Statistical Analysis Plan

Protocol ZP4207-17145

A randomized, double-blind, parallel-group trial to confirm the clinical efficacy and safety of dasiglucagon in the rescue treatment of hypoglycemia in subjects with type 1 diabetes mellitus (T1DM) compared to placebo

Phase 3

Original Protocol:

Version 1.0: 22 June 2018

Version 2.0: 04 July 2018

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Senior Statistician, Statistics

On Behalf of ProSciento Inc.

Version: Final

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This statistical analysis plan has been reviewed and approved by:

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On Behalf of ProScientia Inc.

Table of Contents

List of Abbreviations

Abbreviation	Definition
ADA	Anti-drug antibody
AE	Adverse event
AESI	Adverse events of special interest
ANCOVA	Analysis of covariance
AUC	Area under the concentration-time curve
AUE	Area under the effect curve
BLQ	Below the limit of quantitation
CI	Confidence interval
C_{max}	Maximum plasma concentration
CPH	Cox proportional hazards
ECG	Electrocardiogram
FAS	Full analysis set
HbA1c	Glycated hemoglobin
IV	Intravenous(ly)
KM	Kaplan-Meier
MedDRA	Medical Dictionary for Regulatory Activities
PD	Pharmacodynamics
PK	Pharmacokinetics
PP	Per-protocol
SAE	Serious adverse event
SAP	Statistical analysis plan
SC	Subcutaneous(ly)
T1DM	Type 1 diabetes mellitus
TEAE	Treatment emergent adverse event
t_{max}	Time to the maximum plasma concentration

1 INTRODUCTION

This document describes the statistical methods and procedures to be implemented in the confirmation of clinical safety and efficacy of dasiglucagon in the rescue treatment of hypoglycemia in subjects with type 1 diabetes mellitus (T1DM) compared to placebo. This statistical analysis plan is based on study protocol ZP4207-17145 (Version 2.0, 04 July 2018). If the data suggest and warrant it, deviations from this plan will be considered. However, any deviations from this SAP must be substantiated by sound statistical rationale and documented in the final clinical study report.

2 STUDY OBJECTIVES

2.1 PRIMARY OBJECTIVE

The primary objective of this study is:

- To demonstrate superiority of dasiglucagon compared to placebo following a single subcutaneous (SC) 0.6 mg dose administered to subjects with T1DM with insulin-induced hypoglycemia.

2.2 SECONDARY OBJECTIVES

Secondary objectives of this study are:

- To evaluate the safety, immunogenicity and PK of dasiglucagon following a single SC dose administered to subjects with T1DM with insulin-induced hypoglycemia as compared to placebo.

2.3 TERTIARY/EXPLORATORY OBJECTIVES

Tertiary/Exploratory objective of this study is:

- To serve as a basis for explaining or supporting findings of primary analyses and for suggesting further hypotheses for later research.

3 STUDY OVERVIEW

3.1 STUDY DESIGN

The trial is a multi-center, randomized, parallel-group, double-blind, clinical efficacy and safety trial evaluating rescue treatment of insulin-induced hypoglycemia in subjects with T1DM.

Subjects will be randomized to receive a single SC dose of dasiglucagon 0.6 mg or matching placebo in a ratio of 3:1, as shown in Table 1. It is expected that up to 46 subjects will be randomized to have 40 subjects completing the dosing visit (Visit 2).

Table 1. Randomized Treatment Groups and Dose Regimens

Treatment Group	N	Dose Regimen and Injection Site
Dasiglucagon (liquid formulation, 1.0 mg/mL)	30	0.6 mL delivered in auto-injector
Placebo (liquid formulation)	10	0.6 mL delivered in auto-injector

Each subject will undergo a screening visit (Visit 1) up to 30 days prior to dosing, an overnight in-house visit (Visit 2), where dosing will take place, and a follow-up visit (Visit 3); the duration of participation for the subjects in this trial will approximately be 8 weeks. Additional follow-up visits may be required in case of the development of anti-drug antibodies (ADA). The trial will be conducted at up to 3 sites in North America. The following Figure 1 provides an overview of the trial design.

Figure 1. Trial design

3.2 TRIAL PROCEDURES

A screening visit (Visit 1) will be performed up to 30 days prior to the doing in order to identify eligible subjects. Prior to the in-house period (Visit 2), subjects will discontinue their current insulin therapy in due time to ensure wash-out. Subjects will check-in to the site on Day -1 for an overnight stay. Subjects' eligibility to continue will be checked through assessment of withdrawal criteria. On Day 1, after an overnight fast, and upon check of eligibility according to Dosing Day Criteria, subjects will be randomized to dasiglucagon or placebo. Hypoglycemic induction procedure will take place and the trial drug will be administered when the subject's plasma glucose level is 45-60 mg/dL. During the insulin-induced hypoglycemia, PK and PD samples will be drawn and plasma glucose levels will be monitored closely at site for safety

reasons. After the end of the procedure, subjects may be released from the site if deemed safe by the investigator.

The plasma glucose profile for evaluation of the primary and secondary clinical efficacy (pharmacodynamic) endpoints will be assessed based on plasma concentration data from samples collected at the dosing visit.

The exposure to trial drugs (dasiglucagon or placebo) for evaluation of pharmacokinetics will also be assessed based on plasma concentration data.

The following Table 2 provides an overview of the clinical schedule of procedures.

Table 2. Schedule of Procedures

Visit number	**V1**	**V2**	**V3**
Trial day	**-3**	**-1 and 1**	**28**
Visit type	**Screening**	**In-house stay/ Dosing on Day 1**	**Follow-up**
Window Procedure	**Day -30 to -3**		**+5 days**
Subject related info/assessments			
Informed consent	X		
Inclusion and exclusion criteria	X	X[1,2]	
Demographics	X		
Body measurements	X		
Medical history and concomitant illness	X		
Diabetes diagnosis and current diabetes treatment	X		
Previous and concomitant medication	X	X[1]	X
History of alcohol and drug abuse	X		
Randomization		X[1]	
Withdrawal criteria		X[1]	
Dosing day exclusion criteria		X[1]	
Insulin-induced hypoglycemia		X[1]	
Safety assessments			
Physical examination	X		X
Vital signs	X	X[3]	X
12-lead safety ECG	X	X[4]	X
Local tolerability		X[5]	X
Adverse events	X	X	X
Laboratory			
Biochemistry, hematology, coagulation, HbA1c (coagulation and HbA1c at Visit 1 only)	X	X[6]	X
Urinalysis	X	X[1]	X
Pregnancy test (only women of childbearing potential)	X[7]	X[1,8]	X[8]
Urine drug screen	X	X[1]	

Alcohol breath test	X	X[1]	
PK/Clinical efficacy			
Dasiglucagon PK sampling		X[9]	
PD sampling (plasma glucose)		X[10]	
Other assessments			
Antibodies against dasiglucagon		X[1]	X[11]
Plasma insulin		X[12]	
Trial material			
Administration of trial drug (during hypoglycemic clamp procedure)		X	

ECG = electrocardiogram; HbA1c = glycated hemoglobin; PD = pharmacodynamics; PK = pharmacokinetics.
[1]Prior to the start of the insulin-induced hypoglycemic procedure (randomization is to occur on day 1).
[2]Only check of dosing day exclusion criteria and changes between screening visit and Visit 2.
[3]Prior to the start of the insulin-induced hypoglycemic procedure (within 30 minutes), and at 30, 90 and 300 minutes after dosing.
The actual time of the assessment should not deviate from the nominal time by more than ±10 minutes.
[4]Prior to the start of the insulin-induced hypoglycemic procedure (within 30 minutes), and at 20, 35, 45, 60 and 300 minutes after dosing. The actual time of the assessment should not deviate from the nominal time by more than ±5 minutes.
[5]Local tolerability assessed at 30, 120, and 300 minutes after dosing. The actual time of the assessment should not deviate from the nominal time by more than ±10 minutes.
[6]Prior to the start of the insulin-induced hypoglycemic procedure (within 30 minutes), and at 30 and 300 minutes after dosing. The actual time of the assessment should not deviate from the nominal time by more than ±10 minutes.
[7]Serum pregnancy test.
[8]Urine stick pregnancy test.
[9]Pre-dose, and at 15, 30, 35, 40, 50, 60, 90, and 120 minutes after dosing. The actual time of blood sampling should not deviate from the nominal time by more than ±1 minute. Pre-dose is defined as within 2-5 minutes prior to dosing.
[10]Pre-dose, and at 4, 6, 8, 10, 12, 15, 17, 20, 25, 30, 40, 45, 50, 60, 75, 90 minutes after dosing. The actual time of blood sampling should not deviate from the nominal time by more than ±1 minute. Pre-dose is defined as within 2-5 minutes prior to dosing.
[11]Any subject that tests positive for ADA will be monitored until the ADA levels return to baseline levels.
[12]Pre-dose, and at 30 and 60 minutes after dosing. The actual time of blood sampling should not deviate from the nominal time by more than ±1 minute. Pre-dose is defined as within 2-5 minutes prior to dosing.

3.3 SAMPLE SIZE CONSIDERATION

From phase 2, the median time to an increase in plasma glucose of 20 mg/dL (1.1 mmol/L) of the 0.6 mg dose was approximately 10 minutes. For placebo-treated subjects, we assume that the median time to recovery will be more than 50 minutes. Assuming an exponential time-to-recovery distribution with median times of 10 and 50 minutes, a two-sided log-rank test will be able to detect a difference between dasiglucagon and placebo with 92% power with a follow-up time of 45 minutes at a 5% significance level with 40 subjects randomized 3 to 1 between active and placebo.

4 STUDY ENDPOINTS

4.1 PRIMARY ENDPOINTS

The primary endpoint is:

- Time to plasma glucose recovery. Plasma glucose recovery is defined as first increase in plasma glucose of ≥20 mg/dL (1.1 mmol/L) from baseline without administration of rescue intravenous (IV) glucose.

4.2 SECONDARY ENDPOINTS

Key secondary endpoints are:

- Plasma glucose recovery within 30 minutes, within 20 minutes, within 15 minutes, and within 10 minutes after trial drug injection without administration of rescue IV glucose.
- Plasma glucose changes from baseline at 30 minutes, at 20 minutes, at 15 minutes, and at 10 minutes after trial drug injection or at the time of rescue.

Clinical efficacy (PD) endpoints are:

- Time to first plasma glucose concentration ≥70 mg/dL (3.9 mmol/L) without administration of rescue IV glucose.
- Plasma glucose response as area under the effect curve (AUE) above baseline from time zero to 30 minutes, $AUE_{0\text{-}30min}$.

Exposure (PK) endpoints are:

- Area under the drug concentration curve from time zero to 90 minutes, $AUC_{0\text{-}90min}$.
- Area under the drug concentration curve from time zero to 120 minutes, $AUC_{0\text{-}120min}$.
- Maximum plasma drug concentration (C_{max}).
- Time to maximum plasma drug concentration (t_{max}).

4.3 SAFETY ENDPOINTS

Safety endpoints are:

- Adverse events.
- Clinical laboratory assessments (biochemistry, hematology, urinalysis).

- Local tolerability.
- Number of rescue infusions of IV glucose after trial drug administration.
- Time to first rescue infusion of IV glucose after trial drug administration.

Immunogenicity endpoint is:

- Occurrence of anti-drug-antibodies.

4.4 TERTIARY/EXPLORATORY ENDPOINTS

Tertiary/Exploratory endpoints include:

- Plasma glucose concentration ≥70 mg/dL (3.9 mmol/L) or increase of ≥20 mg/dL (1.1 mmol/L) within 30 minutes after trial drug injection without administration of rescue IV glucose.
- Plasma insulin response as area under the curve above baseline from time zero to 60 minutes, $AUC_{0\text{-}60\ min}$.

5 ANALYSIS POPULATIONS

The following analysis populations will be used in study summaries and analyses: Enrolled, Full analysis set (FAS), Safety, and Per-Protocol (PP).

5.1 ENROLLED

The Enrolled population will consist of all enrolled subjects. The Enrolled population will be used for data listings.

5.2 FULL ANALYSIS SET (FAS)

The Full analysis set (FAS) population will consist of all randomized subjects who receive at least one dose of trial drug. The FAS population will be used for the analysis of the primary endpoint, secondary endpoints, and tertiary endpoints which will be analyzed as randomized (using planned treatment).

5.3 SAFETY

The Safety population will consist of all randomized subjects who receive at least one dose of trial drug. The Safety population will be used for all safety analyses. Summary tables of baseline

and demographic data will also be calculated for the Safety population. Safety analyses will be performed by actual treatment received.

5.4 PER-PROTOCOL (PP)

The Per-Protocol (PP) population will consist all subjects of the FAS for whom no relevant protocol deviations were documented. The decision to exclude a subject or exclude specific data from a subject should be taken at the blinded data review meeting before unblinding, and the exclusion from efficacy or other analyses justified in the signed notes of the meeting or documented in a memo that will be finalized prior to database lock. Supportive analyses of the primary and key secondary efficacy endpoints will be based on the PP population. Additional exclusion and handling of data for the PP analysis is discussed in section 8.1.6.

6 INTERIM ANALYSIS

No interim analyses are planned.

7 STUDY SUBJECTS

7.1 SUBJECT DISPOSITION

Subject disposition will be presented for all randomized subjects. Counts and percentages of subjects who completed or discontinued from the study will be summarized by treatment group. Reasons for early discontinuation include the following categories: safety concern at discretion of investigator, withdrawal of consent by subject, study discontinuation by sponsor and other.

The number and percentage of randomized subjects will also be summarized by investigator site and treatment group.

7.2 DEMOGRAPHICS AND BASELINE CHARACTERISTICS

Demographics and baseline characteristics will only be assessed at screening (Visit 1). Demographics and baseline characteristics data will be summarized using descriptive statistics for, including but not limited to, age, sex, race and ethnicity, height, body weight, and body mass index (BMI). These summaries will be based upon the Safety analysis population.

7.3 CONCOMITANT ILLNESS AND MEDICAL HISTORY

Concomitant illness and medical history will be summarized with descriptive statistics (frequencies and percentages) by concomitant illness (or medical history) code and treatment group for the Safety population.

The date of diagnosis of diabetes will be recorded as will the current diabetes treatment (start date, product name(s), dose(s)) and will be listed.

7.4 INCLUSION/EXCLUSION CRITERIA

Inclusion and exclusion criteria failures will not be summarized but will be listed.

7.5 STUDY DRUG ADMINISTRATION

Subjects will receive their assigned single dose as SC injections. Study drug administration will be summarized and listed for the safety analysis set.

7.6 CONCOMITANT MEDICATION

The WHO DRUG Dictionary (September 2017) will be used to categorize verbatim descriptions of non-study medications into the Anatomic Therapeutic Chemistry (ATC) classification system. Each verbatim name will be classified by anatomical main group (ATC level 1), therapeutic subgroup (ATC level 2), pharmacological subgroup (ATC level 3), chemical subgroup (ATC level 4), and trade name.

The number and percentage of subjects receiving prior concomitant, new concomitant, and any concomitant medications will be summarized by treatment group and ATC classification (ATC level 2 and level 4) for the Safety Population. An additional table for concomitant medications summarized by ATC classification and generic drug name will be provided. In order to characterize the use and the change in the use of anti-diabetic medications, separate summaries will be provided for number of anti-diabetic medications (e.g., 0, 1, 2, ≥3) the subjects received at baseline, and number of subjects who have an increase, decrease, and no change in their diabetes treatment regimen during the 8-week treatment period.

Pre-treatment medications that start and stop prior to receiving randomized trial drug will be listed.

7.7 PROTOCOL DEVIATIONS

Protocol deviations (frequencies and percentages) will be summarized by treatment group for Safety population. All protocol deviations will be reviewed by clinical and statistical personnel to identify notable deviations (those anticipated to have an impact on efficacy or safety findings) prior to database lock. The identified protocol deviations may be used as one of the criteria to determine the subject's eligibility for inclusion in the PP Population.

8 STATISTICAL METHODS OF DATA ANALYSES

8.1 GENERAL CONSIDERATIONS

8.1.1 Statistical Notation and Presentation

In all calculations, zero will be substituted for concentrations below the quantification limit of the assay. All data collected from all subjects will be presented in data listings. Both absolute values and change from baseline values for each subject will be given where applicable. All continuous data will be listed with the same precision as will be presented in the database. Data listings will be sorted by treatment, subject ID and time point. A missing value will be represented by an empty cell and no imputation will be made.

Continuous data will be summarized in tables using number of subjects (n), mean, median, standard deviation (SD), standard error (SE), 95% confidence interval (CI; based on a t distribution if not otherwise stated), minimum and maximum by trial time point. For logarithm-transformed data, the geometric mean and standard error of the geometric mean will also be provided. Negative values and values of zero will be treated as missing when calculating the geometric statistics since these values are undefined when log-transformed. Categorical data will be summarized in two ways, by subject and by time point. Subject data will be summarized using the count of distinct subjects that fall in the category and the percentage of the total number of subjects. Time point data will be summarized using the count of the assessments that fall into the category and the percentage of the total number of assessments. Population counts (either number of subjects or number of time points at the assessment) will be used as the denominator in the calculation of percentages unless otherwise specified.

Min and max values will use the precision of the original value. Means, least squares (LS) means, and medians will be rounded to one decimal place greater than the precision of the original value. SDs, SEs, and 95% confidence intervals (CIs) will be rounded to two decimal places greater than the precision of the original value. Derived PK and PD data, such as Cmax, AUC, and AUE, will be presented with three significant figures. Percentages will be rounded to the nearest tenth. P-values will be presented with four decimal places and values less than 0.0001 will be presented as <.0001.

8.1.2 Hypothesis Testing and Multiplicity Adjustment

Define $median_{dasi}$ and $median_{placebo}$ as the median time to an increase in plasma glucose of 20 mg/dL of the 0.6 mg dose of dasiglucagon and placebo respectively. The hypotheses to be tested for the primary endpoint are:

$$H_0: median_{dasi} = median_{placebo}$$
$$H_a: median_{dasi} \neq median_{placebo}$$

Define $P_{dasi,i}$ and $P_{placebo,i}$, i=30, 20, 15, 10, as the proportion of subjects of dasiglucagon and placebo respectively with plasma glucose recovery within i minutes after trial drug injection without administration of rescue IV glucose. The hypotheses to be tested for the key secondary endpoints 1-4 are:

$$H_{0i}: P_{dasi,i} = P_{placebo,i}$$

$$H_{ai}: P_{dasi,i} \neq P_{placebo,i}$$

where i=30, 20, 15, 10.

Define $\mu_{dasi,i}$ and $\mu_{placebo,i}$, i=30, 20, 15, 10, as the mean plasma glucose change from baseline of dasiglucagon and placebo respectively at i minutes after trial drug injection or at the time of rescue. The hypotheses to be tested for the key secondary endpoints 5-8 are:

$$H_{0i}: \mu_{dasi,i} = \mu_{placebo,i}$$

$$H_{ai}: \mu_{dasi,i} \neq \mu_{placebo,i}$$

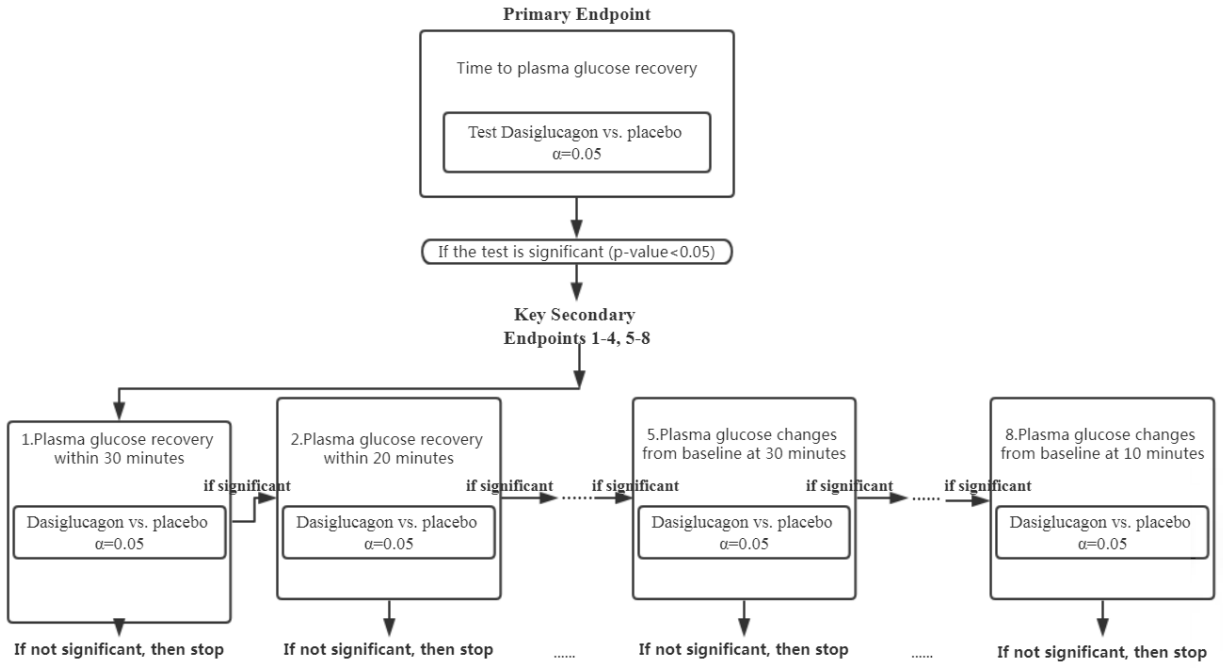
where i=30, 20, 15, 10.

A hierarchical procedure will be applied for the control of multiplicity. For the confirmatory analyses, the following *a priori* defined hierarchical inferential test order will be applied for the control of the type I error rate across the planned multiple comparisons. The primary endpoint and key secondary endpoints will be inferentially evaluated within the FAS in the following order, where inferential statistical testing will proceed at the two-sided 0.05 criterion significance level until the first failure to reject the null hypothesis for a dasiglucagon versus placebo comparison:

- Primary: Time to plasma glucose recovery
- Key secondary endpoints 1-4: Plasma glucose recovery within 30 minutes, within 20 minutes, within 15 minutes, and within 10 minutes after trial drug injection without administration of rescue IV glucose.
- Key secondary endpoints 5-8: Plasma glucose changes from baseline at 30 minutes, at 20 minutes, at 15 minutes, and at 10 minutes after trial drug injection or at the time of rescue.

The process is presented graphically in the following flow chart (Figure 2).

Figure 2. The Hierarchical Procedure of Key Secondary Endpoints



8.1.3 Study Baseline

For a particular study measurement, each subject's baseline value for that measurement will be determined by sorting all scheduled or unscheduled values prior to the dosing of randomized trial drug using the date (or date and collection time for laboratory values). The last value collected in chronological order will be defined as the baseline value for that measurement.

8.1.4 Handling of Multiple Observations

For scheduled visit such as follow-up, the latest measurement within the visit window will be used for data summary and analysis. All measurements will be listed.

8.1.5 Handling of Missing or Partial Dates

Date imputation will only be used for computational purposes; e.g., treatment-emergent status or identifying concomitant medications. Actual data values as they appear in the clinical database will be shown in the subject data listings.

8.1.5.1 Adverse Event Date Imputations

In cases of incomplete dates for adverse events (AEs), the missing component(s) will be assumed as the most conservative value(s) possible. For example, the imputation rule is to conservatively capture AEs with missing start dates as treatment emergent AEs (TEAEs) unless information of AE stop date/time was pre-treatment, which would automatically disqualify the treatment emergent status. Imputation of start dates for AEs with partial start dates is done for the purpose of identifying which AEs are treatment-emergent and to define what their onset dates are for the purpose of time to event analyses. The original values with no imputation applied will be presented in the data listings. The imputation rules follow.

- If "day" is the only missing field, impute the "day" as the randomized dose date if the month and year of randomization are equal to the AE start month and year; otherwise, impute the "day" as the first day of the AE start month.
- If "day" and "month" are missing, impute the "day" and "month" as the day and month of the randomized dose date if "year" is the same as the year of randomization; otherwise, impute January 1 of the non-missing year.
- Missing time will not be imputed. If "time" is missing and the start date is the same as the dose date, then the time is assumed to be after the dose time so that the event will be classified as treatment emergent.
- If the start date is completely missing:
    - and from the end date (either complete or partial date) it can be deduced to be prior to the dose date, then the AE will not be assigned as a TEAE.
    - and from the end date (either complete or partial date) it cannot be deduced to be prior to the dose date, then the AE will be assigned as a TEAE.

8.1.5.2 Medication Date Imputations

Imputation of start and end dates for medications partial dates is done for the purpose of classifying medications. The original values with no imputation applied will be presented in the data listings. The imputation rules follow.

For medications with partial start dates:

- If "day" is the only missing field, impute the "day" as the first of the month.
- If "day" and "month" are the missing fields, impute the "day" and "month" to January 1.
- Missing time will not be imputed. If the medication "time" is missing and the medication start date is the same as:
    - first dose date of randomized trial drug, then the medication is a new concomitant medication.

For a medication that is not checked as ongoing and the start date is completely missing:

- and from the end date (either complete or partial date) it can be deduced to be prior to the dose date of randomized trial drug, then medication is a pre-randomization medication.

8.1.6 Handling of Missing Efficacy Data and Subject Withdrawals

Missing safety data will be treated as missing with no imputation with the exception of date and time information for concomitant medications and adverse events as described in Section 8.1.5.1 and 8.1.5.2.

Missing efficacy data may occur when individuals receiving rescue IV glucose, discontinuation due to treatment or discontinuation in connection with the induced hypoglycemia state. Only if an intermediate assessment is missing independent of rescue treatment, trial drug or an adverse event including hypoglycemia, linear interpolation can be applied to impute a missing value. The same construct of imputation applies for the analysis of the plasma glucose $AUE_{0\text{-}30min}$. In the case of subject withdrawal, no imputation of values for PK or PD measurements will be done. Analyses will be done on valid cases only, i.e., no imputation techniques such as last observation carried forward will be applied.

Prior to database lock, the data will be reviewed during a blinded data review meeting where the decision to exclude subjects or exclude specific data will be made for the per protocol analysis. For the per protocol analysis, if a post dose pharmacodynamic glucose record during the first 45 minutes is out of window by more than 1 minute (i.e. ±2 minutes), then the record will be flagged and the deviation will be identified as an important deviation.

In the situation where data had been collected out of window by more than 1 minute, the following will be applied for the Per Protocol analysis only:

- Out of window records that exceed the window by more than 1 minute will be treated as missing and will be imputed with linear interpolation
- If the out of window record was taken later than expected, then the out of window record and the closest record collected just prior to the scheduled time of the record being imputed may be used for linear interpolation
- If the out of window record was taken earlier than expected, then the out of window record and the closest record collected after the scheduled time of the record being imputed may be used for linear interpolation
- The time point of the record being imputed must be within the time points of the records used for imputation

For the primary analysis using the FAS, the out of window records will be used as observed and will not be linearly interpolated.

8.1.7 Censored Data

Based on the analysis, there are multiple censoring methods to consider during analyses:

- In the primary analysis, subjects who require rescue IV glucose will be censored and time set to 45 min.
    - Sensitivity Analysis 1: subjects requiring rescue IV glucose will not be censored
    - Sensitivity Analysis 2: subjects requiring rescue IV glucose will be censored and time set to the administration of rescue IV glucose.
    - If recovery has not occurred at 45 minutes after study drug injection, censoring will be applied irrespective of the use of rescue IV glucose

- In the analysis of secondary efficacy endpoints, if the ≥70 mg/dL (3.9 mmol/L) endpoint is not met within 45 minutes post-dosing, the time of the last valid plasma glucose measurement up to 45 minutes will be the censoring time.

8.2 EFFICACY ANALYSES

8.2.1 Primary Endpoint

The primary endpoint will be evaluated on the FAS. The primary endpoint is time to plasma glucose recovery. Plasma glucose recovery is defined as first increase in plasma glucose of ≥20 mg/dL(1.1 mmol/L) from baseline without administration of rescue IV glucose. This will be summarized using Kaplan-Meier (KM) estimates stratified by treatment group and injection site.

In the primary analysis, recovery cannot be achieved in those subjects where IV glucose treatment is administered prior to recovery. Those subjects who receive IV glucose before 45 minutes will be censored (i.e. set to 'not recovered') at 45 minutes after dosing. Define T as the actual time to plasma glucose recovery of each subject. Assuming the data of the study are right-censored.

A Kaplan-Meier plot will be used to summarize the time to plasma glucose recovery analysis with the proportion of subjects at risk on the y-axis and time in minutes on the x-axis. Censored observations will be marked on the Kaplan-Meier curve.

The treatment group difference between dasiglucagon and placebo will be evaluated inferentially using a pairwise two-sided log-rank test.

As a supportive analysis to the stratified log-rank test for the time to plasma glucose recovery, dasiglucagon will be compared to placebo using a Wilcoxon test stratified by injection site.

To graphically describe recovery in time, a histogram showing the proportions of patients that recover within each time interval will be created, one for each treatment group.

If the site of injection does not appear to have an effect on time to recovery, the factor can be disregarded and further presentations will not be stratified by injection site.

8.2.1.1 Sensitivity Analyses to the Primary Endpoint

In sensitivity analyses, the time to plasma glucose recovery will be analyzed 1) without censoring those subjects who require rescue IV glucose before 45 minutes and 2) with censoring at the time of administration of rescue IV glucose before 45 minutes.

8.2.1.2 Additional Analyses for Inferences

The primary endpoint will additionally be analyzed using a Cox proportional hazards (CPH) time to event statistical model, if the hazards by treatment group can be assumed proportional using a graphical comparison of log-minus-log survival curves and no more than two-thirds of the recovery times are censored in each treatment group.

Under the proportional hazard assumption, the primary endpoint will be analyzed using a Cox proportional hazards (CPH) time to event statistical model, with treatment group and injection site modeled as categorical effects, and baseline plasma glucose modeled as a continuous covariate.

The treatment group rate ratio (dasiglucagon versus placebo) will be estimated together with the 95% profile confidence intervals.

Treatment group inferences (dasiglucagon vs placebo) will be evaluated using the two-sided likelihood ratio test.

Due to the discrete nature of blood sampling in time, the true recovery time is unmeasured. The method using “exact discrete” partial likelihood for tied failure times will be applied. The factor of injection site will be evaluated in the proportional hazards model by a type 3 test.

To obtain an estimate of the true, but unmeasured, recovery times, a linear interpolation between the time points before and after recovery is observed will be carried out to estimate the patients’ actual time of recovery. These derived recovery times will be described and analyzed in the same manner as for the discrete times.

8.2.2 Key Secondary Endpoints

The key secondary endpoints will be evaluated on the FAS. The key secondary endpoints are in this order:

(1) Plasma glucose recovery within 30 minutes, within 20 minutes, within 15 minutes, and within 10 minutes after trial drug injection without administration of rescue IV glucose.

(2) Plasma glucose changes from baseline at 30 minutes, at 20 minutes, at 15 minutes, and at 10 minutes after trial drug injection or at the time of rescue.

(1) The key secondary incidence variables (plasma glucose recovery within 30 minutes, within 20 minutes, within 15 minutes, within 10 minutes, in hierarchical order for inference) will be analyzed using descriptive statistics (frequencies and percentages) by treatment group.

Inferential treatment group comparisons will be assessed via pairwise tests of independent binomial proportions (dasiglucagon versus placebo) using Fisher's exact test.

(2) The key secondary endpoints of plasma glucose changes from baseline at 30 minutes, at 20 minutes, at 15 minutes, and at 10 minutes after trial drug injection or at the time of rescue, will be analyzed with the plasma glucose changes from baseline at rescue carried forward in those subjects who require rescue IV glucose before the change from baseline in plasma glucose reached ≥20 mg/dL (1.1 mmol/L). Each of these changes from baseline variables will be analyzed using an analysis of covariance (ANCOVA) model, with treatment group (dasiglucagon and placebo) modeled as a fixed effect, and with the baseline plasma glucose modeled as a covariate. The dasiglucagon versus placebo treatment group difference will be evaluated inferentially first for the 30 minute changes from baseline, and then sequentially for the 20 minute, 15 minute, and 10 minute variables, until the first failure to reject.

In addition, line plots of 1) individual and 2) by treatment group mean plasma glucose concentration will be generated.

Sensitivity analyses for the key secondary endpoints will be performed using the PP population using imputation rules described in Section 8.1.6.

8.2.3 Analysis of Clinical Efficacy Endpoints

The secondary clinical efficacy endpoints will be evaluated on the FAS (unless otherwise stated). Secondary clinical efficacy endpoints will be assessed based on plasma concentration data from samples collected at the dosing visit (Visit 2).

Time to first plasma glucose concentration ≥70 mg/dL (3.9 mmol/L) from baseline will be evaluated using a KM approach, with treatment group as a stratification factor, analogous to that used for the primary endpoint analysis. Differences between the KM curves (dasiglucagon versus placebo) will be evaluated inferentially using pairwise two-sided log-rank tests. Those subjects whose time to first plasma glucose concentration ≥70 mg/dL (3.9 mmol/L) is not met within 45 minutes post-dosing will be censored, at the time of the last valid plasma glucose measurement up to 45 minutes. The mathematical procedures and SAS code are the same as that used for the primary endpoint analysis in 8.2.1, except the difference that here it is stratified only by treatment group.

The $AUE_{0\text{-}30min}$ will be calculated as the baseline-adjusted area under the plasma glucose profile from time zero to 30 minutes. Subjects who received rescue IV glucose before 30 minutes will not have their $AUE_{0\text{-}30min}$ calculated. Calculation is determined by Linear Trapezoidal Method:

$$\text{AUE}_{0-30\text{min}} = \sum_{i=1}^{10} \frac{1}{2}[(C_{i-1} - C_0) + (C_i - C_0)](t_i - t_{i-1})$$

where i from 1 to 10 represent the timepoints of PD sampling within 30 minutes, i.e., at 4, 6, 8, 10, 12, 15, 17, 20, 25, 30 minutes after dosing. C_0 is the baseline plasma glucose concentration sampling at t_0 (pre-dose, defined as within 2-5 minutes prior to dosing), and C_i is the plasma glucose concentration at t_i. t_0 is assumed as 0 minutes, and t_i is timepoints of PD sampling represented by i.

The log-transformed AUE endpoint will be analyzed using an ANCOVA model with treatment group as fixed effect and baseline plasma glucose modeled as a covariate. The least squares means treatment group differences (and SE) will be back transformed (anti logged) for presentation as a ratio of the treatment group geometric means, with their corresponding 95% CI and p-value.

8.2.4 Analysis of Exposure Endpoints

Blood sampling for PK of ZP4207-17145 are to be collected at pre-dose, and at 15, 30, 35, 40, 50, 60, 90, and 120 minutes after dosing. Pre-dose is defined as within 2-5 minutes prior to

dosing. The actual time of blood sampling should not deviate from the nominal time by more than ±1 minute. The exposure endpoints are given as below:

- AUC will be derived as the area under the individual plasma dasiglucagon concentration profile for PK from 0 to 90 minutes or last valid measurement if this measurement is assessed sufficiently close to 90 minutes (decision to be taken at the masked data review meeting). The standard trapezoidal method will be used, based on actual rather than nominal time points. Calculation is determined by:

$$\text{AUC}_{0-90\text{min}} = \sum_{i=0}^{7} \frac{1}{2}(C_{i-1} + C_i)(t_i - t_{i-1})$$

where i from 0 to 7 represent the timepoints of blood sampling for PK from 0 to 90 minutes, i.e., at pre-dose, and at 15, 30, 35, 40, 50, 60 and 90 minutes after dosing. C_i is the individual plasma dasiglucagon concentration at i. t_i is timepoints of PK sampling represented by i.

- AUC 0 to 120 minutes or last valid measurement if this measurement is assessed sufficiently close to 120 minutes (decision to be taken at the masked data review meeting) will also be calculated.
- C_{max} will be determined as the maximum of all valid plasma dasiglucagon concentrations.
- t_{max} will be determined as the time point where the maximum of all valid plasma dasiglucagon concentration measurements for each measurement series is observed.

For these exposure endpoints calculations, concentration values that are below the limit of quantitation (BLQ) will be as follows:

- Any post dose BLQ value will be set to missing for the purposes of PK parameter calculation.
- Concentration at pre-dose that are BLQ will be defined as zero for calculation of AUC.

These exposure endpoints will be summarized descriptively for each treatment group. For the descriptive statistics summary. In addition, line plots of 1) individual and 2) by treatment group mean plasma dasiglucagon concentration will be generated.

8.3 SAFETY ANALYSES

8.3.1 Drug Exposure

Drug exposure will be summarized and listed for the safety analysis set.

8.3.2 Adverse Events

AEs will be coded using the latest available version of the MedDRA, the version of which will be provided in the CTR. An overall summary table will be provided showing the number and percentage of subjects with any:

- Treatment-emergent adverse event (TEAE)
- Severe TEAE
- Treatment-emergent SAE
- Drug-related TEAE
- Drug-related severe TEAE
- Treatment-emergent drug-related SAE
- TEAE leading to withdrawal
- TEAE with outcome death
- Adverse Events of Special Interest (AESI)
    - Post-dose clinical signs or measured vital signs indicating a clinically significant drop in blood pressure including signs of orthostatic hypotension, vasovagal responses or bradycardia
    - Post-dose changes in pulse or blood pressure considered an event of hypo- or hypertension as judged by the investigator.

The assessment should be based on signs and symptoms and Investigator clinical judgement. The following definitions can be used as a guidance

- Orthostatic hypotension: decrease in systolic BP at least 20 mmHg (at least 30 mmHg in patients with hypertension) and/or a fall in diastolic blood pressure of at least 10 mmHg and/or increase of heart rate at least 20 BPM within 3 minutes of standing associated with clinical symptoms such as dizziness or fainting
- Vasovagal response: A sudden and clinically significant drop of blood pressure and/or heart rate associated with clinical symptoms such as pale skin and sweating, altered vision, dilated pupils or fainting
- Bradycardia: a drop in heart rate more than 10 BPM from the last pre-dose resting value and a heart rate of less than 50 BPM

8.3.3 Clinical Laboratory Assessments

Clinical laboratory test results will be flagged as to whether the result is below, within or above the respective reference range (Low, Normal, High, and missing). The number of values outside of the reference range will be counted. Observed values and change from baseline will be summarized for continuous assessments. Shift tables will also be generated.

8.3.4 Local Tolerability

The local tolerability at the injection site will be evaluated by means of the following assessments: spontaneous pain, pain on palpation, itching, redness, edema, induration/infiltration, and other. Each of these assessments will be reported on a scale of 0 (none), 1 (mild), 2 (moderate) and 3 (severe). An overall summary table will be provided showing the number and percentage of these assessments.

8.3.5 Other Safety Parameters

Incidence of rescue infusion of IV glucose after trial drug administration will be analyzed using descriptive statistics (frequency and percentage) by treatment group. Inferential treatment group comparisons will be assessed via Fisher's exact test (dasiglucagon versus placebo).

Time to first rescue infusion of IV glucose after trial drug administration will be evaluated using a KM time to event statistical model, with treatment group and injection site as stratification factors. Differences between the KM curves (dasiglucagon versus placebo) will be evaluated inferentially using pairwise two-sided stratified log-rank tests. If the endpoint is never met, the time of the last plasma glucose measurement will be the censoring time.

In addition, a KM plot of time to first rescue will be generated.

A summary of total glucose infused prior to trial drug administration will be summarized by treatment group.

8.3.6. Vital Signs

Vital signs data will be summarized using descriptive statistics by treatment group.

8.3.7. Physical Examination

Physical examination data will be summarized using descriptive statistics by treatment group.

8.3.8. 12-Lead ECG

12-lead ECG data will be summarized using descriptive statistics by treatment group. Shift tables will also be generated.

8.3.9. Immunogenicity Data

Immunogenicity data will be analyzed descriptively by treatment group. No statistical tests are planned. Baseline ADA-positive subjects will be calculated as a percentage of the total number of subjects whose baseline samples were tested for ADA. Post-baseline ADA-positive subjects will be calculated as a percentage of the total number tested for ADA at the follow-up visit. Titer levels will be reported as median and interquartile range.

8.4 OTHER ANALYSES

8.4.1 Subgroup Analyses

Summary tables and KM plots describing the primary endpoint may be created based on age (≤40 years and >40 years), sex or other demographic characteristic(s) may be generated.

8.4.2 Exploratory Analyses

Plasma glucose concentration ≥70 mg/dL (3.9 mmol/L) or increase of ≥20 mg/dL (1.1 mmol/L) within 30 minutes after trial drug injection without administration of rescue IV glucose will be analyzed using descriptive statistics (frequency and percentage) by treatment group.

Plasma insulin response as area under the curve above baseline from time zero to 60 minutes, $AUC_{0\text{-}60\ min}$, will be summarized by treatment group. The standard trapezoidal method will be used, based on actual rather than nominal time points. Calculation is determined by:

$$\text{AUC}_{0-60\text{min}} = \sum_{i=1}^{2} \frac{1}{2}[(C_{i-1} - C_0) + (C_i - C_0)](t_i - t_{i-1})$$

where i from 1 to 2 represent the timepoints of Plasma insulin collecting within 60 minutes, i.e., at 30 and 60 minutes after dosing. C_0 is the baseline plasma insulin collecting at t_0 (pre-dose, defined as within 2-5 minutes prior to dosing), and C_i is the plasma insulin at t_i. t_0 is assumed as 0 minutes, and t_i is timepoints of plasma insulin response represented by i.

In addition, line plots of 1) individual and 2) by treatment group mean plasma insulin concentration will be generated.

9. CHANGES TO PLANNED ANALYSIS IN THE PROTOCOL

Added PK exposure endpoint, AUC 0-120 minutes.

Added a descriptive analysis of total insulin infused and total glucose infused after study drug administration.

Added actual time analysis of primary endpoint using interpolation.

10. STATISTICAL SOFTWARE

All statistical analyses will be performed using SAS® version 9.4.