up to 75 mg LY3522348 QD	4
3	Up to 150 mg LY3522348 QD + Midazolam (Days -1 and 15)	5
4	Up to 300 mg LY3522348 QD + Midazolam (Days -1 and 15)	6

Abbreviation: TBD = To be decided

Actual dose administered will be used for all outputs and CSR.

7. SAMPLE SIZE JUSTIFICATION

The sample size for Parts A and B of the study was chosen to provide sufficient data for evaluating safety, tolerability, and PK parameters, as well as PD and other exploratory objectives of this study.

A maximum of 100 participants will be randomly assigned to study intervention such that approximately:

- 48 evaluable participants complete Part A of the study
- 32 evaluable participants complete Part B of the study.

To ensure that enough participants complete the study, participants who discontinue from the study, for reasons other than an adverse event (AE) suspected to be related to study intervention, may be replaced as agreed between the sponsor and investigator. The replacement participant will assume the randomization schedule of the discontinued participant.

8. DEFINITION OF ANALYSIS POPULATIONS

The "Safety" population will consist of all enrolled participants, irrespective of the completion of all protocol requirements.

The "Pharmacokinetic" population will consist of all subjects who received at least one dose of study drug and have evaluable PK data. If a subject has an AE of vomiting that occurs before 2 times the median t_{max} after dosing, then that subject may be excluded from the PK summary statistics and statistical analysis.

The "Pharmacodynamic" population will consist of all subjects who received at least one dose of study drug or placebo and have evaluable PD data. If a subject has an AE of vomiting that occurs before 2 times the median t_{max} after dosing, then that subject may be excluded from the PD summary statistics and statistical analysis.

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

9. STATISTICAL METHODOLOGY

9.1 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, min, max and N; for log-normal data (e.g. the PK parameters: AUCs and C_{max}) the geometric mean and geometric coefficient of variation will also be presented. For categorical data, frequency count and percentages will be presented. Data listings will be provided for all subjects up to the point of withdrawal, with any subjects excluded from the relevant population highlighted. Summary statistics and statistical analyses will generally only be performed for subjects included in the relevant analysis population. For the calculation of summary statistics and statistical analysis, unrounded data will be used.

Mean change from baseline is the mean of all individual subjects' change from baseline values. Each individual change from baseline will be calculated by subtracting the individual subject's baseline value from the value at the timepoint. The individual subject's change from baseline values will be used to calculate the mean change from baseline using a SAS procedure such as Proc Univariate.

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

9.2 Demographics and Subject Disposition

Subject disposition will be listed. The demographic variables age, sex, race, ethnicity, body weight, height and body mass index will be summarized and listed. All other demographic variables will be listed only.

9.3 Pharmacokinetic Assessment

9.3.1 Pharmacokinetic Analysis

Part A

PK parameter estimates will be determined using non-compartmental procedures in a validated software program (Phoenix WinNonlin Version 8.1 or later).

Plasma concentrations of LY3522348 will be used to determine the following PK parameters, when possible:

Parameter	Units	Definition
AUC(0-6)	µg.h/mL	area under the concentration versus time curve from time zero to 6 h.
AUC(0-24)	µg.h/mL	area under the concentration versus time curve from time zero to 24 hours post-dose during one dosing interval (i.e. 24 h)
AUC(6-12)	µg.h/mL	area under the concentration versus time curve from time 6 h to 12 h.
AUC(12-24)	µg.h/mL	area under the concentration versus time curve from time 12 h to 24 h.
AUC(0-t_{last})	µg.h/mL	area under the concentration versus time curve from time zero to time t, where t is the last time point with a measurable concentration
AUC(0-∞)	µg.h/mL	area under the concentration versus time curve from time zero to infinity
%AUC(t_{last}-∞)	%	percentage of AUC due to extrapolation from the last quantifiable concentration to infinity
C_{max}	µg/mL	maximum observed drug concentration
t_{max}	h	time of maximum observed drug concentration
$t_{1/2}$	h	half-life associated with the terminal rate constant (λz) in non-compartmental analysis
CL/F	L/h	apparent total body clearance of drug calculated after extra-vascular administration
V_Z/F	L	apparent volume of distribution during the terminal phase after extra-vascular administration

Urine concentrations of LY3522348 will be used to determine the following PK parameters, when possible:

Parameter	Units	Definition
A_e(0-24)	mg	amount of drug excreted unchanged between time zero and 24 hours post-dose
F_e(0-24)	%	fraction of dose excreted unchanged between time zero and 24 hours post-dose
CL_r	L/h	renal clearance

Part B

Plasma concentrations of midazolam and 1'-hydroxymidazolam will be used to determine the following PK parameters, when possible:

Parameter	Units	Definition
AUC(0-t_{last})	µg.h/mL	area under the concentration versus time curve from time zero to time t, where t is the last time point with a measurable concentration
AUC(0-∞)	µg.h/mL	area under the concentration versus time curve from time zero to infinity
%AUC(t_{last}-∞)	%	percentage of AUC(0-∞) extrapolated
C_{max}	µg/mL	maximum observed drug concentration
t_{max}	h	time of maximum observed drug concentration
$t_{1/2}$	h	half-life associated with the terminal rate constant (λz) in non-compartmental analysis
CL/F	L/h	apparent total body clearance of drug calculated after extra-vascular administration (midazolam only)
V_Z/F	L	apparent volume of distribution during the terminal phase after extra-vascular administration (midazolam only)
MR_{AUC}		metabolic ratio[a] bases on AUC(0-∞) (1'-hydroxymidazolam only)
MR_{Cmax}		metabolic ratio[a] bases on C_{max} (1'-hydroxymidazolam only)

[a] no molar correction will be applied since the molecular weight of 1'-hydroxymidazolam is within 5% of the molecular weight for midazolam.

Plasma concentrations of LY3522348 will be used to determine the following PK parameters, when possible:

Parameter	Units	Definition
AUC(0-6)	µg.h/mL	area under the concentration versus time curve from time zero to 6 h[a].
AUC(6-12)	µg.h/mL	area under the concentration versus time curve from time 6 h to 12 h[a].
AUC(12-24)	µg.h/mL	area under the concentration versus time curve from time 12 h to 24 h[a].
AUC_{τ}	µg.h/mL	area under the concentration versus time curve during one dosing interval (i.e. 24 h)
$AUC(0\text{-}t_{last})$	µg.h/mL	area under the concentration versus time curve from time zero to time t, where t is the last time point with a measurable concentration[a].
$AUC(0\text{-}\infty)$	µg.h/mL	area under the concentration versus time curve from zero to infinity (after last dose only) [b]
$\%AUC(t_{last}\text{-}\infty)$	%	percentage of AUC due to extrapolation from the last quantifiable concentration to infinity (after last dose only) [b]
C_{max}	µg /mL	maximum observed drug concentration
t_{max}	h	time of maximum observed drug concentration
$t_{1/2}$	h	half-life associated with the terminal rate constant (λz) in non-compartmental analysis
CL/F	L/h	apparent total body clearance of drug calculated after extra-vascular administration
V_Z/F	L	apparent volume of distribution during the terminal phase after extra-vascular administration (LY3522348 only)
R_A		accumulation ratio (based on C_{max} and AUC_{τ})[b]
LI		linearity index[b]

[a] Cohorts 1 and 2 Days 1 and 14, Cohorts 3 and 4 Days 1, Day 14 and 15
[b] Cohorts 1 and 2 Day 14, Cohorts 3 and 4 Day 15.

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 study report.

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 non-bolus pre-dose sampling times which will be set to zero. For non-bolus, multiple dose profiles, the pre-dose time will be set to zero unless a time deviation falls outside of the protocol blood collection time window which is considered to impact PK parameter derivation.

- 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 plasma concentrations above the lower limit of quantification (LLOQ), with at least one of these concentrations following C_{max}. AUC(0-∞) values where the percentage of the total area extrapolated is more than 20% will be flagged. Any AUC(0-∞) 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 subject 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 drug concentration 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). Plasma 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 subject 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 for single-dose data from non-endogenous compounds 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 study report.
- 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 the sampling time window specified in the protocol or ± 10%. 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 study report.

Treatment of Outliers during Pharmacokinetic 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.

<u>Data within an Individual Profile</u>
A value within an individual profile may be excluded from analysis if any of the following criteria are met:

- For PK profiles during multiple dosing, the concentration of the pre-dose sample exceeds all measured concentrations for that individual in the subsequent post-dose samples.

- 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≥6, 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 ≥ 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 report. Approval of the final report will connote approval of the exclusion.

9.3.2 Pharmacokinetic Statistical Methodology

The descriptive statistics for the PK parameters will be provided for each dose level and summarized by part and day. Where appropriate, geometric mean and coefficient of variation will be reported.

Dose proportionality will be assessed separately for Parts A (Day 1), and B (Cohorts 1 and 2 Day 14, Cohort 3 Day 15). Log-transformed C_{max} (both parts), AUC(0- t_{last}) (both parts), AUC(0-∞)

(both parts), AUC(0-24) (Part A only), and AUC_{τ} (Part B only) parameters of LY3522348 after the first dose will be evaluated using a power model (where log-dose acts as an explanatory variable) to estimate ratios of dose-normalized geometric means and corresponding 90% confidence intervals (CIs). The estimated ratio of dose-normalized geometric means of PK parameters between the highest and lowest doses will be used to assess dose proportionality. A subinterval within the highest and lowest doses may also be considered for assessment of dose proportionality using the same approach. Between subject estimates will also be provided.

Example of the SAS code for the analysis:

```
proc mixed data=xxx;
model log_pk = log_dose / alpha=0.1 cl solution outpred=resids ddfm=kr;
estimate 'xx mg' intercept 1 log_dose yy / alpha=0.1 cl; /*Log value of xx*/
estimate 'zz mg - xx mg' log_dose pp / alpha=0.1 cl; /*Difference in log
values of zz and xx*/
ods output solutionf=est;
ods output estimates=estims;
run;
```

For Part B Cohorts 3 and 4, to assess the effect that LY3522348 has on the midazolam and 1'- hydroxymidazolam PK parameters C_{max}, AUC(0- t_{last}), AUC(0- ∞), and MR, a linear fixed effect model will be fit to the logged parameters of those subjects assigned to receive LY3522348. The model will include Day as a fixed effect. The difference in least-squares (LS) day means to compare (Day 15 – Day-1), and, hence, LY3522348 + midazolam to midazolam alone, along with the 90% CIs will be back-transformed to produce the ratio of geometric means and corresponding CIs. The p-value will also be reported.

Example of SAS code as follows:

```
proc mixed data=xxx;
by profile param
class day;
model log_pk = day /residual ddfm=kr;
lsmeans day / cl pdiff alpha=0.1;
ods output lsmeans=lsm diffs=estims;
run;
```

The PK parameter T_{max} for those assigned to receive LY3522348 will be analysed non-parametrically with medians, the median of the differences, comparing Day 15 (test) to Day -1 (reference), and corresponding 90% CI presented alongside the p-value from the Wilcoxon signed-rank test. Example of SAS code as follows:

```
proc univariate data = xxx cipctldf(alpha = 0.1);
 by param;
 var ref test dif;
 ods output quantiles = quant;
 ods output testsforlocation = location;
run;
```

Additionally, to assess the effect that LY3522348 has on the midazolam and 1'- hydroxymidazolam PK parameters C_{max}, AUC(0- t_{last}), AUC(0- ∞), and MR, a linear fixed

effect model will be fit to the logged ratio of Day 15 to Day -1. The model will include treatment as a fixed effect, and baseline as a covariate. The difference in LS treatment means to compare LY3522348 to placebo, along with the 90% CIs will be back-transformed to produce the ratio of geometric means and corresponding CIs. The p-value will also be reported.

Example of SAS code as follows:

```
proc mixed data=xxx;
by profile param
class treat;
model log_ratiopk = treat log_base /residual ddfm=kr;
lsmeans treat / cl pdiff alpha=0.1;
ods output lsmeans=lsm diffs=estims;
run;
```

The PK parameter T_{max} for the ratio of Day 15 to Day -1 will be analysed non-parametrically with medians, the median of the differences, comparing LY3522348 (test) to placebo (reference), and corresponding 90% CI presented alongside the p-value from the Wilcoxon rank-sum test.

Example of SAS code as follows:

```
proc npar1way data = xxx alpha = 0.1;
 class trtan;
 var pk_ratio;
 exact wilcoxon;
 ods output wilcoxontest = out;
run;
```

9.4 Pharmacodynamic Assessment

9.4.1 Pharmacodynamic Analysis

Plasma concentrations of fructose will be used to determine the following PD parameters, when possible:

Parameter	Units	Definition
AUC(0-24)	ng.h/mL	area under the concentration versus time curve from time zero to 24 h
AUC(0-6)	ng.h/mL	area under the concentration versus time curve from time zero to 6 h.
AUC(6-12)	ng.h/mL	area under the concentration versus time curve from 6 h to 12 h.
AUC(12-24)	ng.h/mL	area under the concentration versus time curve from 12 h to 24 h.

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

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 PD Parameter Rules

- Actual sampling times will be used in the final analyses of individual PD parameters.
- AUC parameters will be calculated using linear trapezoidal methods.
- When calculating individual fructose parameters, all concentrations reported as BQL will be set to 0.
- If the data on start or end timepoints (*i.e.,* 0 hr, 6 hr, 12 hr, 24 hr) was not reported for an individual, the mean of the population at that timepoint will be used. If data at any other time point are not reported for an individual, the AUC(0-24) and the partial AUC including the missing time point will not be calculated for that individual.

The individual observed and mean concentration-time profiles of fructose will be plotted by treatment group.

Individual Fructose Concentration vs. Time Profiles

- All concentrations reported as BQL will be set to 0.
- Individual concentrations will be plotted utilizing actual sampling times.

Average Fructose Concentration vs. Time Profiles

- The average concentration profiles will be graphed using scheduled (nominal) sampling times.
- When calculating average concentrations, all individual samples listed as BQL will be set to 0.

- The average concentration profiles will be graphed using arithmetic average concentrations.

- Concentrations excluded from the mean calculation will be documented in the final study report.

9.4.2 Pharmacodynamic Statistical Methodology

PD data will be summarized by day using descriptive statistics. Where appropriate, geometric mean and coefficient of variation will be reported.

In Parts A and B separately, the fructose tolerance test and biomarker parameters AUC(0-24), AUC(0-6), AUC(6-12), and AUC(12-24) will be log-transformed prior to analysis. In Part A, a linear fixed effect model will be fitted to the data. The model will include treatment as a fixed effect. In Part B, a repeated measures linear mixed effect model will be used. The model will include treatment, day, and treatment-by-day interaction as fixed effects, with subject included as a random effect. An unstructured covariance structure will be used to model the correlation between a subject's multiple observations. An alternative structure to be used if the model fails to converge, with an information criterion such as the AIC used to decide which structure is used. The difference in LS treatment means for both (LY3522348– Placebo) and between the different LY3522348 dose levels, along with the 90% CIs, will be back-transformed to produce the ratio of geometric means and the CIs comparing LY3522348 to Placebo by day. The p-value will also be reported.

Example of SAS (for Part B) code as follows:

```
proc mixed data=xxx;
class treatment day subjid;
model PD = treatment day day*treatment/residual ddfm=kr;
repeated day / subject=subjid type=un;
lsmeans treatment*day / cl pdiff alpha=0.1;
ods output lsmeans=lsm diffs=estims;
run;
```

9.5 Safety and Tolerability Assessments

9.5.1 Adverse events

Where changes in severity are recorded in the Case Report Form, 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 an AE that starts before the subject 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 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. TEAEs will be summarized by part, treatment, severity and relationship to the study drug. The frequency (the number of AEs, the number of subjects experiencing an AE

and the percentage of subjects experiencing an AE) of TEAEs will be summarized by part, treatment, Medical Dictionary for Regulatory Activities (MedDRA) version 23.0 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 SAEs will be listed. For Part B, AEs by day of onset will be presented.

Discontinuations due to AEs will be listed.

9.5.2 Concomitant medication

Concomitant medication will be coded using the World Health Organization (WHO) drug dictionary (Version MAR20B3). Concomitant medication will be listed.

9.5.3 Clinical laboratory parameters

All clinical chemistry and hematology data will be summarized by part, parameter and treatment group together with changes from baseline, where baseline is defined as the Day 1 predose assessment, 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 subject data listings.

9.5.4 Serum Creatinine

For Part A, all serum creatinine data will be summarized by treatment, together which change from baseline, where baseline is defined as Day 1 predose. Additionally, the data will be listed.

9.5.5 Glomerular Filtration Rate

For Part A, Glomerular filtration rate will be summarised and listed by treatment, together with change from baseline, where baseline is defined as Day 1 predose. Glomerular filtration rate will be calculated using the CKD-EPI formula[3] which is as follows

$$eGFR = 141 \min(S_{Cr}/k, 1)^{\alpha} \times 141 \times \max(S_{Cr}/k, 1)^{-1.209} \times 0.993^{age} \times 1.018Fem \times 1.159BLA,$$

where S_{Cr} is the standardized serum creatinine in mg/dL, k=0.7 if female, or k=0.9 if male, α=-0.329 if female, or α=-0.411 if male, age is in years, and FEM and BLA are indicator variables that are 1 if the subject is female and black respectively, and 0 if not.

9.5.6 Vital signs

Vital signs data will be summarized by part and treatment group together with changes from baseline, where baseline is defined as the Day 1 predose assessment. Figures of mean vital signs and mean changes from baseline profiles will be presented by treatment.

Values for individual subjects will be listed.

9.5.7 Electrocardiogram

The ECG data will be obtained directly from the 12-lead ECG traces. These data include the pulse rate (PR), QT, respiratory rate (RR), QRS duration and heart rate (HR). In addition, QT interval corrected using Fridericia's formula (QTcF) will be calculated as follows:

$$QTcF = \frac{QT}{\sqrt[3]{\left(60/HR\right)}}$$

The mean of triplicate data will be used for reporting. The ECG data will be summarized by part and treatment group together with changes from baseline, where baseline is defined as the mean of the triplicate Day 1 predose assessments (-1.5, -1 and -0.5 hours). Figures of mean ECG data and mean changes from baseline will be presented by part and treatment. The frequency of subjects with a maximum increase from baseline in QTcF interval will be summarized for each treatment according to the following categories: >30 ms and >60 ms. In addition, the frequency of subjects QTcF postdose values, according to the following categories: >450 ms, >480 ms and >500 ms, will be summarized by Part and treatment.

Plasma PK Concentration versus delta and double delta ECG parameter analysis

A plasma LY3522348 concentration-ECG parameter analysis will be performed to assess the relationship between changes from baseline (mean of Day 1 predose triplicate assessments) in ECG parameters (QTcF, PR, and RR intervals, QRS duration, and HR) and plasma LY3522348 concentrations across all treatments. The change from baseline adjustment will be based on the mean of the individual subject's Day 1 -1.5h, -1h, and -0.5h values. Further details on how these will be calculated:

- Calculate the baseline ECG value for each participant, which is the mean of ECG parameter values of each individual participant over 3 predose time points at day 1.
- Calculate the change from baseline at each timepoint for each individual participant.
- Calculate the mean ECG parameter value across all participants at baseline.
- For each participant subtract the mean ECG parameter value from their own individual observed ECG parameter value. This will be each participant's centered ECG parameter value.

The relationship between LY3522348 concentrations and ECG parameters will be explored graphically by plotting delta ECG parameter values against LY3522348 concentrations, including all post dosing timepoints.

A mixed effects analysis model will be employed with change from baseline in ECG parameter as the dependent variable, LY3522348 concentration and baseline ECG parameter value as continuous covariates, treatment, time, and day (Part B only) as categorical factors, and a random intercept and slope per subject. Treatment will be fitted as a binary variable (Placebo, or LY3522348). The model (for Part A) will have the form

$$\Delta ECG_{ijk} = (\theta_0 + \eta_{0,i}) + \theta_{1j} TRT_j + (\theta_2 + \eta_{2,i}) C_{ijk} + \theta_{3k} TIME_k + \theta_4 (ECG_{i,j,k=0} - \overline{ECG_{k=0}}) + \varepsilon_{ijk},$$

where ΔECG_{ijk} is the change from baseline in ECG parameter for subject i on treatment j (Placebo, or LY3522348) at time k, θ_0 is the population mean intercept in the absence of treatment effect, $\eta_{0,i}$ is the random effect associated with the intercept term θ_0 for each subject, θ_{1j} is the fixed effect categorical variable associated with treatment TRT_j , θ_2 is the population mean slope of the assumed linear association between concentration and ΔECG_{ijk}, $\eta_{2,i}$ is the random effect associated with the slope θ_2 for each subject, C_{ijk} is the concentration for subject i in treatment j and time k, θ_{3k} is the fixed effect associated with time k, θ_4 is the fixed effect associated with baseline $ECG_{i,j,k=0}$, $\overline{ECG_{k=0}}$ is the overall mean of baseline $ECG_{i,j,k=0}$ of all subjects, and ε_{ijk} is the residual error. It will be assumed the random effects are multivariate Gaussian distributed with mean vector **0** and an unstructured covariance matrix G, whereas the residuals, ε_{ijk}, are Gaussian distributed with mean 0 and variance r.

In Part B, the model will change to

$$\Delta ECG_{ijkl} = (\theta_0 + \eta_{0,i}) + \theta_{1j} TRT_j + (\theta_2 + \eta_{2,i}) C_{ijkl} + \theta_{3k} TIME_k + \theta_{4l} DAY_l + \theta_5 TIME_k \times DAY_l + \theta_6 (ECG_{i,j,k=0,l=0} - \overline{ECG_{k=0,l=0}}) + \varepsilon_{ijkl},$$

where ΔECG_{ijkl} is the change from baseline in ECG parameter for subject i on treatment j (Placebo, or LY3522348) at time after dose k, on day l. θ_0 is the population mean intercept in the absence of treatment effect, $\eta_{0,i}$ is the random effect associated with the intercept term θ_0, θ_{1j} is the fixed effect categorical variable associated with treatment TRT_j , θ_2 is the population mean slope of the assumed linear association between concentration and ΔECG_{ijk}, $\eta_{2,i}$ is the random effect associated with the slope θ_2, C_{ijkl} is the concentration for subject i in treatment j, time k, on day l, θ_{3k} is the fixed effect associated with time k on same day as dose, θ_{4l} is the fixed effect associated to day l, θ_5 is the fixed effect associated with the interaction between time and day, θ_6 is the fixed effect associated with baseline $ECG_{i,j,k=0,l=0}$, $\overline{ECG_{k=0,l=0}}$ is the overall mean of $ECG_{i,j,k=0,l=0}$ (the mean of all the baseline ECG parameter values, at day 0), and ε_{ijkl} is the residual error. It will be assumed the random effects are multivariate Gaussian distributed with mean vector **0** and an unstructured covariance matrix G, whereas the residuals, ε_{ijkl}, are Gaussian distributed with mean 0 and variance r. If the model fails to converge, a different covariance structure may be used, random effects may reduce to fixed effects, or the model may be reduced to that of Part A.

The predicted mean change from baseline and placebo-corrected change from baseline in ECG parameter (ΔECG and ΔΔECG respectively) at the observed geometric mean C_{max} of each treatment (slope estimate * C_{max} + treatment effect) and two-sided 90% CI at different dose levels will be calculated. Residual plots will be produced to assess the adequacy of the model.

Example of SAS code (for Part A) as follows:

```
proc mixed data=xxx;
by param;
class treat time subject;
model ΔECG = treat time baseline_ECG PKconc / solution cl alpha=0.1 ddfm=kr;
```

```
random intercept PKconc / type=un subject=subject solution;
estimate 'Placebo ' intercept 1 treat 1 0 PKconc 0/ CL alpha=0.1;
estimate 'XX mg LY3522348' intercept 1 treat 0 1 PKconc [cmax XXmg] / CL
alpha=0.1;
estimate 'YY mg LY3522348' intercept 1 treat 0 1 PKconc [cmax YYmg] / CL
alpha=0.1;
estimate 'YY mg LY3522348- Placebo' treat -1 1 PKconc [cmax XXmg] / CL
alpha=0.1;
ods output covparms=covp(where=(covparm="Residual"));
ods output solutionF=sol;
ods output estimates=estim;
run;
```

Example of SAS code (for Part B) as follows:

```
proc mixed data=xxx;
by param;
class treat time day subject;
model ΔECG = treat time day baseline_ECG PKconc / solution cl alpha=0.1
ddfm=kr;
random intercept PKconc / type=un subject=subject solution;
estimate 'Placebo ' intercept 1 treat 1 0 PKconc 0/ CL alpha=0.1;
estimate 'XX mg LY3522348' intercept 1 treat 0 1 PKconc [cmax XXmg] / CL
alpha=0.1;
estimate 'YY mg LY3522348' intercept 1 treat 0 1 PKconc [cmax YYmg] / CL
alpha=0.1;
estimate 'YY mg LY3522348- Placebo' treat -1 1 PKconc [cmax XXmg] / CL
alpha=0.1;
ods output covparms=covp(where=(covparm="Residual"));
ods output solutionF=sol;
ods output estimates=estim;
run;
```

9.5.8 Hepatic Monitoring

If a subject experiences elevated alanine aminotransferase ≥3× upper limit of normal (ULN), alkaline phosphatase ≥2× ULN, or elevated total bilirubin ≥2× ULN, liver 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 subjects' liver disease history and associated person liver disease history data will be listed. Any concomitant medications that have potential for hepatotoxicity, including acetaminophen will be listed. Results from any hepatic monitoring procedures, such as a magnetic resonance elastography 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 by treatment 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 subject data listings.

9.5.9 Other assessments

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

9.5.10 Safety and Tolerability Statistical Methodology

No inferential statistical analyses are planned.

10. INTERIM ANALYSES

No interim analyses are planned for this study.

11. CHANGES FROM THE PROTOCOL SPECIFIED STATISTICAL ANALYSES

There were no changes from the protocol specified statistical analyses.

12. 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.

3. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med. 2009;150(9):604-612

13. DATA PRESENTATION

13.1 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. N 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.

13.2 Missing Data

Missing data will not be displayed in listings.

13.3 Insufficient Data for Presentation

Some of the TFLs may not have sufficient numbers of subjects 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.”

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