Statistical Analysis Plan I8Z-MC-APCA

A Randomized, 9-Way, Single-Dose, Crossover Study to Evaluate the Pharmacokinetics, Pharmacodynamics, Safety, and Tolerability of LY3185643 and rGlucagon in Healthy Subjects

NCT02951780

Approval Date: 19-Oct-2016

STATISTICAL ANALYSIS PLAN

A Randomized, 9-Way, Single-Dose, Crossover Study to Evaluate the Pharmacokinetics, Pharmacodynamics, Safety, and Tolerability of LY3185643 and rGlucagon in Healthy Subjects

Statistical Analysis Plan Status: Final
Statistical Analysis Plan Date: 18-October-2016

Study Drug: LY3185643

Sponsor Reference: I8Z-MC-APCA
Covance CRU Study: 1000071-8340902

Clinical Phase I

Approval Date: 19-Oct-2016 GMT

1. TABLE OF CONTENTS

2. ABBREVIATIONS

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

AE	Adverse event
AUC	Area under the concentration versus time curve
C_{max}	Maximum observed drug concentration
CI	Confidence interval
CRF	Case Report Form
CSR	Clinical Study Report
CRU	Clinical Research Unit
CV	Coefficient of variation
EC	Early Clinical
ECG	Electrocardiogram
e.g.	For example (Latin: *exempli gratia*)
ICH	International Council on Harmonisation
MedDRA	Medical Dictionary for Regulatory Activities
PD	Pharmacodynamic
PK	Pharmacokinetic
SAP	Statistical Analysis Plan
SC	Subcutaneous
SD	Standard deviation
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

3. INTRODUCTION

This SAP has been developed after review of the Clinical Study Protocol (final version dated 17 August 2016).

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, PD and PK analyses of data. In general, the analyses are based on information from the protocol, unless they have been modified by agreement between Eli Lilly and Company and Covance Early Clinical (EC) Biometrics. 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 signed off prior to first subject administration for this study. 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 between Eli Lilly and Company and Covance EC Biometrics 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 Council 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

Primary Objective

The primary objective of this study is to evaluate the PK and PD profiles of LY3185643 and rGlucagon after single doses of study treatment.

Secondary Objective

To evaluate the safety and tolerability of LY3185643 after single doses of study treatment.

Exploratory Objective

To evaluate immunogenicity of LY3185643 after multiple single doses of study treatment.

5. STUDY DESIGN

This study is a Phase 1, single-center, subject- and investigator-blind, randomized, 9-way, 3-period crossover study in healthy subjects to evaluate the PK and PD of LY3185643 and rGlucagon after subcutaneous (SC) administration.

Up to 27 healthy men and women may be enrolled to target approximately 18 subjects to complete the study. Each subject will receive 3 doses on each of 3 dosing days (total of 9 doses: 5 doses of LY3185643 and 4 doses of rGlucagon) administered SC. Doses will be administered in a 9-way complete crossover design in 3 periods, and subjects will be randomized to predefined treatment sequences. Completers are defined as subjects who have received all 9 doses.

Subjects will be required to attend the Clinical Research Unit (CRU) on at least 5 occasions:

- 1 screening visit (may occur up to 28 days before randomization)
- 3 treatment periods
- 1 follow-up visit (at least 30 days after completing the last dose of study treatment)

Each treatment period will consist of 3 days (Days -1 to 2). Subjects should return for the next period with a minimum of 3 days between treatment periods. On Day -1, subjects will be admitted to the CRU. Subjects will be provided a carbohydrate-rich meal in the evening at approximately 10:00 PM and will remain fasted until completion of the study procedures on Day 1. On Day 1, study drug will be administered SC as follows:

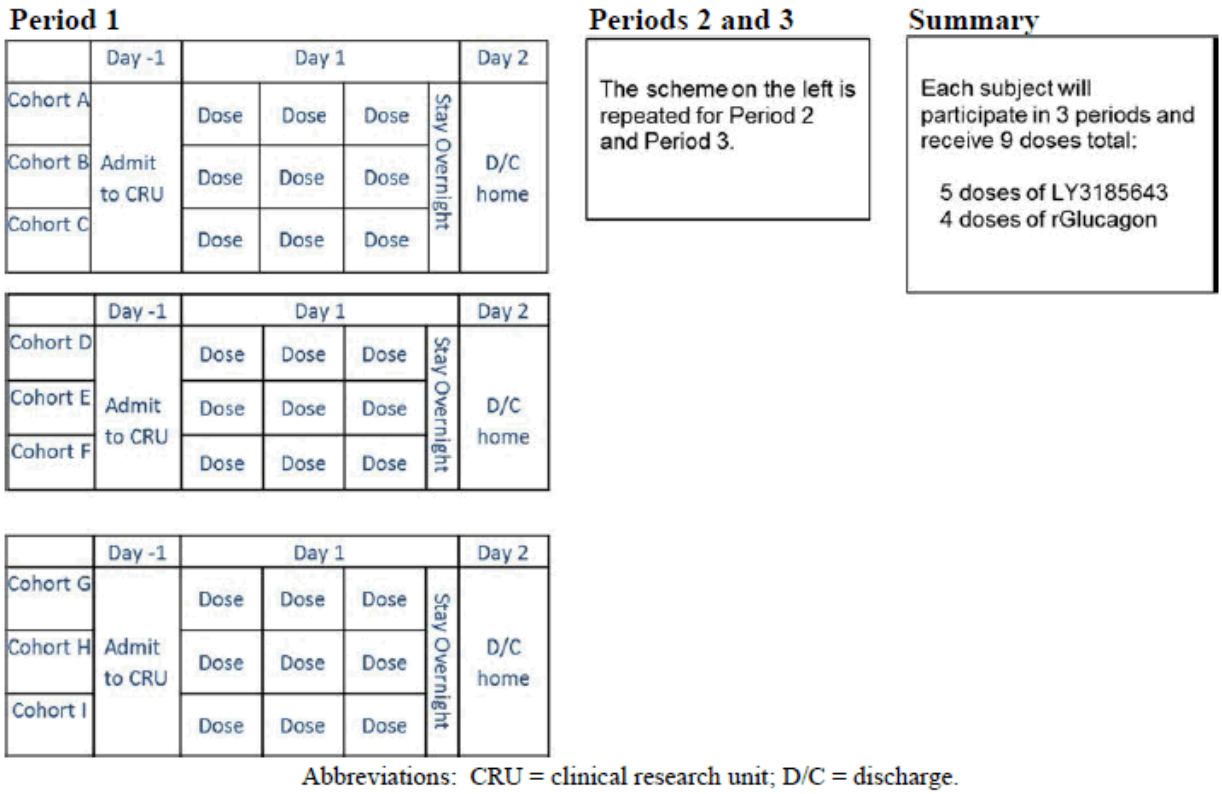
- Dose 1: morning of Day 1
- Dose 2: approximately 4 hours after administration of the first dose
- Dose 3: approximately 4 hours after administration of the second dose

Blood samples will be collected for the determination of concentrations of LY3185643, glucagon, glucose, C-peptide, and for immunogenicity as shown in the Schedule of Activities. Blood samples for measurement of glucose concentrations using YSI glucose analyzer will also be collected.

Safety data will include clinical examinations, vital signs, electrocardiograms (ECGs; including telemetry, holter monitoring), clinical laboratory tests, concomitant medications and a record of adverse events (AEs).

All study procedures will end approximately 4 hours after the third dose. Subjects will be provided dinner and stay overnight in the CRU for observation. Subjects will be discharged the following day (Day 2) after completion of all assessments as shown in the Schedule of Activities.

Figure 1 illustrates the study design:



Abbreviations: CRU = clinical research unit; D/C = discharge.

Figure 1.

Approximately 18 subjects are expected to complete the study. Each subject will be randomized to 1 of 9 treatment sequences comprising single SC doses of LY3185643 or rGlucagon (Table 1). Subjects who discontinue may be replaced to target at least 18 com pleters; the replacement subject will be assigned the treatment sequence of the discontinued subject and complete all 3 treatment periods (total of 9 doses).

Cohort[a]	Treatment Period 1			Treatment Period 2			Treatment Period 3		
A	Dose 1	Dose 9	Dose 7	Dose 6	Dose 4	Dose 8	Dose 5	Dose 3	Dose 2
B	Dose 2	Dose 7	Dose 8	Dose 4	Dose 5	Dose 9	Dose 6	Dose 1	Dose 3
C	Dose 3	Dose 8	Dose 9	Dose 5	Dose 6	Dose 7	Dose 4	Dose 2	Dose 1
D	Dose 4	Dose 6	Dose 2	Dose 3	Dose 9	Dose 1	Dose 8	Dose 7	Dose 5
E	Dose 5	Dose 4	Dose 3	Dose 1	Dose 7	Dose 2	Dose 9	Dose 8	Dose 6
F	Dose 6	Dose 5	Dose 1	Dose 2	Dose 8	Dose 3	Dose 7	Dose 9	Dose 4
G	Dose 7	Dose 1	Dose 4	Dose 9	Dose 2	Dose 6	Dose 3	Dose 5	Dose 8
H	Dose 8	Dose 2	Dose 5	Dose 7	Dose 3	Dose 4	Dose 1	Dose 6	Dose 9
I	Dose 9	Dose 3	Dose 6	Dose 8	Dose 1	Dose 5	Dose 2	Dose 4	Dose 7

Note: This is an example table; subjects will be assigned a treatment sequence according to the actual treatment schedule provided to the site.

Dose assignment: Dose 1 = 10 µg LY3185643; Dose 2 = 25 µg LY3185643; Dose 3 = 50 µg LY3185643; Dose 4 = 100 µg LY3185643; Dose 5 = 200 µg LY3185643; Dose 6 = 10 µg rGlucagon; Dose 7 = 25 µg rGlucagon; Dose 8 = 50 µg rGlucagon; Dose 9 = 200 µg rGlucagon.

[a] In each cohort, LY3185643 and rGlucagon will be administered.

Table 1.

6. TREATMENTS

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

Study Treatment Name	Treatment order in TFL
{Insert brief description of indication} 3185643	1
{Insert brief description of indication} 3185643	2
{Insert brief description of indication} 3185643	3
{Insert brief description of indication} 3185643	4
{Insert brief description of indication} 3185643	5
10 µg rGlucagon	6
25 µg rGlucagon	7
50 µg rGlucagon	8
200 µg rGlucagon	9

7. SAMPLE SIZE JUSTIFICATION

The sample size is customary for Phase 1 studies evaluating safety, PK, and/or PD parameters and is considered sufficient to evaluate the primary objective of this study.

Subjects who discontinue the study may be replaced so that at least 18 subjects complete the study. Replacement subjects will be assigned the treatment sequence of the subject that discontinued the study.

8. DEFINITION OF ANALYSIS POPULATIONS

The "Safety" population will consist of all subjects who received at least one dose of study drug, and have at least one postdose safety assessment.

The "Pharmacokinetic" population will consist of all subjects who received at least one dose of study drug and have evaluable PK data.

The "Pharmacodynamic" population will consist of all subjects who received at least one dose of study drug and have evaluable PD data.

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: area under the concentration versus time curve [AUCs] and maximum observed drug concentration [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 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 9.3 or greater.

9.2 Demographics and Subject Disposition

Subject disposition will be listed. The demographic variables age, sex, race, ethnicity, country of enrolment, site ID, body weight, height and body mass index will be summarized and listed.

9.3 Pharmacokinetic Assessment

9.3.1 Pharmacokinetic Analysis

PK parameter estimates for LY3185643 and rGlucagon will be calculated by standard noncompartmental methods of analysis.

The primary parameters for analysis will be C_{max} and AUC. Other noncompartmental parameters, such as $t_{1/2}$, apparent clearance, and apparent volume of distribution, may be reported. Population PK and exposure-response analyses may be performed using PK, PD and safety data.

PK parameters for LY3185643 and rGlucagon will be summarized for each dose group. Concentration-time profiles (mean and individual) will be generated. All PK data analyses will be the responsibility of the Lilly team.

9.3.2 Pharmacokinetic Statistical Methodology

PK parameter estimates (C_{max} and AUC) will be provided to Covance to delineate effects of dose proportionality for LY3185643 and rGlucagon separately using a power model as follows:

$$\log(PK_i) = \alpha + \beta.\log(dose_i) + \varepsilon_i$$

Where *i* represents the *i*th subject, PK represents a PK parameter (AUC or C_{max}), and ε_i is a residual error term. The estimated ratios of dose-normalized means

$$\hat{R}_{dnm} = \left(\frac{h}{l}\right)^{\hat{\beta}-1}$$

and the corresponding 90% confidence intervals will be reported, where *h* and *l* denote the highest and lowest doses, respectively.

An example of the SAS code that will be used is as follows:

```
/* Lack of fit test */
proc mixed data=<indata>;
   class trtmnt vol;
   model l_pk = logdose trtmnt / htype=1 ddfm=kr;
   random intercept / subject=vol;
   ods output tests1=tests;
run;

/* Power model */
```

```
proc mixed data=<indata>;
   class vol;
   model l_pk = logdose / solution cl alpha=0.1 oupred=resids ddfm=kr;
   estimate ' x mg' intercept 1 logdose a / alpha=0.1 cl; /* a=Log value of x*/
   estimate ' y mg' intercept 1 logdose b / alpha=0.1 cl; /* b=Log value of y*/
   estimate ' z mg' intercept 1 logdose c / alpha=0.1 cl; /* c=Log value of z*/
   estimate 'z mg - x mg' logdose d / alpha=0.1 cl; /* d = Difference in log values of highest
   and lowest dose*/
   random intercept / subject=vol;
   ods output solutionf=estimate;
   ods output estimates=estims;
run;
```

Where l_pk is the log-transformed (base e) PK parameter.

Additional analyses may be conducted as appropriate.

9.4 Pharmacodynamic Assessment

9.4.1 Pharmacodynamic Analysis

The PD time-action and response of LY3185643 and rGlucagon will be compared to establish the relative potency between LY3185643 and rGlucagon.

The primary PD response variables of interest include t_{max}, C_{max}, change from baseline in C_{max} and change from baseline to each time point, and incremental AUCs for glucose measured by YSI glucose analyzer and C-peptide. Similar PD response variables using laboratory measured glucose will also be estimated.

9.4.2 Pharmacodynamic Statistical Methodology

All PD parameters including the baseline-corrected parameters will be summarized by dose group and tabulated. The individual observed and mean (by dose group) time profile of the PD measurements after dosing will be plotted.

For these parameters, a linear model will be fitted to account for the PD difference between LY3185643 and rGlucagon. The model is detailed below. In addition, partial AUC for glucose over appropriate time intervals will be calculated using the trapezoidal rule. The AUC for each subject after each dose will also be baseline-adjusted. Baseline for each dose will be defined as the average of predose values for that dose.

For each PD parameter, the linear model that is proposed to account for differences between LY3185643 and rGlucagon is:

$$\boldsymbol{Y_{kl} = \beta_0 + \beta_1 d_{kl} + \beta_2 d_{kl} * Z_{kl} + u_k + e_{kl}} \qquad (1)$$

where k denotes the subject index, l denotes the period index, d_{kl} is the dose in mg during each period, Z_{kl} is the treatment indicator (0 for rGlucagon and 1 for LY3185643), $u_k \sim \text{NI}(0, \sigma_{u^2})$ denotes the between subject error, and $e_{kl} \sim \text{NI}(0, \sigma_{e^2})$ denotes the within-subject error. The

notation "NI" means "Normally Independent." There is no term for the treatment group indicator Z_{kl} because at dose = 0 the response should be the same for both treatment groups. Setting the mean responses for each treatment equal to each other, it follows that:

$$\beta_0 + \beta_1 D_1 + \beta_2 D_1 = \beta_0 + \beta_1 D_0$$

where D_0 denotes the rGlucagon dose and D_1 denotes the LY3185643 dose. It follows that $D_1 = \lambda D_0$ where $\lambda = \beta_1/(\beta_1 + \beta_2)$ is the conversion factor that determines how many mg of LY3185643 are equivalent to one mg of rGlucagon. The model (1) can be reparameterized as a nonlinear mixed model:

$$Y_{kl} = \beta_0 + \beta_1 d_{kl} + (1-\lambda)/\lambda\, \beta_1 d_{kl} * Z_{kl} + u_k + e_{kl}.$$

Then, the conversion factor λ (the point estimate and the 90% confidence interval) will be directly estimated from this nonlinear model. This model may be extended to allow for multiple parameter values, if deemed appropriate.

9.4.3 Glucose and C-peptide samples

Glucose concentrations from the PD sample and the YSI glucose analyzer samples, along with C-peptide data will be summarized by treatment together with changes from baseline, where baseline is defined as Day 1 predose of each dose within each period. The glucose and C-peptide data will be listed for individual subjects. In addition, partial AUC for the lab measured glucose and C-peptide over appropriate time intervals will be calculated using the trapezoidal rule. The AUC for each subject after each dose will also be baseline-adjusted. Baseline for each dose will be defined as the average of predose values for that dose.

9.4.4 Immunogenicity

The frequency of antibody formation to LY3185643 will be determined. If a neutralization assay is performed, the frequency of neutralizing antibodies will be determined. The relationship between the presence (or absence) of antibodies and AEs will be assessed. Likewise, the relationship between the presence of antibodies and the PK parameters and PD response to LY3185643 will be assessed. Any inferential analyses to explore the relationship between the presence (or absence) of antibodies and AEs will be the responsibility of the Lilly team.

9.5 Safety and Tolerability Assessments

9.5.1 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 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 treatment-emergent AE 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. Treatment-emergent AEs will be summarized by 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 treatment-emergent AEs will be summarized by treatment, Medical Dictionary for Regulatory Activities (MedDRA) version 19.0 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 . Any serious AEs will be tabulated.

9.5.2 Concomitant medication

Concomitant medication will be coded using the World Health Organization drug dictionary (Version March 2016). Concomitant medication will be listed.

9.5.3 Clinical laboratory parameters

All clinical chemistry, hematology and urinalysis data will be listed. Additionally clinical chemistry, hematology and urinalysis data outside the reference ranges will be listed.

Values for any clinical chemistry, hematology and urinalysis values outside the reference ranges will be flagged on the individual subject data listings.

9.5.4 Vital signs

Vital signs data will be summarized by treatment together with changes from baseline, where baseline is defined as Day 1 predose of each dose within each period. Figures of mean vital signs and mean changes from baseline profiles will be presented by treatment. Furthermore, values for individual subjects will be listed.

Vital signs data will be listed for individual subjects.

9.5.5 Holter monitoring

Continous 12-lead Holter recordings will be measured on Day 1 to extract 15 ECGs at -5, 30, 60, 120 and 180 minutes postdose for each Day 1 dose administration.

All Holter ECG parameters (PR, RR, QT, QTcF, QRS, Heart rate) will be summarized by treatment together with changes from baseline, where baseline is defined as Day 1 predose of each dose within each period. All Holter ECG data will be listed for individual subjects.

All Holter ECG interpretations will be from the central lab will be listed for individual subjects.

Exposure-response analysis of the change from baseline QTcF versus the drug concentration will be performed.

9.5.6 Glucose

Glucose will also be available for safety evaluation as well as glucose collected as part of the PD objective. Glucose will be summarized by treatment together with changes from baseline, where

baseline is defined as Day 1 predose of each dose within each period. The glucose data will be listed for individual subjects.

9.5.7 Other assessments

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

9.5.8 Safety and Tolerability Statistical Methodology

No inferential statistical analyses are planned.

10. INTERIM ANALYSES

No interim statistical analyses are planned.

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.

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 centre of the table, such as, “No serious adverse events occurred for this study.”

Leo Document ID = 3887a8d9-7221-4e8c-84b4-4b1be7f57c99

Approver: PPD
Approval Date & Time: 19-Oct-2016 15:55:06 GMT
Signature meaning: Approved

Approver: PPD
Approval Date & Time: 19-Oct-2016 16:01:55 GMT
Signature meaning: Approved

Approver: PPD
Approval Date & Time: 19-Oct-2016 17:35:53 GMT
Signature meaning: Approved