## 16.1 TRIAL INFORMATION

### 16.1.1 PROTOCOL AND PROTOCOL AMENDMENTS

This appendix includes

| **Document** | **Date, Version** |
|---|---|
| Clinical Trial Protocol | 18 January 2017, Version 1 |
| Amendment 01 | 08 May 2017 |
| Clinical Trial Protocol | 08 May 2017, Version 2 |
| Amendment 02 | 21 August 2017 |
| Clinical Trial Protocol | 21 August 2017, Version 3 |

# Clinical Trial Protocol

***A phase 3, Randomized, Double-Blind, Parallel Group Safety Trial to Evaluate the Immunogenicity of Dasiglucagon Compared to GlucaGen® Administered Subcutaneously in Patients with Type 1 Diabetes Mellitus (T1DM)***

**Sponsor code: ZP4207-16136**
**SynteractHCR: ZEA-DNK-01711**
**EudraCT number: 2017-000062-30**

Coordinating investigator: Linda Morrow, MD
Prosciento
855 Third Avenue
91911 Chula Vista
CA, USA

Sponsor: Zealand Pharma A/S
Smedeland 36
2600 Glostrup, Copenhagen
Denmark

Version: final version 1
Date: 18 January 2017

***GCP statement***
*This trial will be performed in compliance with Good Clinical Practice (GCP), the Declaration of Helsinki (with amendments) and local legal and regulatory requirements.*

## 1. Signatures and agreement with protocol

**Title:** A phase 3, randomized, double-blind, parallel group safety trial to evaluate the immunogenicity of dasiglucagon compared to GlucaGen® administered subcutaneously in patients with type 1 diabetes mellitus (T1DM)

We, the undersigned, agree to conduct this trial according to the Trial Protocol.

We agree that the trial will be carried out in accordance with Good Clinical Practice (GCP), with the Declaration of Helsinki (with amendments) and with the laws and regulations of the countries in which the trial takes place.

Coordinating investigator

Linda Morrow, MD
Prosciento
855 Third Avenue
91911, Chula Vista, CA, USA

[redacted] Date [redacted] Signature

Statistician

[redacted]
Senior Director Biometrics EMEA
SynteractHCR Deutschland GmbH
Albrechtstr. 14
80636 Munich, Germany

[redacted] Sign [redacted]

Sponsor's representative

[redacted] MSc (Pharm)
Clinical Trial Manager
Zealand Pharma
Smedeland 36
2600 Glostrup, Denmark

[redacted] Date [redacted] Signature

[redacted] MD, PhD
Medical Director
Zealand Pharma
Smedeland 36
2600 Glostrup, Denmark

[redacted] Date [redacted] Signature

**Title:** A phase 3, randomized, double-blind, parallel group safety trial to evaluate the immunogenicity of dasiglucagon compared to GlucaGen® administered subcutaneously in patients with type 1 diabetes mellitus (T1DM)

I agree to conduct this trial according to the Trial Protocol.

I agree that the trial will be carried out in accordance with Good Clinical Practice (GCP), with the Declaration of Helsinki (with amendments) and with the laws and regulations of the countries in which the trial takes place.

Investigator

| Date | Signature |
|---|---|

Name and address

## 2. Trial synopsis

| | |
|---|---|
| **Title of the trial:**<br>A phase 3, randomized, double-blind, parallel group safety trial to evaluate the immunogenicity of dasiglucagon*) compared to GlucaGen® administered subcutaneously in patients with type 1 diabetes mellitus (T1DM)<br>* Dasiglucagon is the proposed international nonproprietary name | |
| **EudraCT number:**<br>2017-000062-30 | **Protocol codes:**<br>Sponsor: ZP4207-16136<br>SynteractHCR: ZEA-DNK-01711 |
| **Sponsor or sponsor's representative in the European Union:**<br>Zealand Pharma A/S, Smedeland 36, 2600 Glostrup (Copenhagen), Denmark | |
| **Coordinating investigator:**<br>Linda Morrow, MD, Prosciento, 855 Third Avenue, 91911, Chula Vista, CA, USA | |
| **Trial center(s):**<br>2 centers in the EU, 2 centers in the US, and 3 centers in Canada | |
| **Planned trial period:**<br>First Patient First Visit: March 2017<br>Last Patient First Visit: September 2017 | **Phase of Development:**<br>Phase 3 |
| **Objectives:**<br>The primary objective is to evaluate the immunogenicity of repeated single doses of dasiglucagon following subcutaneous (s.c.) administration compared with s.c. GlucaGen in T1DM patients.<br>The secondary objective is to evaluate the safety and tolerability of repeated single doses of dasiglucagon following s.c. administration compared with s.c. GlucaGen in T1DM patients. | |
| **Trial design:**<br>This is a randomized, double-blind, parallel group trial comparing the immunogenicity of either dasiglucagon or GlucaGen administered to euglycemic T1DM patients. Patients will be randomized 1:1 to receive 3 s.c. injections of dasiglucagon or GlucaGen with 1 week between doses. Patients will be followed for at least 3 months from the day of the first dose to assess any immune response. A total of 90 patients are expected to complete the trial. Handling, preparation and administration of trial medication will be done by unblinded trial personnel. All trial assessments will be done by blinded trial personnel. However, exposure assessments and anti-drug antibody (ADA) assessments will be performed by unblinded personnel at the specialty laboratories, to make sure that dasiglucagon or GlucaGen administration is matched with the applicable bioanalytical assay. | |
| **Planned number of patients:**<br>90 completed patients (45 completed patients per treatment group). Prematurely discontinued patients will be replaced in order to reach 90 completed patients. It is expected that 112 patients in total will be randomized and treated.<br>To qualify as completed, the patient must be dosed according to protocol and have blood drawn for the planned anti-drug antibody (ADA) analyses. | |
| **Medical condition or disease under investigation:**<br>Given the role of the immune system in the pathogenesis of T1DM, the present trial is conducted in patients with T1DM. There are no data indicating an altered immune response with varying blood glucose levels. Therefore, for the safety and well-being of the patients, they will not be brought into hypoglycemia prior to dosing. Patients with previous exogenic glucagon exposure will not be excluded from the trial, but the information on previous glucagon administration will be recorded. | |
| **Inclusion criteria:**<br>To be included in the trial, patients have to fulfill all of the following criteria:<br>1. Informed consent obtained before any trial-related activities (trial-related activities are any procedure that would not have been performed during normal management of the patient)<br>2. Availability for the entire trial period<br>3. Age between 18 and 70 years, both inclusive<br>4. Male or female patients with T1DM for at least 1 year. Diagnostic criteria as defined by the | |

American Diabetes Association

5. Hemoglobin A1c ($HbA_{1c}$) <10%
6. Stable anti-diabetic treatment for at least 1 month (e.g. within 10% insulin dose adjustment)
7. A female participant must meet one of the following criteria
   a. Participant is of childbearing potential and agrees to use one of the accepted contraceptive regimens throughout the entire duration of the trial from screening until last follow-up visit. An acceptable method of contraception includes at least one of the following:
      i. Abstinence from heterosexual intercourse
      ii. Systemic contraceptives (birth control pills, injectable/implant/ insertable hormonal birth control products, transdermal patch); if the participant is using systemic contraceptives, she must use an additional form of acceptable contraception (iii or iv, below)
      iii. Intrauterine device (with and without hormones)
      iv. Condom with spermicide

   or

   b. Participant is of non-childbearing potential, defined as surgically sterile (i.e. has undergone complete hysterectomy, bilateral oophorectomy, or tubal ligation), or in a menopausal state (at least 1 year without menses)
8. A male must be surgically sterilized or willing to refrain from sexual intercourse from screening and until last follow-up visit or, if sexually active, uses a condom and partner practices contraception during the trial from screening and until the last follow-up visit

**Exclusion criteria:**

Patients meeting any of the following criteria during screening evaluations will be excluded from trial participation:

1. Previous administration of dasiglucagon (previously referred to as ZP4207)
2. Known or suspected allergy to trial medication(s) or related products
3. History of anaphylaxis or symptoms of severe systemic allergy (such as angioedema)
4. Previous participation (randomization) in this trial
5. Females who are pregnant according to a positive pregnancy test, actively attempting to get pregnant, or are lactating
6. Patients on a closed loop artificial pancreas
7. Receipt of any investigational drug within 3 months prior to screening
8. Active malignancy within the last 5 years
9. Congestive heart failure, New York Heart Association class II-IV
10. Inadequately treated blood pressure as defined as systolic blood pressure ≥180 mmHg or diastolic blood pressure ≥110 mmHg at screening
11. Current bleeding disorder, including use of anticoagulant treatment
12. Known presence or history of pheochromocytoma (i.e. adrenal gland tumor) or insulinoma (i.e. insulin-secreting pancreas tumor)
13. Known or suspected HIV infection
14. Use of a systemic beta-blocker drug, indomethacin, warfarin or anticholinergic drugs in the previous 28 days before Day 1 of this trial
15. Use of systemic corticosteroids, anti-inflammatory biological agents, kinase inhibitors or other immune modulating agents within the last 3 months prior to screening
16. Aspartate aminotransferase (AST) or alanine aminotransferase (ALT) >2.5 X the upper limit of normal (ULN), bilirubin >1.5 X ULN, estimated glomerular filtration rate (eGFR) <30 mL/min/1.73$m^2$ according to the Modification of Diet in Renal Disease (MDRD) Study definition
17. Clinically significant abnormal ECG at screening as evaluated by Investigator
18. Donation of blood or plasma in the past month, or in excess of 500 mL within 12 weeks prior to screening
19. Active substance or alcohol abuse
20. Patients with mental incapacity or language barriers that preclude adequate understanding or cooperation, who are unwilling to participate in the trial, or who in the opinion of the Investigator

should not participate in the trial
21. Surgery or trauma with significant blood loss within the last 2 months prior to screening
22. Any condition interfering with trial participation or trial endpoints or that could be hazardous to the patient

**Test product, dose and mode of administration:**
Dasiglucagon: 0.6 mg; liquid formulation, 1 mg/mL in prefilled syringes containing 0.6 mL

**Reference product, dose and mode of administration:**
GlucaGen, 1 mg; powder and solvent for reconstitution as 1 mL solution for injection (recombinant glucagon hydrochloride, Novo Nordisk)

**Duration of treatment:**
Patients will receive 3 s.c. injections of trial medication (dasiglucagon or GlucaGen) with 1 week between each dosing.
Prior to administration of trial medication patients must reach a target plasma glucose level of 70-150 mg/dL. Plasma glucose levels may be adjusted by s.c. administration of a fast-acting insulin analog or by glucose ingestion.

**Criteria for evaluation:**

**Immunogenicity:**

**Primary endpoint:**

- Overall ADA incidence
  This will be calculated as a percentage of the combined results of treatment-induced ADA-positive patients and treatment-boosted ADA-positive patients and the total number of evaluable patients, excluding baseline-positive patients without any samples available after drug administration.

**Key secondary endpoints:**

- Treatment-induced ADA
  Incidence calculated as a percentage of the total number of evaluable patients that were ADA negative at baseline and ADA positive after drug administration and the total number of evaluable patients, excluding baseline positive patients without any samples available after drug administration.
- Treatment-boosted ADA
  Incidence calculated as percentage of baseline ADA-positive patients with significant increases (≥5-fold) in ADA titer after drug administration and the total number of evaluable patients, excluding baseline-positive patients without any samples available after drug administration.

**Secondary endpoints:**

Characterization of ADA response:

- Incidence and titer of neutralizing activity of ADA positive patients
- Incidence of cross-reactivity of ADA positive patients towards endogenous glucagon
- Kinetics of ADA:
  The timing and duration of detected ADA response

Safety:

- The incidence, type and severity of AEs
- Changes from baseline in clinical laboratory parameters
- Changes from baseline in vital signs
- Clinically meaningful changes from baseline in physical examination and electrocardiogram (ECG)

Exposure endpoints, after administration of first and third doses of trial medication:

- Plasma dasiglucagon and glucagon concentrations from 0-90 min after dosing will be

evaluated based on the following endpoints: $AUC_{0\text{-}30min}$, $AUC_{0\text{-}90\ min}$, $C_{max}$, $t_{max}$

Pharmacodynamics, after administration of first and third doses of trial medication:

- Plasma glucose profiles over the period from 0-90 min after dosing will be evaluated based on the following endpoints: $AUE_{0\text{-}30min}$, $AUE_{0\text{-}90\ min}$, $CE_{max}$, $t_{max}$

**Statistical methods:**
All statistical analysis will be descriptive. Continuous variables will be summarized with means, standard deviations, medians, minimums, and maximums, and valid cases. Other summaries (e.g. quartiles, 95% confidence intervals) may be used as appropriate. Categorical variables will be summarized by counts and by percentage of patients in corresponding categories.

**Sample size calculation:**
The purpose of the present trial is to generate data describing the immunogenic potential of dasiglucagon, when used as a rescue therapy from severe hypoglycemia and with reference to the immunogenic potential of GlucaGen. The sample size is based on generating data to show that the 90% confidence of the ADA incidence is no worse than 15% as the predefined acceptability criterion and with a reference to similar data being generated for GlucaGen.
Currently, no ADA incidences have been detected in the completed clinical trials, where up to 5 repeated doses of dasiglucagon have been administered to the same patients within a week. Across the two phase 1 clinical trials and a completed phase 2 pharmacokinetic/pharmacodynamic trial, a total of 141 subjects have been exposed to dasiglucagon and no incidences of ADA development have been observed. The obtained data indicate that dasiglucagon has a low risk for induction of ADAs in the investigated settings and as a consequence the sample size is based on showing that the ADA incidence is no worse than the predefined margin of 15%.

**Table 2-1: Flow chart**

| Trial period | Screening | Treatment | | | Follow-up | | |
|---|---|---|---|---|---|---|---|
| Visit number | V1 | V2 | V3 | V4 | V5 | V6 | V7 (EoT) |
| Trial day | -3 | 0 | 7 | 14 | 35 | 60 | 104 |
| Visit window (days) | -30 to - -3 | | ±1 | ±1 | ±2 | ±5 | ±10 |
| **Patient related info/assessments** | | | | | | | |
| Informed consent | X[1] | | | | | | |
| Inclusion/exclusion criteria | X | X[2,3] | | | | | |
| Demography | X | | | | | | |
| Body measurements | X | | | | | | |
| Medical history | X | | | | | | |
| Concomitant illness | X | | | | | | |
| Prior medications | X | | | | | | |
| Concomitant medication | X | X | X | X | X | X | X |
| History of alcohol/drug abuse | X | | | | | | |
| Randomization | | X | | | | | |
| Withdrawal criteria | | X | X | X | X | X | |
| Dosing day exclusion criteria | | X | X | X | | | |
| **Safety assessments** | | | | | | | |
| Physical examination | X | | | | | | X |
| Vital signs | X | X[4] | | X[4] | X | | X |
| ECG | X | X[4] | | X[4] | X | | X |
| Local tolerability | | X[5] | X[5] | X[5] | | | |
| Adverse events | X | X | X | X | X | X | X |

| Trial period | Screening | Treatment | | | Follow-up | | |
|---|---|---|---|---|---|---|---|
| Visit number | V1 | V2 | V3 | V4 | V5 | V6 | V7 (EoT) |
| Trial day | -3 | 0 | 7 | 14 | 35 | 60 | 104 |
| Visit window (days) | -30 to - -3 | | ±1 | ±1 | ±2 | ±5 | ±10 |
| **Laboratory** | | | | | | | |
| Hematology, biochemistry, coagulation | X[4] | X[4] | | X[4] | X | | X |
| Pregnancy test (women only) | X | | | | | | |
| Urinalysis | X | X[2] | | X[2] | | | X |
| Urine drug screen | X[6] | X[2,6] | X[2,6] | X[2,6] | | | |
| Alcohol breath test | X | X[2] | X[2] | X[2] | | | |
| **Exposure and pharmacodynamics (PD)** | | | | | | | |
| Dasiglucagon /glucagon | | X[7] | | X[7] | | | |
| Plasma glucose | | X[8] | | X[8] | | | |
| **Other assessments** | | | | | | | |
| Antibodies against dasiglucagon /glucagon | | X[2] | X[2] | X[2] | X | X | X |
| **Trial material** | | | | | | | |
| Administration of trial medication | | X[9] | X[9] | X[9] | | | |

ECG = electrocardiogram; EoT = End of Trial; ; PD = pharmacodynamics; V = visit

[1] At least 1 day before the screening visit (Visit 1)

[2] Pre-dose

[3] Only check of changes between the screening visit and V2.

[4] Coagulation parameters are measured at screening visit only. On dosing days Visit 2 and 4, vital signs, ECG's and blood samples are collected pre-dose, and at 30 and 90 min post-dosing.

[5] Local tolerability assessed at 0.5 and 2 h post-dose

[6] Urine drug screen will be performed at trial site for visits 1-4

[7] Pre-dose, 5, 10, 30, 60, and 90 min post-dosing. The actual time of blood sampling for exposure should not deviate from the nominal time by more than ±1 min. Pre-dose is defined as within 5 min prior to dosing.

[8] Pre-dose, 5, 10, 30, 60, and 90 min post-dosing. The actual time for blood sampling for plasma glucose should not deviate from the nominal time by more than ±1 min. Pre-dose is defined as within 5 min prior to dosing.

[9] Prior to administration of trial medication patients must reach a target plasma glucose level of 70-150 mg/dL. Plasma glucose levels may be adjusted by subcutaneous (s.c.) administration of a fast-acting insulin analog or by glucose ingestion. Patients must be fasting for 90 min after administration of trial medication, and will be treated individually to alleviate any potential side effects. Patients will be observed for at least 5 h post-dose.

## 3. Table of contents

**Appendices:**

(1) List of names and addresses

**Index of in-text tables**



**Index of in-text figures**

## 4. List of abbreviations and definition of terms

### 4.1 Abbreviations

| | |
|---|---|
| ADA | Anti-drug Antibody |
| AE | Adverse Event |
| ALT (SGPT) | Alanine Aminotransferase (Serum Glutamic Pyruvic Transaminase) |
| AST (SGOT) | Aspartate Aminotransferase (Serum Glutamic Oxaloacetic Transaminase) |
| BG | Blood Glucose |
| CA | Competent Authority (Directive 2001/20/EC) |
| CFR | Code of Federal Regulations |
| CRF/eCRF | Case Report Form/Electronic Case Report Form |
| CI | Confidence Interval |
| CRO | Contract Research Organization |
| CSII | Continuous Subcutaneous Insulin Infusion |
| CTA | Clinical Trial Authorization (Directive 2001/20/EC) |
| EC | Ethics Committee |
| ECG | Electrocardiogram |
| EDC | Electronic Data Capture |
| eGFR | Estimated Glomerular Filtration Rate |
| EoT | End of Trial |
| EudraCT | European Clinical Trials Database |
| FAS | Full Analysis Set |
| FDA | Food and Drug Administration |
| gamma-GT | gamma-Glutamyltransferase |
| GCP | Good Clinical Practice |
| $HbA_{1c}$ | Hemoglobin $A_{1c}$ |
| HEENT | Head, Ears, Eyes, Nose, Throat |
| HIV | Human Immunodeficiency Virus |
| ICF | Informed Consent Form |
| ICH | International Conference on Harmonization |
| IEC | Independent Ethics Committee |
| IM | Intramuscular(ly) |
| IMP | Investigational Medicinal Product |
| IND | Investigational New Drug |
| INR | International Normalized Ratio |
| IRB | Institutional Review Board |
| i.v. | Intravenous(ly) |
| IWRS | Interactive Web Response System |
| LOCF | Last Observation Carried Forward |
| MDRD | Modification of Diet in Renal Disease |
| MedDRA | Medical Dictionary for Regulatory Activities |
| Nab | Neutralizing Antibody |
| PG | Plasma Glucose |
| ODM | Operational Data Model |
| PD | Pharmacodynamic(s) |

| | |
|---|---|
| PK | Pharmacokinetic(s) |
| PPS | Per Protocol Set |
| PT | Preferred Term |
| SAP | Statistical Analysis Plan |
| SAS | Safety Analysis Set |
| SAE | Serious Adverse Event |
| SC | Safety Committee |
| s.c. | Subcutaneous(ly) |
| SOC | System Organ Class |
| SOP | Standard Operating Procedure |
| T1DM | Type 1 Diabetes Mellitus |
| T2DM | Type 2 Diabetes Mellitus |
| ULN | Upper Limit of Normal |
| V | Visit |
| ZP4207 | Dasiglucagon |

**Plasma concentrations of dasiglucagon/GlucaGen**

| | |
|---|---|
| $AUC_{0\text{-}30min}$ | Area under the plasma concentration curve from administration to observed concentration 30 min |
| $AUC_{0\text{-}90\ min}$ | Area under the plasma concentration curve from administration to observed concentration at 90 min |
| $C_{max}$ | Maximum plasma concentration |
| $t_{max}$ | Time until $C_{max}$ is reached |

**Plasma glucose concentrations**

| | |
|---|---|
| $AUE_{0\text{-}30min}$ | Area under the effect curve from administration to 30 min |
| $AUE_{0\text{-}90\ min}$ | Area under the effect curve from administration to 90 min |
| $CE_{max}$ | Change from baseline plasma glucose to maximum plasma glucose measured post dose |
| $t_{max}$ | Time to maximum effect |

## 4.2 Definitions of terms

Definition of the end of the trial: The trial ends with the last visit of the last patient participating in the trial.

# 5. Introduction

## 5.1 Background of the trial

### Hypoglycemia

Hypoglycemia in patients with diabetes is defined as episodes of an abnormally low plasma glucose concentration.[1] This is a common, unpredictable, and potentially dangerous side effect of treatment of diabetes mellitus with especially insulin or sulfonylureas. It is more frequent in patients with profound endogenous insulin deficiency, such as occurs in type 1 diabetes mellitus (T1DM) and advanced type 2 diabetes mellitus (T2DM). Treatment of T2DM with insulin causes hypoglycemia progressively and more frequently over time, whereas in T1DM, hypoglycemia is experienced throughout the course of established disease.

Symptoms and signs of hypoglycemia are not specific. Patients undergoing a hypoglycemic episode experience unpleasant symptoms such as anxiety, sweating, hunger, tremors, palpitations, paresthesia, nausea and pallor. Depending on its severity, the hypoglycemia may lead to mild confusion, behavioral changes, loss of consciousness, seizures, coma, and death.[2]

The incidence of hypoglycemic events or even the fear of hypoglycemia influences patients' adherence to prescribed treatment regimens for diabetes mellitus. This leads to inadequate glycemic control, which in turn may lead to an increased risk of diabetic complications.

### Glucagon

Glucagon is a naturally occurring hormone, secreted from the alpha cells of the pancreatic islets. Glucagon plays a central role in the regulation of glucose homeostasis and is the counterpart of insulin for controlling blood glucose levels (i.e. it acts in opposition to insulin in terms of effects on blood glucose levels). Glucagon stimulates hepatic glycogenolysis and gluconeogenesis in hypoglycemic states, thereby restoring glucose homeostasis. Glucagon receptor agonism has also been shown to exert effects on lipid metabolism, energy balance, body adipose tissue mass and food intake. Insulin decreases blood glucose levels and cases of hypoglycemia can be reversed by glucagon. Therefore, glucagon is indicated for the treatment of severe hypoglycemia.

Antibodies against therapeutic peptides like glucagon and analogues hereof may develop when injected subcutaneously. Although important, glucagon is not considered to have a critical endogenous function since other counter regulatory hormones are also induced during hypoglycemia (e.g. growth hormone, cortisol, and epinephrine). In addition, results from 3 mouse models defective in various pathways of the glucagon signaling have confirmed that glucagon action is dispensable for their development and survival. Also, in non-clinical toxicity studies performed with dasiglucagon (see below), no consequences of ADA formation have currently been observed. In summary, glucagon appears to have a partly redundant endogenous function. These non-clinical data are of importance when evaluating the consequences of ADA formation.

### Dasiglucagon

Dasiglucagon (ZP4207) is a stable peptide analog of human glucagon, available in a ready-to-use liquid formulation and is in development for the treatment of severe hypoglycemia in insulin dependent patients with diabetes mellitus. Dasiglucagon is a specific and full glucagon receptor agonist designed to mimic the effects of glucagon, having a fast absorption and elimination (minutes). It is a peptide of 29 amino acids, with 7 amino acid substitutions compared to native glucagon. The main purpose of the substitutions is to increase the physical and chemical stability of the glucagon analog compared to marketed glucagon products such as Lilly Glucagon or GlucaGen®. Dasiglucagon exhibits improved physical and chemical stability and is available in an aqueous solution at neutral pH.[3]

Three clinical trials have been completed with dasiglucagon, a first human dose trial (ZP4207-14013), a multiple-dose dose-escalation trial (ZP4207-15007) to evaluate the safety, tolerability, pharmacokinetics (PK) and pharmacodynamics (PD) of dasiglucagon, and a phase 2 crossover trial to assess the pharmacokinetics and pharmacodynamics of a single dose of an optimized formulation of dasiglucagon administered subcutaneously (s.c.) in patients with T1DM (ZP4207-15126).[3]

*Pharmacokinetics and pharmacodynamics of dasiglucagon*

The results of the phase 1 and 2 clinical trials confirm dose-proportionality for dasiglucagon pharmacokinetics, which is characterized by a fast absorption with a peak plasma concentration obtained after 35 minutes. Thereafter, the plasma concentration rapidly declines with an average half-life of 28 minutes. The median time to the maximum plasma concentration ($C_{max}$) was later for dasiglucagon than for GlucaGen (35 versus 20 minutes). Doses of 0.3 mg dasiglucagon and 0.5 mg GlucaGen and also 0.6 mg dasiglucagon and 1.0 mg GlucaGen were similar with regard to $C_{max}$. For $C_{max}$, the results indicated that 0.3 mg dasiglucagon was comparable to 0.5 mg GlucaGen (90% confidence interval (CI): 0.8167; 1.0068) and 0.6 mg dasiglucagon was comparable to 1.0 mg GlucaGen (90% CI: 0.8850; 1.1991).[3] At these dose levels, the total exposures ($AUC_{0\text{-}inf}$) were higher for dasiglucagon compared to GlucaGen.

At all dose levels in the phase 2 trial, all patients achieved a plasma glucose level of at least 70 mg/dL as well as an increase in plasma glucose by at least 20 mg/dL within 30 min post-dose. The maximal observed time to reach the 20 mg/dL plasma glucose increase ranged from 15 to 27 minutes across doses and decreased as the dose increased. The pharmacodynamic responses of 0.6 mg of dasiglucagon and 1.0 mg of GlucaGen were comparable.[3]

*Safety of dasiglucagon*

The safety data for dasiglucagon did not give rise to any relevant safety concerns for dasiglucagon beyond those related to the pharmacological effect of glucagon agonism. All adverse events (AEs) recorded in the trials were of mild or moderate severity. The most frequently reported systemic AE was nausea, which is a known side effect following administration of glucagon. Headache was the next most frequently reported event, occurring in all dose groups in the phase 2 trial. Injection site reactions were observed only sporadically after administration with either dasiglucagon or GlucaGen® and all were mild and transient. The most frequent injection site reaction was erythema, occurring in all treatment groups, including the placebo group, irrespective of dose. Therefore, the phase 1 and 2 results and the safety profile described to date do not give rise to specific safety concerns. For further information, please refer to the Investigator Brochure.[3]

*Immunogenicity of dasiglucagon*

To date, in the 3 clinical trials performed with dasiglucagon (described above), there have not been any anti-drug antibody (ADA) occurrences in a total of 141 subjects exposed to 1 or more doses. Data from the non-clinical toxicology program (7 non-clinical toxicity studies) showed that ADAs were detected in mice, rats, and dogs, and were most frequent in animals in the highest dasiglucagon dose groups. A fraction of the ADAs from rats and dogs were able to cross-react with native glucagon. However, the ADAs did not appear to be associated with changes in the safety or toxicity profiles compared to ADA-negative animals. In rats, the average exposure (AUC) was increased following 13 and 26 weeks of treatment in dose groups with higher ADA frequency (≥8 mg/kg/day). The ADA frequency and titers of consistently ADA-positive rats were reduced from 13 to 26 weeks of treatment, indicating a transient response in most animals. It therefore appears that dasiglucagon has a low risk for induction of ADAs. A fraction of the antibodies detected in the non-clinical toxicity studies was found to cross-react with glucagon without advert clinical or toxicity findings. These data suggest that dasiglucagon and glucagon share epitopes and potential ADAs induced by dasiglucagon in humans may have the ability to cross-react with glucagon.[3]

Although glucagon is an important hormone for controlling blood glucose levels it is considered to have a partially redundant endogenous function since hypoglycemia can also be corrected by other means. The overall immunogenicity risk of dasiglucagon in a clinical context is therefore considered to be low and the potential effects of induced ADAs judged to be of limited clinical consequence.

As dasiglucagon contains 7 amino acid substitutions compared to native glucagon and historic data indicate that the immune system's tolerance to glucagon can be impaired in the intended target population, there is an inherent risk for the induction of an ADA response against dasiglucagon. However, other product specific characteristics, i.e. dasiglucagon being a chemical synthetized product without host cell contaminants, a reduced potential for aggregation, a physiological compatible formulation, and a high bioavailability with a short half-life, are all in favor of reducing the risk of dasiglucagon to induce an unwanted immune response. Considering the intended indication, in which dasiglucagon is administered as a single-dose rescue treatment on an infrequent basis (0.21 to 1.6 episodes per patient per year),[4,5] the induction of a high titer ADA response with effects on clinical safety seems unlikely. Overall, the risk of dasiglucagon to induce an ADA response is considered low. The present trial aims to evaluate that immunogenicity risk in patients with T1DM administered multiple s.c. doses of dasiglucagon.

### 5.2 Trial rationale

Dasiglucagon is in clinical development as a rescue treatment for severe hypoglycemia in patients with insulin-dependent diabetes mellitus. Immune responses to therapeutic peptides and proteins may develop after subcutaneous administration and could potentially adversely affect their safety and efficacy. The potential formation of high titer ADAs to dasiglucagon, although considered unlikely in a rescue indication, could influence the efficacy of dasiglucagon, either indirectly by interacting with the pharmacokinetics of dasiglucagon or directly by neutralizing the glucagon response.

In recent years, regulatory agencies have outlined and recommended the use of a risk-based approach in the evaluation and mitigation of immune responses to therapeutic compounds that may adversely affect their safety and/or efficacy. Both transient and persistent antibody response should be combined to determine the overall immunogenicity of a product. Persistent antibodies are of importance since patients with persistent antibodies could experience clinical adverse reactions affecting safety and efficacy, while a transient antibody response can resolve without further consequence.

The present trial aims to evaluate that immunogenicity risk with an assessment of the occurrence of ADAs and neutralizing ADAs, and of cross-reactivity with native glucagon, following repeated single doses of dasiglucagon by s.c. administration in T1DM patients. The comparator in this trial is GlucaGen, a recombinant human glucagon approved for the treatment of the severe hypoglycemic reactions that may occur in the management of insulin-treated children and adults with diabetes mellitus.

### 5.3 Assessment of anticipated benefits and risks

As glucagon and its analogs belong to a well-known drug class with a known mode of action, dasiglucagon is not expected to be a high-risk molecule.

Treatment with an investigational medicinal product may result in undesired effects or complaints. Undesired effects and complaints such as nausea, vomiting, and diarrhea are known AEs occurring with glucagon administration. Similar AEs have also been observed to a limited degree in the 3 clinical studies conducted with dasiglucagon. As with every novel drug substance, new and as yet unknown side effects also may occur.

There are limited data available to assess the immunogenic potential of glucagon products available on the market, but the data available indicate that marketed glucagon only has a small

immunogenic potential. Based on the 3 clinical studies conducted with dasiglucagon to date (see Section 5.2), no anti-dasiglucagon or anti-glucagon antibodies have been detected.

In relation to diabetes therapy, development of insulin-antibodies is documented more substantially and the route of administration has been shown to influence the immunogenic potential of insulins. However, antibodies against insulin do not generally have an impact on insulin action and are thus not clinically relevant. In terms of consequence, development of high titer antibodies against dasiglucagon could, in theory reduce the activity of endogenous glucagon, which, in theory, could influence hypoglycemic episodes. Limited suppression of glucagon would, however, not be considered critical, since low glucose levels can also be corrected by other means, including oral intake of glucose and the action of other endogenous hormones such as oxyntomodulin and epinephrine.

Overall, dasiglucagon is judged to be a low-risk molecule, based upon the available clinical data as well as the outcome of the risk-based approach to the immunogenicity assessment. In line with the primary objective of this trial to assess the immunogenicity of dasiglucagon, sampling for measurement of antibodies against dasiglucagon will take place prior to first dosing (Visit 2), pre-dose at subsequent visits during the treatment period (Visits 3 and 4), and at all Follow-up visits (at 35, 60, and 104 days after the first dose of trial medication; i.e. at Visits 5, 6, and 7 (End of Trial [EoT] visit), respectively).

Administration of dasiglucagon may be associated with a risk of allergic reactions similar to those observed for other therapeutic peptides or proteins. Patients with known or suspected allergies to the trial medications or related products will be excluded from the trial. Mild or moderate allergic reactions may include symptoms of rash, fever, flu-like symptoms, nausea, headache, and myalgia. Acute generalized hypersensitivity reactions are usually very rare but may include symptoms of flushing, sweating, dizziness, change in blood pressure and difficulties in breathing. No severe acute hypersensitivity reactions have been observed in the 3 clinical trials conducted with dasiglucagon. Direct access to resuscitation equipment is ensured at the clinical trial sites.

With the exception of medical examinations, a patient participating in this trial is not likely to derive any personal health-related benefits. The results of the trial may contribute to the future use of dasiglucagon in patients with diabetes mellitus experiencing severe hypoglycemic reactions.

Overall, the benefit to risk ratio for patients entering the ZP4207-16136 trial is considered acceptable.

## 6. Trial objectives

**Primary objective**

- To evaluate the immunogenicity of repeated single doses of dasiglucagon following s.c. administration compared with s.c. GlucaGen in T1DM patients.

**Secondary objective**

- To evaluate the safety and tolerability of repeated single doses of dasiglucagon following s.c. administration compared with s.c. GlucaGen in T1DM patients.

**Primary endpoint:**

- Overall ADA incidence
  This will be calculated as a percentage of the combined results of treatment-induced ADA-positive patients and treatment-boosted ADA-positive patients and the total number of evaluable patients, excluding baseline-positive patients without any samples available after drug administration.

**Key secondary endpoints:**

- Treatment-induced ADA
  Incidence calculated as a percentage of the total number of evaluable patients that were ADA negative at baseline and ADA positive after drug administration and the total number of evaluable patients, excluding baseline positive patients without any samples available after drug administration.
- Treatment-boosted ADA
  Incidence calculated as percentage of baseline ADA-positive patients with significant increases (≥5-fold) in ADA titer after drug administration and the total number of evaluable patients, excluding baseline-positive patients without any samples available after drug administration.

**Secondary endpoints:**

Characterization of ADA response:

- Incidence and titer of neutralizing activity of ADA positive patients
- Incidence of cross-reactivity of ADA positive patients towards endogenous glucagon
- Kinetics of ADA:
  The timing and duration of detected ADA response

Safety:

- The incidence, type and severity of AEs
- Changes from baseline in clinical laboratory parameters
- Changes from baseline in vital signs
- Clinically meaningful changes from baseline in physical examination and electrocardiogram (ECG)

Exposure endpoints, after administration of first and third doses of trial medication:

- Plasma dasiglucagon and glucagon concentrations from 0-90 min after dosing will be evaluated based on the following endpoints: $AUC_{0\text{-}30min}$, $AUC_{0\text{-}90\ min}$, $C_{max}$, $t_{max}$

Pharmacodynamics after administration of first and third doses of trial medication:

- Plasma glucose profiles over the period from 0-90 min after dosing will be evaluated based on the following endpoints: $AUE_{0\text{-}30min}$, $AUE_{0\text{-}90\ min}$, $CE_{max}$, $t_{max}$

# 7. Investigational plan

## 7.1 Overall trial design and plan

This is a randomized, double-blind, parallel group trial comparing the immunogenicity of 3 fixed doses of either dasiglucagon or GlucaGen administered to euglycemic T1DM patients.

Patients with T1DM will be randomized 1:1 to receive 3 s.c. injections of either dasiglucagon (0.6 mg) or GlucaGen (1 mg), with 1 week between each dose. Patients will be followed for at least 3 months from the day of the first dose to assess any immune response. Patients with previous exogenic glucagon exposure will not be excluded from the trial, but the information on previous glucagon administration will be recorded to enable subgroup analyses. A total of 90 patients are expected to participate in and complete the trial (45 in each treatment arm). To qualify as completed, the patient must be dosed according to protocol and have blood drawn for the ADA analyses (as scheduled in

). Prematurely discontinued patients will be replaced in order to reach 90 completed patients. It is expected 112 patients in total will be randomized and treated.

For the safety and well-being of the patients, they will not be brought into hypoglycemia prior to dosing. Prior to administration of trial product patients must reach a target plasma glucose level of 70-150 mg/dL.

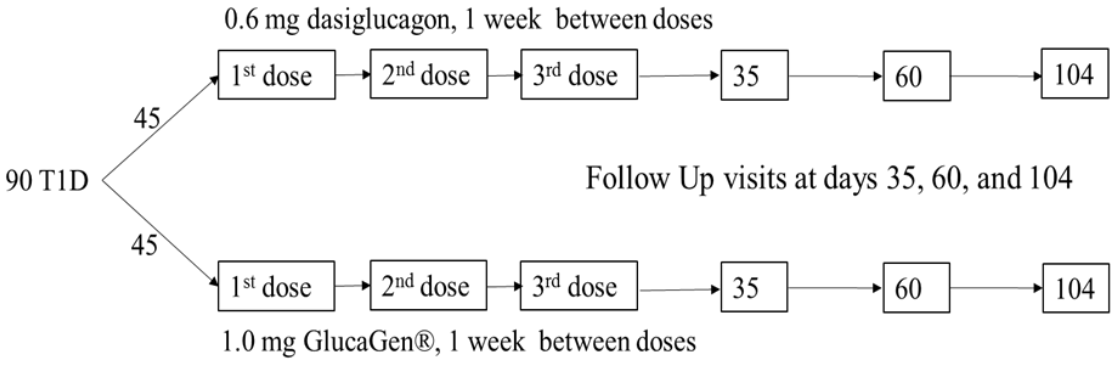
The trial will include the following periods (as illustrated in Figure 7-1, below).

- A screening period from Day -30 to Day -3
- A treatment period, from Day 0 (day of randomization) to Day 14 (day of third and final dosing with trial medication), with s.c. trial medication administered on Day 0, Day 7, and Day 14. Handling, preparation and administration of trial medication will be done by unblinded trial personnel. All trial assessments will be done by blinded trial personnel.
- A follow-up period, from the end of the Treatment Period, with follow-up visits at Day 35, Day 60, and Day 104 (the EoT visit)

Time windows for each trial visit are given in

.

**Figure 7-1 Overview of the trial design**




An overview on the trial procedures is given in the flow chart (Table 2-1). Patients should be seen for all visits on the designated day or as close to it as possible.

### 7.2 Discussion of trial design and choice of control groups

The trial will be randomized and double-blind to increase trial validity and to reduce bias during evaluation of assessments with the two treatments. Since the 2 trial medications are not identical in appearance (dasiglucagon is a liquid formulation and GlucaGen is available as a powder for reconstitution), the handling, preparation and administration of trial medication will be done by unblinded trial personnel who will not be involved in other trial procedures and assessments. All trial assessments performed at the trial site will be done by blinded trial personnel. However, exposure assessments and ADA assessments will be performed by unblinded personnel at the specialty laboratories, to make sure that dasiglucagon or GlucaGen administration is matched with the applicable bioanalytical assay.

Euglycemic patients with T1DM will be randomized 1:1 in order to evaluate the immunogenicity of dasiglucagon compared to GlucaGen. The randomized, double-blind, parallel group design, with administration of 3 fixed consecutive doses of randomized trial medication (dasiglucagon or GlucaGen) to the same patient will allow characterization of immunogenic potential and a relative comparison of immunogenicity between the 2 products. Treatment with the 3 repeated doses (each separated by 1 week), with follow-up visits at Days 35 (where potential immune responses are known to be most pronounced), and at 60 and 104 days after the first dose (following the patient for 2-3 antibody half-lives after expected peak titer), is deemed relevant and sufficient for evaluating any immunogenic response to treatment. Patients that test positive for ADA will be monitored until the ADA levels return to baseline, and samples from the ADA positive patients will be tested for neutralizing potential in an Nab (neutralizing antibody) assay.

Dasiglucagon and GlucaGen will be administered at fixed doses independent of body weight because this is the intended therapeutic dosing regimen in the emergency treatment of hypoglycemia. The selected dose of 1 mg GlucaGen is the recommended dose for treatment of severe hypoglycemia. Based on pre-clinical and clinical studies, it has been demonstrated that 0.6 mg of dasiglucagon results in an initial pharmacodynamic response (i.e. acute glucose mobilization) comparable to 1 mg GlucaGen (see also Section 5.1).

For the safety and well-being of the patients, they will not be brought into hypoglycemia prior to dosing. However, very high blood glucose levels at dosing will potentially impact the reporting of nausea and other associated AE's. Therefore, to enable a more precise safety assessment, patients are required to be dosed while at a normal blood glucose level, and a pre-treatment plasma glucose level of 70-150 mg/dL will be targeted. Plasma glucose levels may be adjusted by s.c. administration of a fast-acting insulin analog or by glucose ingestion. Even with this precaution, it cannot be excluded that a higher frequency of nausea may be anticipated, if this AE is caused by hyperglycaemia.

The safety profile described to date does not give rise to specific safety concerns. In previous studies, dasiglucagon was associated with the AE's nausea, a known side effect following administration of glucagon, headache, and injection site reactions (erythema).

### 7.3 Selection of trial population

Dasiglucagon is indicated for treatment of severe hypoglycemia in patients with T1DM. Given the role of the immune system in the pathogenesis of T1DM, the present trial aims to evaluate the immune response of patients with T1DM taking repeated single doses of s.c. dasiglucagon compared to GlucaGen.

There are no data indicating an altered immune response with varying blood glucose levels, therefore, for the safety and well-being of the patients, they will not be brought into a hypoglycemic state prior to dosing. Patients with previous exogenic glucagon exposure will not be excluded from the trial, but the information on previous glucagon administration will be recorded to enable subgroup analyses.

The trial will enroll patients in centers in the EU, in the US, and in Canada.

### 7.3.1 Inclusion criteria

To be included in the trial, patients have to fulfill all of the following criteria:

(1) Informed consent obtained before any trial-related activities (trial-related activities are any procedure that would not have been performed during normal management of the patient)
(2) Availability for the entire trial period
(3) Age between 18 and 70 years, both inclusive
(4) Male or female patients with T1DM for at least 1 year. Diagnostic criteria as defined by the American Diabetes Association
(5) Hemoglobin $A_{1c}$ ($HbA_{1c}$) <10%
(6) Stable antidiabetic treatment for at least 1 month (e.g. within 10% insulin dose adjustment)
(7) A female participant must meet 1 of the following criteria

   a. Participant is of childbearing potential and agrees to use one of the accepted contraceptive regimens throughout the entire duration of the trial from screening until last follow-up visit. An acceptable method of contraception includes at least one of the following:

      i. Abstinence from heterosexual intercourse

      ii. Systemic contraceptives (birth control pills, injectable/implant/ insertable hormonal birth control products, transdermal patch); if the participant is using systemic contraceptives, she must use an additional form of acceptable contraception (iii or iv, below)

      iii. Intrauterine device (with and without hormones)

      iv. condom with spermicide

   or

   b. Participant is of non-childbearing potential, defined as surgically sterile (i.e. has undergone complete hysterectomy, bilateral oophorectomy, or tubal ligation), or in a menopausal state (at least 1 year without menses).

(8) A male must be surgically sterilized or willing to refrain from sexual intercourse from screening and until last follow-up visit or, if sexually active, uses a condom and partner practices contraception during the trial from screening and until the last follow-up visit.

### 7.3.2 Exclusion criteria

Patients meeting any of the following criteria during screening evaluations will be excluded from trial participation:

(1) Previous administration of dasiglucagon (previously referred to as ZP4207).
(2) Known or suspected allergy to trial medication(s) or related products
(3) History of anaphylaxis or symptoms of severe systemic allergy (such as angioedema)
(4) Previous participation (randomization) in this trial
(5) Females who are pregnant according to a positive pregnancy test, actively attempting to get pregnant, or are lactating
(6) Patients on a closed loop artificial pancreas
(7) Receipt of any investigational drug within 3 months prior to screening
(8) Active malignancy within the last 5 years
(9) Congestive heart failure, New York Heart Association class II-IV

(10) Inadequately treated blood pressure as defined as systolic blood pressure ≥180 mmHg or diastolic blood pressure ≥110 mmHg at screening.
(11) Current bleeding disorder, including use of anticoagulant treatment
(12) Known presence or history of pheochromocytoma (i.e. adrenal gland tumor) or insulinoma (i.e. insulin-secreting pancreas tumor)
(13) Known or suspected HIV infection
(14) Use of a systemic beta-blocker drug, indomethacin, warfarin or anticholinergic drugs in the previous 28 days before Day 1 of this trial)
(15) Use of systemic corticosteroids, anti-inflammatory biological agents, kinase inhibitors or other immune modulating agents within the last 3 months prior to screening
(16) Aspartate aminotransferase (AST) or alanine aminotransferase (ALT) >2.5 X the upper limit of normal (ULN), bilirubin >1.5 X ULN, estimated glomerular filtration rate (eGFR) <30 mL/min/1.73m$^2$ according to the Modification of Diet in Renal Disease (MDRD) Study definition
(17) Clinically significant abnormal ECG at screening, as evaluated by Investigator
(18) Donation of blood or plasma in the past month, or in excess of 500 mL within 12 weeks prior to screening
(19) Active substance or alcohol abuse
(20) Patients with mental incapacity or language barriers that preclude adequate understanding or cooperation, who are unwilling to participate in the trial, or who in the opinion of the Investigator should not participate in the trial
(21) Surgery or trauma with significant blood loss within the last 2 months prior to screening
(22) Any condition interfering with trial participation or trial endpoints or that could be hazardous to the patient

### 7.3.3 Premature removal from trial

Participation in the trial is strictly voluntary. A patient has the right to withdraw from the trial at any time and for any reason. If he/she chooses to withdraw, the investigator must be informed immediately. The investigator has the right to terminate participation of any patient at any time if the investigator deems it in the patient's best interest. The reason and circumstances for premature discontinuation will be documented in the electronic Case Report Form (eCRF).

#### 7.3.3.1 Possible reasons for patient discontinuation

A patient will be discontinued if the following applies:

- If a protocol deviation occurs which, in the clinical judgment of the Investigator, can invalidate the assessment of ADA responses to dasiglucagon or glucagon, the patient will be withdrawn by the Investigator
- AEs that are considered unacceptable by the patient or the Investigator

If discontinuation occurs following administration of any trial medication, the patient will be asked to return and participate in the complete follow-up visit at trial Day 104.

If trial participation is terminated due to an AE possibly related to the trial medication (including reference product) or trial examinations, the patient must be followed up by additional examinations according to the medical judgment of the investigator until the abnormal condition is resolved or the investigator deems further observations or examinations to be no longer medically indicated.

Patients, who meet one or more of the following dosing day exclusion criteria at a dosing visit, will be excluded from the dosing visit, but can be rescheduled to one of the following days (maximum 3 days postponement). Each dosing visit can only be rescheduled once.

- Strenuous exercise within 4 days prior to dosing, as judged by the Investigator. Strenuous exercise is not allowed during the treatment period of the trial
- Clinically significant illness that may interfere with trial objectives or impose a risk to patients, as judged by the Investigator
- Consumption of alcohol within 24 h prior to dosing visit, or positive results from an alcohol breath test
- Changes in medical history or concomitant medication resulting in fulfillment of clinical exclusion criteria, as judged by the Investigator

A total of 90 patients must complete the trial. To qualify as completed, the patient must be dosed according to protocol and have blood drawn for the ADA analyses described in the protocol. Discontinued patients will be replaced in order to reach 90 completed patients.

#### 7.3.3.2 Center discontinuation

The center can be closed and the trial terminated for the following reasons:

- The center is unlikely to be able to recruit sufficient patients within the agreed time frame
- The center does not respond to trial management requests
- Repeat protocol violations

#### 7.3.3.3 Trial termination

The sponsor reserves the right to modify or terminate the trial at any time. Possible reasons for termination are:

- Safety reasons – the incidence of AEs in this or any other trial using the same trial medication indicates a potential health risk for the patients.
- New scientific knowledge becomes known that makes the objectives of the trial no longer feasible/valid
- Unsatisfactory enrolment of patients

### 7.3.4 Replacement of patients

Patients prematurely withdrawn from the trial will be replaced in order to reach 90 completed patients. Replacement patients will receive the same treatment allocation (i.e. 3 doses of dasiglucagon or GlucaGen) as the patients they are replacing.

## 7.4 Investigational medicinal product(s)

### 7.4.1 Identity of investigational medicinal product(s)

The identity of the investigation products is summarized in Table 7-1.

**Table 7-1: Identity of investigational products**

| | **Test product** | **Reference product** |
|---|---|---|
| Name | Dasiglucagon | GlucaGen® |
| Active substance | ZP4207 | Recombinant glucagon hydrochloride |
| Formulation | Liquid formulation, 0.6 mL | Powder and solvent for reconstitution as 1 mL solution for injection |
| Strength | 1 mg/mL | 1 mg |
| Container | Single use pre-filled syringe | Powder and solvent for reconstitution packed together in a plastic box. A “hypo-kit” |
| Manufacturer | Zealand Pharma A/S, Glostrup (Copenhagen), Denmark | Novo Nordisk A/S, Bagsværd, Denmark |
| Storage requirements | Store between 2 and 8°C | Store between 2 and 8°C |

Handling, preparation and administration of trial medication will be done by unblinded trial personnel. All trial assessments on the trial site will be done by blinded trial personnel. However, exposure assessments and ADA assessments will be performed by unblinded personnel at the specialty laboratories, to make sure that dasiglucagon or GlucaGen administration is matched with the applicable bioanalytical assay.

The test product will be packed by the sponsor. The information on the labels will be in the local language. The label on the product will be compliant with local laws and regulations.

### 7.4.2 Treatments administered

Dasiglucagon is a stable peptide analog of human glucagon in a ready-to-use liquid formulation indicated for treatment of severe hypoglycemia in insulin dependent patients with diabetes mellitus. Dasiglucagon is in clinical development and has no marketing authorization as yet. GlucaGen is approved in the EU and US and is indicated for treatment of severe hypoglycemic reactions, which may occur in the management of insulin-treated children and adults with diabetes mellitus.

Patients in this trial have not previously been treated with dasiglucagon (ZP4207) and will be randomly assigned (1:1) to receive 1 of the following trial medications:

- 0.6 mg dasiglucagon
- 1 mg GlucaGen

Prior to administration of trial medication at all dosing visits patients must reach a target plasma glucose level of 70-150 mg/dL. Plasma glucose levels may be adjusted by s.c. administration of a fast-acting insulin analog if blood glucose exceeds150 mg/dL or by glucose ingestion if blood glucose is below 70 mg/dL.

Following the first (Visit 2) and third (Visit 4) dose administration, patients must be fasting for 90 min after dosing. Following the 90-min blood sample draw after the administration of the first (Visit 2) and third (Visit 4) dose administration, patients may be treated individually in order to

alleviate any potential side effects in order to minimize withdrawals and consequently reduce the amount of missing data. This treatment can also be instituted immediately after the second dose administration (Visit 3), as pharmacodynamics will not be assessed at this visit.

The following treatment modalities may be used, as considered appropriate by the investigator:

- patients will be allowed to eat and drink moderately to make them feel comfortable
- a moderate and individualized corrective dose of insulin to convert the induced hyperglycemia to euglycemia, after agreement with the investigator
- antiemetic treatment e.g. in the form of an 8-mg slow intravenous (i.v.) dose of ondansetron (Zofran®) or alternatively a 10-mg slow i.v. dose of metoclopramide (Primperan®)

Patients must be monitored for at least 5 h after dosing at the clinical site for safety observations, including blood glucose monitoring.

Patients will not be discharged until they are considered stable and with a blood glucose level in the range of 70-180 mg/dL. Before discharge, the investigator will provide instructions to the patients on management of their blood glucose levels. Each trial medication will be administered s.c. 3 times in total, with 7 days between dosing (i.e. dosing occurs at Days 0, 7, and 14; Visits 2, 3, and 4).

### 7.4.3 Selection of doses in the trial

The selected dose of 1 mg GlucaGen is the recommended dose for treatment of severe hypoglycemia. Based on pre-clinical and clinical studies, it has been demonstrated that 0.6 mg of dasiglucagon results in an initial pharmacodynamic response (i.e. acute glucose mobilization) comparable to 1 mg GlucaGen.

### 7.4.4 Treatment compliance

All trial medications will be prepared and administered by unblinded trial personnel.

### 7.4.5 Method of assigning patients to treatments or treatment sequences

Patients who meet all inclusion and none of the exclusion criteria and have given written informed consent will be randomized in a 1:1 ratio to either dasiglucagon or GlucaGen via an Interactive Web Response System (IWRS) that will assign a kit number to one of the 2 aforementioned treatment arms.

Patients with previous exogenic glucagon exposure will not be excluded from the trial, but the information on previous glucagon administration will be recorded, to enable subgroup analyses.

In the event of an emergency, e.g., when it becomes necessary for the investigator to know which treatment the patient is taking, the code for that patient can be broken by the investigator, preferably after consultation with the medical monitor. Emergency code breaks can be performed using the IWRS.

### 7.4.6 Blinding

This is a double-blind trial. Since dasiglucagon is available as a liquid formulation and GlucaGen is available as a powder for reconstitution, and they are therefore not identical in appearance, unblinded trial personnel will be responsible for handling, preparing, and administering the trial medication. To maintain double-blind conditions, all trial assessments at the trial site will be done by blinded trial personnel not involved in the administration of trial medications. However, exposure assessments and ADA assessments will be performed by unblinded personnel at the specialty laboratories, to make sure that dasiglucagon or GlucaGen administration is matched with the applicable bioanalytical assay.

### 7.4.7 Drug accountability and disposal

Handling, preparation and administration of trial medication will be done by unblinded trial personnel. Each center will keep accurate records of the trial supplies received, stored, and dispensed, using appropriate forms. The trial supplies will be stored under appropriate conditions, locked and with restricted access.

All unused supplies and all empty and partially empty containers of trial medication will be stored until the trial closure visit has been performed and then sent to the sponsor.

### 7.4.8 Prior and concomitant therapy

Prior glucagon exposure will be recorded in the eCRF at screening. All concomitant medications will be recorded in the eCRF at each visit.

Patients using any new concomitant medication resulting in fulfillment of a dosing day exclusion criterion will be excluded from the dosing visit, but can be rescheduled to one of the following days. Each dosing visit can only be rescheduled once. See Section 7.3.3.1 for possible reasons for patient discontinuation.

### 7.4.9 Treatment after end of trial

Not applicable in this trial.

## 7.5 Assessments and schedule of measurements (overview)

The following assessments and measurements will be carried out at the times specified in the trial flow chart (Table 2-1).

Informed consent will be obtained prior to any trial-related procedures; see Section 11.3.

### 7.5.1 Screening examination

At screening (Visit 1), the following assessments will take place:

- Informed consent
- Check of patient eligibility
- Demographics
- Body measurement
- Medical history
- History of alcohol/drug abuse
- Concomitant illnesses
- Prior medications
- Concomitant medications
- Physical examination
- Vital signs
- ECG
- AEs
- Hematology, biochemistry, coagulation
- Pregnancy test (women only)
- Urinalysis
- Urine drug screen (trial site testing)
- Alcohol breath test

### 7.5.2 Instructions to patients prior to dosing

On dosing days, patients are allowed to consume a small meal for breakfast with corresponding administration of rapid acting insulin to address the meal and in accordance with their normal management of blood glucose levels.

Use of long-acting insulin and rate of basal continuous subcutaneous insulin infusion (CSII) will be continued according to normal glycemic management of the individual patients during dosing days.

### 7.5.3 Procedures and assessments during the treatment period of the trial

At Visit 2 (Day 0) patient eligibility is rechecked (check of changes between the screening visit and Visit 2) and patients eligible to participate will be randomized to treatment with dasiglucagon or GlucaGen.

Additionally, during the treatment period of the trial, at Visits 2 and 4 (Days 0 and 14), the following assessments will take place:

- Concomitant medication
- Check of withdrawal criteria
- Check of dosing day exclusion criteria
- Vital signs (pre-dose, and at 30 and 90 min post-dosing)
- ECG (pre-dose, and at 30 and 90 min post-dosing)
- Local tolerability (at 0.5 and 2 h post-dosing)
- AEs
- Hematology and biochemistry (pre-dose, and at 30 and 90 min post-dosing)
- Urinalysis (pre-dose)
- Urine drug screen (pre-dose) at trial site
- Alcohol breath test (pre-dose)
- Dasiglucagon/glucagon plasma concentrations
  (Pre-dose, 5, 10, 30, 60, and 90 min post-dosing. The actual time of blood sampling for exposure should not deviate from the nominal time by more than ±1 min. Pre-dose is defined as within 5 min prior to dosing.)
- Plasma glucose concentration
  (Pre-dose, 5, 10, 30, 60, and 90 min post-dosing. The actual time for blood sampling for plasma glucose should not deviate from the nominal time by more than ±1 min. Pre-dose is defined as within 5 min prior to dosing.)
- Antibodies against dasiglucagon/glucagon (pre-dose)
- Administration of trial medication
  Prior to administration of trial medication patients must reach a target plasma glucose level of 70-150 mg/dL. Plasma glucose levels may be adjusted by s.c. administration of a fast-acting insulin analog or by glucose ingestion. Patients must be fasting for 90 min after administration of trial medication, and can be treated individually to alleviate any potential side effects. See section 7.4.2. Patients will be observed for at least 5 h post-dose.

At Visit 3 of the treatment period (Day 7), only the following assessments will take place

- Concomitant medication
- Check of withdrawal criteria
- Check of dosing day exclusion criteria
- Local tolerability (at 0.5 and 2 h post-dosing)
- AEs

- Urine drug screen (pre-dose) at trial site
- Alcohol breath test (pre-dose)
- Antibodies against dasiglucagon/glucagon (pre-dose)
- Administration of trial medication
  Prior to administration of trial medication patients must reach a target plasma glucose level of 70-150 mg/dL. Plasma glucose levels may be adjusted by s.c. administration of a fast-acting insulin analog or by glucose ingestion. Patients are NOT required to be fasting after administration of trial medication, and can be treated individually to alleviate any potential side effects immediately following dosing. See section 7.4.2. Patients will be observed for at least 5 h post-dose.

### 7.5.4 Follow-up period of the trial

After the final dose of trial medication (at Visit 4, Day 14, described above), patients will be followed up until Day 104 (Visit 7). During the follow-up period, visits take place at Days 35, 60, and 104 (Visits 5, 6, and 7).

At Visits 5 and 6 during the follow-up period, the following assessments will take place:

- Concomitant medication
- Check of withdrawal criteria
- AEs
- Antibodies against dasiglucagon/glucagon

Additionally, the following assessments will take place at Visit 5 only:

- Vital signs
- ECG
- Hematology and biochemistry

At Visit 7, the final visit of the follow-up period and of the trial (EoT visit), the following assessments will take place:

- Concomitant medication
- Physical examination
- Vital signs
- ECG
- AEs
- Hematology and biochemistry
- Urinalysis
- Antibodies against dasiglucagon/glucagon

### 7.5.5 Final examination at the end of the trial

The final visit of the trial is Visit 7 (Day 104 of the follow-up period; EoT visit). See Section 7.5.4 for further details.

### 7.5.6 Additional (safety) examinations

If there are any unclear symptoms or observations the responsible physician in charge may perform further medical examinations, other than outlined in this protocol, including further clinical laboratory tests, in order to clarify the relevance or to diagnose symptoms.

### 7.5.7 Safety laboratory tests

Samples obtained will be prepared and transferred to the appropriate laboratory by SynteractHCR according to Standard Operating Procedures. The parameters listed in Section 7.6.4.1 will be determined using standard methods.

The total volume of blood sampled per patient for safety analyses is 68 mL.

## 7.6 Immunogenicity, pharmacokinetic, pharmacodynamic, tolerability and safety measurements

Details of sampling for immunogenicity testing, plasma glucose concentrations, plasma trial medication concentrations, and safety laboratory testing are provided in a laboratory manual (see the Investigator site file).

### 7.6.1 Immunogenicity measurements

Antibodies against dasiglucagon/glucagon will be measured at all visits after screening. During the treatment period (Visits 2, 3, and 4) samples will be collected pre-dose.

The clinical ADA assays have been validated in accordance with existing guidelines and recommendations.[6,7,8,9,10]

Confirmed positive anti- dasiglucagon antibody samples, (treatment-induced or treatment-boosted) from anti-dasiglucagon antibody-positive patients will be evaluated for binding titer neutralizing potential and titer as well as cross-reactivity towards endogenous glucagon.

No further serum sampling will be needed as the ADA samples can be used for Nab analysis.

The *in vitro* neutralizing effect of antibodies will be measured using an assay[9,12] based on glucagon receptor transfected human embryonic kidney cells. The calculated sensitivity in previous studies was about 51.8 ng/mL. The assay was also validated for recombinant glucagon with similar results.[11,12] In case of a positive result in the Nab assay, a titer estimation will be performed. The cell-based Nab analyses will be performed by a special laboratory, BioAgilytix, Durham, NC, USA.

*In vivo* neutralizing effect will be evaluated by comparing PK and PD endpoints between ADA positive and ADA negative patients, further investigation may be performed by correlating ADA titer with PK and PD endpoints.

Residual and additional antibody serum samples may be stored until approval of market authorization by the health authorities. Further characterization of the antibody response may be requested by the health authorities.

### 7.6.2 Plasma concentrations of dasiglucagon and GlucaGen

The exposure to trial medication (dasiglucagon or GlucaGen) will be assessed based on plasma concentration data ($AUC_{0\text{-}30min}$, $AUC_{0\text{-}90\ min}$, $C_{max}$, $t_{max}$) from samples collected at Visits 2 and 4 (after administration of the first and third doses of trial medication).

Samples will be collected pre-dose, and at 5, 10, 30, 60, and 90 min post-dosing. The actual time of blood sampling for exposure should not deviate from the nominal time by more than ±1 min. Pre-dose is defined as within 5 min prior to dosing.

### 7.6.3 Pharmacodynamic measurements

The plasma glucose profile will be assessed based on plasma concentration data ($AUE_{0\text{-}30min}$, $AUE_{0\text{-}90\ min}$, $CE_{max}$, $t_{max}$) from samples collected at dosing Visits 2 and 4 (first and third dosing visit).

Samples will be collected pre-dose, and at 5, 10, 30, 60, and 90 min post-dosing. The actual time of blood sampling for evaluation of plasma glucose should not deviate from the nominal time by more than ±1 min. Pre-dose is defined as within 5 min prior to dosing.

#### 7.6.4 Safety and tolerability measurements

##### 7.6.4.1 Safety laboratory tests

Routine safety laboratory tests will be performed centrally. Samples for safety laboratory parameters will be collected at Visits 1, 2, 4, 5, and 7. Samples for urinalysis will be collected at Visits 1, 2, 4, and 7. The following parameters will be determined:

- Clinical chemistry: sodium, potassium, calcium, glucose, urea, creatinine, total bilirubin, AST (SGOT), ALT (SGPT), gamma-GT, alkaline phosphatase, total protein, C-reactive protein, $HbA_{1c}$, C-peptide
- Hematology: hemoglobin, red blood cell count (erythrocytes), hematocrit, platelet count (thrombocytes), total white blood cell count (leucocytes)
- Coagulation: international normalized ratio (INR), fibrinogen (at screening visit only)
- Urinalysis: pH, blood (leukocytes and erythrocytes), protein, glucose, ketones, nitrite

A pregnancy test will be performed at screening (Visit 1) for women only.

Alcohol breath tests and urine drug screen will be performed at Screening (Visit 1) and at Visits 2, 3, and 4.

For further details, please refer to the laboratory manual.

##### 7.6.4.2 Safety examinations

Physical examination is performed at Screening (Visit 1) and EoT (Visit 7).

AEs are assessed at all visits. Local tolerability is assessed at all dosing visits (Visits 2, 3 and 4. ECG and vital signs are assessed at Screening (Visit 1) Visits 2, 4, 5, and 7.

- Physical examination includes examination of the following body systems: head, ears, eyes, nose, throat (HEENT), including the thyroid gland; heart, lung, chest; abdomen; skin; musculoskeletal system; nervous system; lymph node
- Vital signs include: pulse rate and blood pressure in a sitting position after 5 min, body temperature
- Local tolerability: skin reactions will be assessed at the injection site at 0.5 and 2 h post-dosing. Skin reactions will be reported as AEs (see Section 8).
- 12-lead ECG. Details from ECG assessments will be recorded, including PR, QRS and QT intervals.

## 8. Adverse events

### 8.1 Definitions

**Adverse Event (AE)**

An AE is any untoward medical occurrence in a trial patient administered an investigational medicinal product (IMP) and which does not necessarily have a causal relationship with the treatment. An AE can therefore be any unfavorable and unintended sign (for example, an abnormal laboratory finding), symptom, or disease temporally associated with the use of an IMP, whether or not considered related to the IMP.

Note: This includes events from the first trial related activity after the patient has signed the informed consent.

AEs include:

- A clinically significant worsening of a concomitant illness.
- A clinical laboratory abnormality which is clinically significant, i.e. any abnormality that suggests a disease and/or organ toxicity and is of a severity that requires active management. Active management includes active treatment or further investigations, for example change of medicine dose or more frequent follow-up due to the abnormality.
- Hypoglycemic episodes (see Section 8.4)
- Injection site reactions

The following should not be recorded as AEs, if recorded at screening (on Screening Form or CRF):

- Pre-planned procedure, unless the condition for which the procedure was planned has worsened from the first trial related activity after the patient has signed the informed consent.
- Pre-existing conditions found as a result of screening procedures (pre-existing conditions should be reported as medical history or concomitant illness).

**Serious adverse event (SAE)**

A SAE is any untoward medical occurrence that at any dose:

- results in death
- is life-threatening
- requires inpatient hospitalization or prolongation of existing hospitalization
- results in persistent or significant disability or incapacity
- is a congenital anomaly or birth defect
- is medically important
  Medical judgement must be exercised in deciding whether an AE is believed to be 'medically important'. Medically important event may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above.

**Suspected unexpected serious adverse reactions (SUSAR)**

An AE, fulfilling one of the criteria of seriousness and being assessed as related to IMP application, the nature or severity of which is not consistent with the applicable reference document (e.g. ZP4207 IB[3] or package leaflet/SmPC for an approved product such as GlucaGen[13]).

**Intensity of an adverse event**

The maximum intensity (severity) of all AEs must be assessed by the Investigator and documented. Severity should be graded when the AE outcome is known:

Mild: A type of adverse event that is usually transient and may require only minimal treatment or therapeutic intervention. The event does not generally interfere with usual activities of daily living.

Moderate: A type of adverse event that is usually alleviated with additional specific therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort but poses no significant or permanent risk of harm to the research participant.

Severe: A type of adverse event that interrupts usual activities of daily living, or significantly affects clinical status, or may require intensive therapeutic intervention.

A ‘severe’ reaction does not necessarily deem the AE as ‘serious’ and a SAE may not be ‘severe’ in nature.

**Causality relationship to trial medication**

The causality of each AE should be assessed by the Investigator according to the following classification:

Probable: Good reason and sufficient documentation to assume a causal relationship.

Possible: A causal relationship is conceivable and cannot be dismissed.

Unlikely: The event is most likely related to etiology other than the trial product.

Not related: No relationship to trial product

**Outcome of an adverse event**

The outcome of all AEs must be assessed by the Investigator and documented by his/her staff. The following definitions should be used:

| | |
|---|---|
| Recovered/resolved: | The patient has fully recovered, or by medical or surgical treatment the condition has returned to the level observed at the first trial-related activity after the patient signed the informed consent. |
| Recovering/resolving: | The condition is improving and the patient is expected to recover from the event. This term is only applicable if the patient has completed the trial. |
| Recovered/resolved with sequelae: | The patient has recovered from the condition, but with lasting effect due to a disease, injury, treatment or procedure. If a sequela meets an SAE criterion, the AE must be reported as an SAE. |
| Not recovered/ not resolved: | The condition of the patient has not improved and the symptoms are unchanged. |
| Fatal: | This term is only applicable if the patient died from a condition related to the reported AE. Outcomes of other reported AEs in a patient before he/she died should be assessed as "recovered/resolved", "recovering/resolving", "recovered/ resolved with sequelae" or "not recovered/not resolved". An AE with fatal outcome must be reported as an SAE. |
| Unknown: | This term is only applicable if the patient is lost to follow-up. |

## 8.2 Collection, recording and reporting of adverse events

All events meeting the definition of an AE must be collected and reported from the first trial related activity after the patient has signed the informed consent until the end of the post-treatment follow-up period. At each contact with the site (visit or telephone, excluding safety visits, where the patient is not seeing the Investigator or his staff (e.g. visits to the laboratory)) the patient must be asked about AEs. All AEs, either observed by the Investigator or reported by the patient, must be recorded by the Investigator and evaluated.

The Investigator should record the diagnosis, if possible. If no diagnosis can be made the Investigator should record each sign and symptom as individual AEs.

All AEs must be recorded by the Investigator. One single Adverse Event Form must be used per AE from start to resolution. For SAEs, the Serious Adverse Event Form must also be completed.

AE information should include the following:

- Date and time of onset and resolution
- Date and time of Investigator's first information on the (S)AE
- Seriousness
- Severity
- Causal relationship with IMP
- Interruption or withdrawal of treatment with IMP and other measures taken
- Outcome

All AEs are coded; details are described in the trial specific Data Management Plan.

The Investigator must report initial information in writing (fax or email) on all SAEs to the responsible medical monitor of the Sponsor (Zealand) and to the Sponsor's responsible pharmacovigilance unit (PharmaLex) immediately (within 24 hours) after obtaining knowledge about the event.

Name: [redacted]

Company: PharmaLex (formerly Lindeq)

Address: Agern Allé 24, DK-2970 Hørsholm, Denmark

Tel: [redacted] (8 a.m. to 4 p.m.)
[redacted] (outside 8 a.m. to 4 p.m.)

Fax: [redacted]

E-mail: drugsafety@lindeq.com

In addition, and meeting the same timeline, Investigators have to report all SAEs to Zealand by forwarding the SAE form electronically (e.g. in PDF format) within 24 hours to the representatives of Zealand.

Names: [redacted] and [redacted]

Address: Smedeland 36, DK-2600 Glostrup, Denmark

Tel: [redacted] / [redacted]

E-mails: [redacted] / [redacted]

It is the responsibility of PharmaLex to report all SUSARs that occur in this trial to the Competent Authorities and IRBs/IECs in accordance with the local requirements in force and ICH guideline for GCP. The trial monitor must be informed accordingly.

### 8.3 Follow-up of adverse events

All AEs that are ongoing at the end of the patient's participation in the study will be followed-up until the event is resolved or reaches a satisfactory outcome as deemed by the Investigator.

Follow-up actions for all SAEs will be performed according to appropriate clinical care practices and may depend on the nature of the event. These will be determined after internal review and/or sponsor review.

The follow up information should only include new (updated and/or additional) information that reflects the situation at the time of the Investigator's signature.

Follow-up information on (S)AEs will be updated using the (S)AE Form. If a non-serious event becomes serious during the follow-up the AE Form and SAE Form have to be used and reporting timelines follow those of a SAE.

The Investigator must forward follow-up information on SAEs and if previously non-serious AEs become SAEs to the Sponsor immediately (within 24 hours) after obtaining knowledge about the new information.

### 8.4 Hypoglycemia

Hypoglycemia will be regarded as an AE and recorded and documented on an AE Form.

Hypoglycemia is defined as a fall of plasma glucose below 3.9 mmol/L (70 mg/dL).

During the dosing visits, mild to moderate symptoms of hypoglycemia, or a blood glucose (BG) value less than a threshold of <2.8 mmol/L (50 mg/dL), corresponding to a plasma glucose (PG) value of <3.1 mmol/L (56 mg/dL), will be treated by i.v. glucose solution at the investigator's discretion according to best available medical practice. Treatment is to be repeated until BG value stabilized above the threshold limit again. BG measurements will only be done due to safety concerns. Insulin-induced hypoglycemia should be recorded as an AE.

### 8.5 Pregnancy

Female patients must be instructed to notify the Investigator immediately if they become pregnant or if they suspect to be pregnant during the trial. The Sponsor has a responsibility to monitor the outcome of all pregnancies reported during the clinical trial.

Male patients must be instructed to notify the Investigator immediately if their partner becomes pregnant or suspects to be pregnant. The Sponsor has a responsibility to monitor the outcome of all pregnancies reported during the clinical trial. During an information meeting at the trial site, the patient's partner will be fully informed by a physician of her participation in capturing the outcome data, and will be asked to provide her written consent.

The Investigator must report all information on pregnancies on the initial pregnancy form. The completed initial pregnancy form must be forwarded to the Sponsor according to the procedure stated in Section 8.2. Any (S)AEs in the mother, as well as other pregnancy complications (ectopic pregnancy) fulfilling the criteria of an (S)AE, must be reported on the (S)AE form.

The following must be collected in the initial pregnancy form:

- Medical history of the mother
- Family history
- Course of the pregnancy, including expected delivery date

The Investigator must follow the pregnancy until the pregnancy outcome and the newborn infant(s) until the age of 1 month. The Investigator must collect information on the pregnancy and pregnancy complications as well as the pregnancy outcome including the health of the newborn infant(s) on the pregnancy outcome form. The completed pregnancy outcome form must be forwarded to the Sponsor according to the procedure stated in Section 8.2. Any (S)AEs in the newborn must be reported on the (S)AE form.

The SAEs that must be reported including abnormal outcome - such as congenital anomalies, fetal death and termination of pregnancy (spontaneous or elective abortion), including any anomalies of the fetus observed at gross examination or during autopsy.

The following must be collected in the pregnancy outcome form:

- Course of the pregnancy
- Outcome of the pregnancy
- Condition of the newborn
- Any AEs in the newborn infant must be followed till the age of 1 month

### 8.6 Precautions

Normal precautions taken for a human trial, including the provision of emergency equipment, will be taken during this trial. Qualified and well trained physicians and medical staff will instruct the patients. During a patient's participation in the trial, the Investigator should ensure that adequate medical care is provided to the patients for any AEs, including clinically significant laboratory values related to the trial. The Investigator should inform the patient when medical care is needed for intercurrent illnesses of which the Investigator becomes aware.

For further information on safety precautions for ZP4207 and GlucaGen, please refer to the current version of the Investigator's Brochure[3] and the SmPC for GlucaGen[13], respectively.

### 8.7 Safety Committee

The internal Zealand Pharma Safety Committee (SC) is constituted to perform ongoing safety surveillance of clinical trials with dasiglucagon, including this trial.

If safety signals are observed, either based on reported SAEs, periodic review of laboratory parameters, review of all AEs reported between the SC meetings, or on notification of significant findings, the SC will take appropriate measures to safeguard the patients.

The SC convenes every quarter to review relevant safety information, including AEs and laboratory data.

# 9. Data management and quality control

## 9.1 Case report forms

All the information collected during the trial will be recorded in the eCRFs, which are identified by patient number. Suitable eCRFs will be designed by SynteractHCR. The investigator will ensure that the eCRFs are correctly completed. All key pages will be signed or initialed by the investigator, signifying agreement with and responsibility for the recorded data. Key pages are the following: end-of-visit form, AE-reporting form, medication form, concomitant medication form and trial-closure form.

A trial specific list should be generated prior to the performance of the trial, which specifies which data fields in the Case Report Form will be used by the medical personnel for direct entry during the performance of the trial. This means that these data fields in the CRF are source documents. For the other CRF data fields, the source data will be found in other documents (such as patients' files, worksheets, etc.), that is, for these CRF data fields the "other" documents are the source documents. Should such a list be created, it should be submitted to the authorities together with the protocol.

## 9.2 Quality control

The investigator will permit trial-related monitoring, IEC review, and regulatory inspections, providing direct access to source data /documents. Sponsor-authorized quality assurance personnel may carry out audits for which the investigator must provide support.

The trial will be supervised by a monitor from SynteractHCR. The trial monitor will contact the investigator regularly to discuss the progress of the trial and to check the trial documents including the informed consent forms for completeness and consistency.

The trial monitor or a representative of the sponsor will cross-check the data entered in the eCRFs with the source data at the trial site and observe the trial procedures in order to verify adherence to the trial protocol.

The eCRFs will be checked for completeness and correctness by the monitor and data management department of SynteractHCR according to the SynteractHCR SOPs and any queries will be resolved by the investigator.

All of the clinical data will be captured via electronic data capture (EDC) using a web-based tool. The software Marvin from the company XClinical (www.xclinical.com/) is the preferred EDC software. Marvin is compliant with all legislation relevant to electronic data capture (FDA 21 CFR Part 11, GCP).

The investigator site staff will enter and edit the data via a secure network, with secure access features (username and password). A complete electronic audit trail will be maintained. The investigator will approve the data using an electronic signature (Ref: 21 CFR Part 11), and this approval is used to confirm the accuracy of the data recorded.

eCRFs will be used for all patients. The investigator's data will be accessible from the investigator's site throughout the trial. The electronic CRFs must be kept current to reflect patient status at each phase during the course of the trial. The electronic CRF will not capture personalized data. The investigator must make a separate confidential record of personalized details (name and initials) on the patient identification and enrollment log. All changes to data are done by the investigator through the EDC system.

It is the responsibility of the Principal Investigator of the respective site to ensure that all patient discontinuations or changes in trial or other medications entered on the patient's eCRF are also made on the patient's medical records.

The eCRFs for any patient leaving the trial should be completed at the time of the final visit or shortly thereafter.

### 9.3 Data management

Data management will be performed according to SynteractHCR SOPs.

# 10. Statistical methods and determination of sample size

## 10.1 Statistical and analysis plan

A separate Statistical Analysis Plan (SAP) will be finalized that details the planned statistical analysis and may include necessary adaptations to the planned statistical analysis before unblinding of the data.

Any deviations from the planned analyses will be described and justified in the final integrated trial report.

### 10.1.1 General considerations

All data obtained in this trial and documented in the eCRFs will be listed and summarized with statistics or frequency tables as appropriate. In case of termination of the trial, all data collected up to that time point will be included into the analysis.

Raw data listings and summary tables will be generated using the software SAS© Version 9 or higher.

Continuous variables will be summarized with means, standard deviations, medians, minimums, and maximums and valid cases.

Other summaries (e.g. quartiles, 95% confidence intervals) may be used as appropriate. Categorical efficacy and safety variables will be summarized by counts and by percentage of patients in corresponding categories.

### 10.1.2 Classification of patients to subsets

For the statistical analysis the randomized patients will be divided up into the following datasets:

The following definitions are applicable:

| | |
|---|---|
| **Safety analysis set (SAS)** | all patients who were randomized and received at least one dose of trial medication |
| **Full analysis set (FAS)** | all patients of the SAS with at least one measurement of the ADA titer |
| **Per Protocol set (PPS)** | all patients of the FAS for whom no relevant protocol deviations were documented |

The analysis of the primary endpoint will be based on the FAS. A secondary analysis of the primary endpoint will be based on the PPS. Safety analysis will be based on the SAS.

The decision whether a protocol deviation is relevant or not for the exclusion of patients from the PPS set will be made case-by-case in a data review meeting.

### 10.1.3 Immunogenicity data

All immunogenicity parameters will be analyzed descriptively per treatment group. No statistical tests are planned. Baseline ADA-positive patients will be calculated as a percentage of the total number of patients whose baseline samples were tested for ADA. Titer will be reported as median and interquartile range.

95% confidence intervals for incidence rates and for rate differences will be given.

Secondary immunogenicity parameters will be described with appropriate descriptive statistics for dichotomous, categorical or continuous variables.

#### 10.1.4 Exposure and PD endpoints

Plasma dasiglucagon and glucagon concentrations 0-90 min from dosing: $AUC_{0\text{-}30min}$, $AUC_{0\text{-}90\ min}$, $C_{max}$, and $t_{max}$ will be summarized with descriptive statistics.

Plasma glucose profiles 0-90 min from dosing: $AUE_{0\text{-}30min}$, $AUE_{0\text{-}90\ min}$, $CE_{max}$, and $t_{max}$ will be summarized with descriptive statistics.

#### 10.1.5 Safety data

**Clinical laboratory data**

Clinical laboratory test results will be marked whether the result is below, within or above the respective reference range. The number of values outside of the reference range will be counted.

**Adverse events**

Adverse events will be tabulated by system organ class (SOC) and preferred term (PT) after medical coding using the Medical Dictionary for Regulatory Activities (MedDRA). AE summary tables will include counts and percentages of patients who experienced adverse events summarized by system organ class (SOC) and preferred term (PT).

**Other safety data**

Vital signs, physical examination, ECG and local tolerability data will be summarized with descriptive statistics.

#### 10.1.6 Further data

Baseline and demographic data will be summarized using descriptive statistics. Baseline ADA-positive patients will be calculated as a percentage of the total number of patients whose baseline samples were tested for ADA.

All other data obtained in this trial and documented in the eCRF will be listed.

#### 10.1.7 Withdrawals, drop-outs and missing data

In the case of drop-outs, no imputation of values for immunogenicity measurements will be done. Analysis is done on valid cases only i.e. no imputation technique like LOCF (last observation carried forward) will be applied.

#### 10.1.8 Baseline and center comparisons

Demographic and other baseline characteristics will be compared.

#### 10.1.9 Subgroup analysis

No subgroup analysis is currently planned.

#### 10.1.10 Interim analysis

No interim analysis is currently planned.

### 10.2 Determination of sample size

The purpose of the present trial is to generate data describing the immunogenic potential of dasiglucagon, when used as a rescue therapy from severe hypoglycemia and with reference to the immunogenic potential of GlucaGen. The sample size is based on generating data to show that the 90% confidence of the ADA incidence is no worse than 15% as the predefined acceptability criterion and with a reference to similar data being generated for GlucaGen.

Currently, no ADA incidences have been detected in the completed clinical trials, where up to 5 repeated doses of dasiglucagon have been administered to the same patients within a week. Across the two phase 1 clinical trials and a completed phase 2 PK/PD trial, a total of 141 subjects have been exposed to dasiglucagon and no incidences of ADA development have been observed. The obtained data indicate that dasiglucagon has a low risk for induction of ADAs in the investigated settings and as a consequence the sample size is based on showing that the ADA incidence is no worse than the predefined margin of 15%.

# 11. Ethics and regulations

## 11.1 Independent ethics committees and competent authorities

The clinical trial authorization (CTA) granted by the competent authority (CA) and a favorable opinion from the relevant independent ethics committee(s) (IEC(s)) /Institutional Review Board (IRB) will be obtained prior to the start of the trial. The local authorities will be notified about the trial as required by law.

The CA and the EC/IRB will be notified about the end of the trial and a report summarizing the trial results will be sent to the CA and the EC within one year after the end of the trial. If the trial is terminated early, the CA and the EC will be notified within 15 days.

The IECs and/or IRBs met the requirements of the International Conference on Harmonisation (ICH) Harmonised Tripartite Guideline for Good Clinical Practice (GCP) and local legislation. They also met the requirements of 21 CFR 312.3.

## 11.2 Ethical conduct of the trial

The trial will be conducted in accordance with the ethical principles set forth in the Declaration of Helsinki (including amendments).

## 11.3 Patient information and consent

Written informed consent will be obtained from all patients prior to entry into the trial. The investigator will explain to each patient orally and in writing (patient information sheet) the nature, significance, risks and implications of the trial before inclusion. In particular, the patients will be informed about the following:

- the possibility of withdrawing from the clinical trial at any time by revoking the consent and without any resulting disadvantage
- how personal and health-related data will be collected and used during the trial
- the patient must be informed that his/ her medical records may be examined by authorized monitors or Clinical Quality Assurance auditors appointed by the Sponsor, by appropriate IEC members, and by inspectors from regulatory authorities.

All patients will receive a copy of the patient information sheet and a copy of their signed and dated informed consent form.

All patients will be insured against injury caused by their participation in the trial according to legal requirements. They will be informed about the insurance and the resulting obligations on their part.

## 11.4 Legal and regulatory requirements

This trial will be carried out in accordance with:

- ICH guidelines for GCP, United States investigational new drug (IND) regulations (21 CFR 312), the regulations on electronic records and electronic signature (21 CFR 11), the most recent guidelines of the Declaration of Helsinki, and the relevant laws and regulations of the country in which the trial takes place.
- Standard operating procedures (SOPs for clinical investigation and documentation in force at SynteractHCR)

# 12. Trial administration

## 12.1 Responsibilities

Zealand A/S is the sponsor of this trial. SynteractHCR, a contract research organization (CRO), will organize the performance of this trial.

A list with the names and addresses of the responsible institutions and persons is provided in Appendix 1 of this protocol.

## 12.2 Protocol deviations

The investigator agrees to conduct the trial in compliance with the protocol. Prospective protocol deviations or waivers will not be granted for this trial.

Any deviation from the clinical trial protocol in the conduct of the clinical trial will be notified to the Monitor on an ongoing basis.

## 12.3 Protocol changes

Amendments to this trial protocol may be made following the procedures specified by local laws and regulations. Substantial amendments to this trial protocol may be implemented only if the approval of the CA(s) and a favorable opinion of the ethics committee(s) have been obtained.

Substantial amendments to the conduct of the clinical trial may arise from changes to the protocol or from new information relating to the scientific documents in support of the trial. Amendments to the trial are regarded as “substantial” where they are likely to have a significant impact on:

- the safety, physical health and mental integrity of the patients;
- the scientific value of the trial;
- the conduct or management of the trial; or
- the quality or safety of any investigational medicinal product used in the trial.

If a new event occurs related to the conduct of the trial or the development of the investigational product, which may affect the safety of the patients, the sponsor and the investigator will take appropriate safety measures to protect the patients against any immediate hazard. The sponsor will immediately inform the CA(s) and ethics committee(s) of the new events and the measures taken.

## 12.4 Publication of results

The original eCRFs and the data generated from the eCRFs or otherwise obtained during the trial under this trial protocol will become the property of the sponsor. Publication of the results of this trial by SynteractHCR or the investigator is possible only after written consent has been obtained from the sponsor. Any material intended for publication will be given to the sponsor at least 4 weeks before submission for publication. The sponsor will have the right to comment on the intended publication and to take any reasonable measures for patent protection.

## 12.5 Clinical trial report

After completion of the trial, the results will be tabulated, evaluated and issued as a complete final clinical trial report according to the ICH-E3 Note for guidance on structure and content of clinical trial reports.

The sponsor will send a summary of this clinical trial report to the EC and CA within one year after the end of the trial.

### 12.6 Retention of trial records

Records and documents pertaining to the conduct of the trial and the distribution of the investigational product (e.g. ICFs, laboratory slips, medication inventory records, and other pertinent information) must be retained by the Investigator according to local requirements.

To meet regulatory requirements, the eCRF data will be electronically stored at sites. The CDISC ODM (see http://www.cdisc.org/ for details) will be used to store and archive all electronic data at the sites. Since CDISC ODM is also the source for the EDC-web-based system, no transcription of data is necessary. CDISC ODM is a platform-independent standardized data format including the complete trial metadata and audit trail. The data can be reviewed at a later stage using off-the-shelf tools. CDISC provides a complete CDISC ODM Viewer for these purposes. If needed, PDF-files can be created from the ODM file.

## 13. References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13. SPC Novo Nordisk GlucaGen® HypoKit 1 mg powder and solvent for solution for injection. http://www.medicines.org.uk/emc/medicine/4258/SPC/GlucaGen+Hypokit+1+mg

# APPENDIX 1:

List of names and addresses

**List of names and addresses**

| | |
|---|---|
| Sponsor | Zealand Pharma A/S<br>Smedeland 36<br>2600 Glostrup, Copenhagen<br>Denmark |
| Contract Research Organization (CRO) | SynteractHCR Deutschland GmbH<br>Albrechtstr. 14<br>80636 Munich, Germany |
| Coordinating investigator | Linda Morrow, MD<br>Prosciento<br>855 Third Avenue<br>91911 Chula Vista, CA, USA |
| Trial monitor | To be decided<br>SynteractHCR Deutschland GmbH<br>Albrechtstr. 14<br>80636 Munich, Germany |
| Statistician | [redacted]<br>Senior Director Biometrics EMEA<br>SynteractHCR Deutschland GmbH<br>Albrechtstr. 14<br>80636 Munich, Germany |
| Sponsor representative | [redacted]<br>Clinical Trial Manager<br>Zealand Pharma A/S<br>Smedeland 36, 2600 Glostrup, Denmark<br>Tel: [redacted]<br>E-mail: [redacted] |
| Sponsor's medical expert | [redacted]<br>Medical Director<br>Zealand Pharma A/S<br>Smedeland 36, 2600 Glostrup, Denmark<br>Tel: [redacted]<br>E-mail: [redacted] |
| Sponsor representative for pharmacovigilance | [redacted]<br>PharmaLex A/S Agern Allé 24, 2970 Hørsholm, Denmark<br>Tel: [redacted] (8 a.m. to 4 p.m.)<br>[redacted] (outside 8 a.m. to 4 p.m.)<br>Fax: [redacted]<br>E-mail: drugsafety@lindeq.com |
| Clinical laboratory | *To be determined* |
| Analytical laboratory (ZP4207 PK, ZP4207 ADA, Glucagon ADA) | York Bioanalytical Solutions (YBS)<br>[redacted] (ZP4207 Bioanalysis)<br>[redacted] (ADA analyses)<br>Cedar House |

Northminster Business Park
Northfield Lane
York, YO26 6QR
United Kingdom
E-mail: [redacted]
E-mail: [redacted]

(ZP4207 and Glucagon Nab) [redacted]

BioAgilytix Labs
2300 Englert Drive
Durham, NC 27713
USA
[redacted]

# CLINICAL TRIAL PROTOCOL AMENDMENT

**A phase 3, Randomized, Double-Blind, Parallel Group Safety Trial to Evaluate the Immunogenicity of Dasiglucagon and GlucaGen® Administered Subcutaneously in Patients with Type 1 Diabetes Mellitus (T1DM)**

**Sponsor code: ZP4207-16136**
**SynteractHCR: ZEA-DNK-01711**
**EudraCT number: 2017-000062-30**

| | |
|---|---|
| Sponsor: | Zealand Pharma A/S,<br>Smedeland 36,<br>2600 Glostrup,<br>DENMARK |
| Clinical Research Organization: | SynteractHCR Deutchland GmbH,<br>Albrechtstr. 14,<br>80636 Munich<br>GERMANY |
| Study Drug Name: | Dasiglucagon |
| Development Phase: | 3 |
| Amendment Number: | 01 |
| Amendment Date: | May 8th 2017 |
| Type of Amendment: | Substantial |
| Amendment to protocol: | Final version 1, dated January 18th 2017 |

The study will be conducted according to the protocol and in compliance with Good Clinical Practice (GCP), with the Declaration of Helsinki [1] and with other applicable regulatory requirements.

## SIGNATURE PAGE

Declaration of Sponsor or Responsible Medical Officer

**Title: A phase 3, Randomized, Double-Blind, Parallel Group Safety Trial to Evaluate the Immunogenicity of Dasiglucagon and GlucaGen® Administered Subcutaneously in Patients with Type 1 Diabetes Mellitus (T1DM)**

This trial protocol amendment number 1 was subjected to critical review. The information it contains is consistent with current knowledge of the risks and benefits of the investigational product, as well as with the moral, ethical and scientific principles governing clinical research as set out in the 2008 revision of the Declaration of Helsinki [1] and the guidelines on Good Clinical Practice (GCP) [2].

[redacted] _____ [redacted]
Date

Title: Clinical Project Manager
Institution: Zealand Pharma a/s
Smedeland 36, 2600 Glostrup, Denmark

[redacted] _____ [redacted]
Date

Title: Medical Director
Institution: Zealand Pharma a/s
Smedeland 36, 2600 Glostrup, Denmark

[redacted] _____ [redacted]
Date

Title: Head of Clinical [redacted]pment
Institution: Zealand Pharma a/s
Smedeland 36, 2600 Glostrup, Denmark

**Declaration of the Investigator**

**Title: A phase 3, Randomized, Double-Blind, Parallel Group Safety Trial to Evaluate the Immunogenicity of Dasiglucagon and GlucaGen® Administered Subcutaneously in Patients with Type 1 Diabetes Mellitus (T1DM)**

All documentation for this trial that is supplied to me and that has not been previously published will be kept in the strictest confidence. This documentation includes the protocol, and this trial protocol amendment number 1, Investigator's Brochure, Case Report Forms (CRFs), and other scientific data.

The trial will not be commenced without the prior written approval of a properly constituted Institutional Review Board (IRB) or Independent Ethics Committee (IEC). No changes will be made to the trial protocol without the prior written approval of the Sponsor and the IRB or IEC, except where necessary to eliminate an immediate hazard to the patients.

I have read and understood and agree to abide by all the conditions and instructions contained in this protocol amendment.

Responsible Investigator of the local trial centre

_____ _____

Signature Date

_____

Name (block letters)

_____

Title (block letters)

_____

Institution (block letters)

_____

Phone number

## Table of Contents

## 1 Amendment type

This is substantial amendment number 01 to trial protocol version 1, dated 18 January 2017.

## 2 Rationale for amendment

The amendment will add precision to the description of the overall rationale of the trial. In addition, details pertaining to safety assessment of the patients are revised. Furthermore, the amendment serves to correct changes, errors or inconsistencies in the description of the operational set up of the trial.

## 3 Summary of Amendment

With this substantial amendment number 01, the following changes are made to the original protocol:

- Clarification on the statistical method and anti-drug antibody (ADA) assays
- Update of exclusion criterion on blood pressure
- Update of exclusion criterion on alcohol/drug abuse
- Specification of prohibited concomitant medication
- Additional ECG assessment added
- Clinical event of interest added
- Treatment options for patients experiencing hypo- or hyperglycemia prior dosing
- Monitoring of patients' electrolyte levels
- Monitoring of potential pregnancies
- Additional visits required for patients discontinuing treatment prematurely
- Specification on time windows for assessments
- Specification of the requirements at the dosing visits
- Responsibility of unblinded trial personnel
- Randomization of replacement patients
- Clarification to the reporting of (Serious) Adverse Events
- Clarification on case report forms
- Subgroup analysis added to the statistical section
- Administrative changes

Section 4 of this amendment will describe the overall changes and rationales.

Section 5 of this amendment will list the actual changes in the protocol text reflecting both superseded and new wording.

# 4 Changes and rationale:

## 4.1 Clarification on the statistical method and anti-drug antibody (ADA) assays

The primary objective has been updated to clarify that GlucaGen is considered a reference product and the objective of the trial is to evaluate the immunogenicity of both dasiglucagon and GlucaGen. It has been specified that the ADA assays to be used in this trial are both validated, but specific for dasiglucagon and GlucaGen, respectively. Hence, the performance of the assays are not directly comparable and a formal statistical comparison or non-inferiority testing is not performed. The rationale for the sample size calculation has been adapted accordingly. These clarifications have been implemented throughout the protocol including the protocol title.

The rationale for the update is to clarify that the trial is not a non-inferiority trial and no formal statistical testing will be performed.

## 4.2 Update of exclusion criterion on blood pressure

Exclusion criterion number 10 has been adjusted to exclude patients with a systolic blood pressure ≥160 mmHg or diastolic blood pressure ≥90 mmHg at screening.

The rationale for narrowing the limit of allowed blood pressure values at screening is to only include patients of good health, apart from having type 1 diabetes, in the trial.

## 4.3 Update of exclusion criterion on alcohol/drug abuse

Exclusion criterion number 19 has been updated to exclude patients with a significant history of alcohol or drug abuse or a positive alcohol or drug test at screening. Further, the limits for current alcohol consumption has been added.

In addition, it has been specified in the protocol which drugs the drug test will screen for and that a positive drug test during the trial will be a withdrawal criteria.

The rationale of the update is to ensure patients with a history or current abuse of alcohol and/or drugs are not included in the trial.

## 4.4 Specification of prohibited concomitant medication

An additional exclusion criterion has been added to exclude patients using any non-prescription or prescription drugs known to cause QT prolongation. It has further been specified that metoclopramide (Primperan®) is the only allowed antiemetic treatment that can be used at the dosing visits; 2, 3, and 4.

The rationale for the addition is due to the sporadic QTcB changes post dasiglucagon/GlucaGen administration observed in the phase 2 trial.

## 4.5 Additional ECG assessment added

ECG will be measured at 20, 35, 45 and 60 minutes post dosing at all three dosing visits; 2, 3, and 4.

The rationale for the change is due to the sporadic QTcB changes post dasiglucagon/GlucaGen administration observed in the phase 2 trial. The updated time points for ECG assessments are to cover the range of $t_{max}$ for dasiglucagon and GlucaGen at all three dosing visits.

### 4.6 Monitoring of patients' electrolyte levels

It has been added to exclusion criterion 16 that patients with altered electrolytes values of clinical relevance for cardiac conduction will be excluded from the trial. Further, the investigator must ensure that patients are actively treated to correct any electrolyte imbalances following potential events of vomiting at the dosing visits.

The rationale for the change is to ensure that patients do not have any electrolyte imbalances that could potentially affect the cardiac conduction and the QT interval.

### 4.7 Clinical event of interest added

Changes in blood pressure and/or pulse after dosing will be considered a clinical event of interest. Additional vital sign assessments have been added.

The rationale for the addition is due to the nonclinical and clinical findings in healthy volunteers of hemodynamic changes with dasiglucagon. Due to these findings additional information on such events will be captured in a specific form in the case report form.

### 4.8 Treatment of patients experiencing hypo- or hyperglycemia prior dosing

It has been specified that at all three dosing visits; 2, 3, and 4, the investigator should administer corrective doses of insulin if a patient has a plasma glucose >150 mg/dL before dosing. Likewise if a patient has a plasma glucose <70 mg/dL before dosing glucose ingestion should be provided to the patient.

The rationale for this addition is to clarify how the investigator should ensure patients have a plasma glucose level on 70-150mg/dL before dosing.

### 4.9 Monitoring of potential pregnancies

For women of childbearing potential a urine stick pregnancy test will be performed at the site prior dosing at all three dosing visits; 2, 3, and 4.

If a female patient becomes pregnant in the period between the screening visit and the last dosing visit, she must be discontinued from trial treatment.

The rationale for the change is to ensure that patients who have become pregnant in the period following the screening visit are not exposed to trial medication.

### 4.10 Additional visits required for patients discontinuing treatment prematurely

A patient prematurely discontinuing treatment will be asked to attend all three follow up visits; 5, 6, and 7, to have all follow up assessments performed. Only in case a patient withdraw consent or is excluded before exposure to trial product the follow up visits can be omitted.

The rationale for this addition is to avoid missing data for the primary and key secondary endpoints.

### 4.11 Specification on time windows and order of assessments

Time window allowance has been added to collection time of safety blood samples, vital signs and local tolerability assessments. Further, the time for obtaining informed consent has been specified as well as the time of randomization.

The rationale for the addition is to clarify the time and timing for the assessments to take place.

### 4.12 Specification of the requirements at the dosing visits

The requirements for fasting at the dosing visits; 2, 3, and 4, have been specified. Further, the instructions to patients prior dosing have been made more clear.

The rationale for the addition is to clarify the procedures around the dosing visits.

### 4.13 Responsibility of unblinded trial personnel

The responsibility of the unblinded trial personnel has been clarified.

The rationale for the addition is to clarify the procedures to maintain the double blinding.

### 4.14 Randomization of replacement patients

Replacement patients will not necessarily be randomized to the same treatment as the patient they are replacing.

The rationale is that it is not technically possible nor needed to assign the replacement patients to the same treatment as the patient they are replacing.

### 4.15 Clarification to the reporting of (Serious) Adverse Events

It has been clarified that the investigator only needs to send the SAE form to the Pharmacovigilance unit (Safety CRO). Further, the text has been updated to make it more simple.

The rationale for the changes is to make the process for SAE reporting more simple and to make the text more clear.

### 4.16 Clarification on case report forms

Minor clarifications have been made to the section on case report forms. E.g. it has been specified that no end-of-visit form will be used in the trial.

The rational for the change is to correct minor errors and make the section more clear.

### 4.17 Subgroup analyses added to statistical section

It has been added that subgroup analyses by gender, age and race will be performed for the primary endpoint. Further details if needed will be provided in the statistical analysis plan.

The rationale for including this in the protocol is due to regulatory requirements.

### 4.18 Administrative changes

- IND and dasiglucagon SUB numbers have been added
- Coordinating investigator and other trial personnel have been updated (changes also applicable for Appendix 1)

## 5 Amended text

This section will list the actual changes in the protocol text reflecting both the original and the new wording.

New wording is marked in *underlined italics* and deleted wording is marked with ~~strikethrough~~.

### 5.1 Clarification on the statistical method and anti-drug antibody (ADA) assays

Front page:

Updated text:

A phase 3, Randomized, Double-Blind, Parallel Group Safety Trial to Evaluate the Immunogenicity of Dasiglucagon *and* ~~compared to~~ GlucaGen® Administered Subcutaneously in Patients with Type 1 Diabetes Mellitus (T1DM)

Section 1. Signatures and agreement with protocol

Updated text:

**Title:** A phase 3, Randomized, Double-Blind, Parallel Group Safety Trial to Evaluate the Immunogenicity of Dasiglucagon *and* ~~compared to~~ GlucaGen® Administered Subcutaneously in Patients with Type 1 Diabetes Mellitus (T1DM)

Section 2. Trial synopsis

Title of the trial

Updated text:

A phase 3, Randomized, Double-Blind, Parallel Group Safety Trial to Evaluate the Immunogenicity of Dasiglucagon *and* ~~compared to~~ GlucaGen® Administered Subcutaneously in Patients with Type 1 Diabetes Mellitus (T1DM)

Objectives

Updated text:

The primary objective is to evaluate the immunogenicity of repeated single doses of dasiglucagon *and GlucaGen* following subcutaneous (s.c.) administration ~~compared with s.c. GlucaGen~~ in T1DM patients.

Trial design

Updated text:

This is a randomized, double-blind, parallel group trial *evaluating* ~~comparing~~ the immunogenicity of either dasiglucagon or GlucaGen administered to euglycemic T1DM patients.

Statistical methods:

Updated text:

All statistical analysis will be descriptive *i.e. no formal statistical testing will be performed*. Continuous variables will be summarized with means, standard deviations, medians, minimums, and maximums, and valid cases.

Sample size calculation:

Updated text:

The purpose of the present trial is to generate data describing the immunogenic potential of dasiglucagon *and GlucaGen*~~, when used as a rescue therapy form severe hypoglycemia and with reference to the immunogenic potential of GlucaGen. The sample size is based on generating data to show that the 90% confidence of the ADA incidence is no worse than 15% as the predefined acceptability criterion and with a reference to similar data being generated for GlucaGen~~. *The ADA assays to be used in this trial are both validated, but specific for GlucaGen and dasiglucagon, respectively, and the performance of the assays are thus not directly comparable. As a consequence, a formal comparison or non-inferiority analysis will not be performed.*

Currently, no ADA incidences have been detected in the completed clinical trials, where up to 5 repeated doses of dasiglucagon have been administered to the same patients within a week. Across the two phase 1 clinical trials and a completed phase 2 pharmacokinetic/pharmacodynamic trial, a total of 141 subjects have been exposed to dasiglucagon and no incidences of ADA development have been observed. The obtained data indicate that dasiglucagon has a low risk for induction of ADAs in the investigated settings and as a consequence ~~the sample size is based on showing that the ADA incidence is no worse than the predefined margin of 15%.~~*a meaningful sample size to compare both treatments cannot be estimated.*

*The sample size is therefore based on a certain precision of the confidence interval for the overall ADA incidence if no events are observed, respectively to ensure a certain probability for observing one event.*

*When no events are observed, to obtain an upper bound of 0.050 on the 90.0% confidence interval for the probability of such a rare event, would require a sample size of 45. Respectively, accepting a chance of observing at least one event of 90% and an actual probability of the event of 5% leads to a sample size of 45 patients completing the trial. In order to account for drop-outs, It is expected that 112 patients in total will be randomized and treated.*

Section 5.2 Trial rationale

Updated text:

The present trial aims to evaluate that immunogenicity risk with an assessment of the occurrence of ADAs and neutralizing ADAs, and of cross-reactivity with native glucagon, following repeated single doses of dasiglucagon by s.c. administration in T1DM patients. The *reference product* ~~comparator~~ in this trial is GlucaGen, a recombinant human glucagon approved for the treatment of the severe hypoglycemic reactions that may occur in the management of insulin-treated children and adults with diabetes mellitus. *Given the differences in ADA assay for dasiglucagon and GlucaGen, a formal statistical comparison or non-inferiority testing is not performed.*

Section 6 Trial objectives

Updated text:

Primary objective

- To evaluate the immunogenicity of repeated single doses of dasiglucagon *and GlucaGen* following s.c. administration ~~compared with s.c. GlucaGen~~ in T1DM patients.

Section 7.2 Discussion of trial design and choice of control groups

Updated text:

Euglycemic patients with T1DM will be randomized 1:1 in order to evaluate the immunogenicity of dasiglucagon *and* ~~compared to~~ GlucaGen. The randomized, double-blind, parallel group design, with administration of 3 fixed consecutive doses of randomized trial medication (dasiglucagon or GlucaGen) to the same patient will allow characterization of immunogenic potential *for* ~~and a relative comparison of the immunogenicity between~~ the 2 products.

Section 7.3 Selection of trial population

Updated text:

Dasiglucagon is indicated for treatment of severe hypoglycemia in patients with T1DM. Given the role of the immune system in the pathogenesis of T1DM, the present trial aims to evaluate the immune response of patients with T1DM taking repeated single doses of s.c. dasiglucagon *and* ~~compared to~~ GlucaGen.

Section 10.1.1 General Considerations

New text:

*All statistical analysis will be descriptive i.e. no formal testing will be performed.*

Section 10.1.2 Classification of patients to subsets

Updated text:

The following definitions are applicable:

| | |
|---|---|
| **Safety analysis set (SAS)** | all patients who were randomized and received at least one dose of trial medication |
| **Full analysis set (FAS)** | all patients of the SAS with at least one measurement of the ADA titer *at baseline* |
| **Per Protocol set (PPS)** | all patients of the FAS for whom no relevant protocol deviations were documented |

Section 10.2 Determination of sample size

Updated text:

The purpose of the present trial is to generate data describing the immunogenic potential of dasiglucagon *and GlucaGen*.~~, when used as a rescue therapy form severe hypoglycemia and with reference to the immunogenic potential of GlucaGen. The sample size is based on generating data to show that the 90% confidence of the ADA incidence is no worse than 15% as the predefined acceptability criterion and with a reference to similar data being generated for GlucaGen~~.*The ADA assays to be used in this trial are both validated, but specific for GlucaGen*

*and dasiglucagon, respectively, and the performance of the assays are thus not directly comparable. As a consequence, a formal comparison or non-inferiority analysis will not be performed.*

Currently, no ADA incidences have been detected in the completed clinical trials, where up to 5 repeated doses of dasiglucagon have been administered to the same patients within a week. Across the two phase 1 clinical trials and a completed phase 2 pharmacokinetic/pharmacodynamic trial, a total of 141 subjects have been exposed to dasiglucagon and no incidences of ADA development have been observed. The obtained data indicate that dasiglucagon has a low risk for induction of ADAs in the investigated settings and as a consequence ~~the sample size is based on showing that the ADA incidence is no worse than the predefined margin of 15%.~~*a meaningful sample size to compare both treatments cannot be estimated.*

*The sample size is therefore based on a certain precision of the confidence interval for the overall ADA incidence if no events are observed, respectively to ensure a certain probability for observing one event.*

*When no events are observed, to obtain an upper bound of 0.050 on the 90.0% confidence interval for the probability of such a rare event, would require a sample size of 45. Respectively, accepting a chance of observing at least one event of 90% and an actual probability of the event of 5% leads to a sample size of 45 patients completing the trial. In order to account for drop-outs, It is expected that 112 patients in total will be randomized and treated.*

### 5.2 Update of exclusion criterion on blood pressure

Section 2. Trial synopsis, Exclusion criteria

Updated text:

10. Inadequately treated blood pressure as defined as systolic blood pressure ≥*160* ~~180~~ mmHg or diastolic blood pressure ≥*90* ~~110~~ mmHg at screening

*Section 7.3.2 Exclusion criteria*

Updated text:

10. Inadequately treated blood pressure as defined as systolic blood pressure ≥*160* ~~180~~ mmHg or diastolic blood pressure ≥*90* ~~110~~ mmHg at screening

### 5.3 Update of exclusion criteria on alcohol/drug abuse

Section 2. Trial synopsis, Exclusion criteria

Updated text:

19. ~~Active substance of alcohol abuse.~~ *A positive result in the alcohol and/or urine drug screen at the screening visit. Significant history of alcoholism or drug abuse as judged by the investigator or consuming more than 24 g alcohol per day for men, or more than 12 g alcohol per day for women.*

*Section 7.3.2 Exclusion criteria*

Updated text:

19. ~~Active substance of alcohol abuse.~~ *A positive result in the alcohol and/or urine drug screen at the screening visit. Significant history of alcoholism or drug abuse as judged by the*

*investigator or consuming more than 24 g alcohol per day for men, or more than 12 g alcohol per day for women.*

Section 7.3.3.1 Possible reasons for patient discontinuation

Updated text:

A patient will be discontinued if the following applies:

- If a protocol deviation occurs which, in the clinical judgment of the Investigator, can invalidate the assessment of ADA responses to dasiglucagon or glucagon, the patient will be withdrawn by the Investigator
- AEs that are considered unacceptable by the patient or the Investigator
- *Positive result from an urine drug screen test*

Section 7.6.4.1 Safety laboratory tests

Updated text:

Alcohol breath tests and urine drug screen *(amphetamine, cocaine, MDMA, methamphetamine, opiate/morphine, marihuana)* will be performed at Screening (Visit 1) and at Visits 2, 3, and 4.

### 5.4 Specification of prohibited concomitant medication

Section 2. Trial synopsis, Exclusion criteria

New text:

*23. Use of prescription or non-prescription medications known to cause QT prolongation*

Section 7.3.2. Exclusion criteria

New text:

*23. Use of prescription or non-prescription medications known to cause QT prolongation*

Section 7.4.2 Treatments administered

Updated text:

- antiemetic treatment ~~e.g.~~ in the form of ~~an 8 mg slow intravenous (i.v.) dose of ondansetron (Zofran®) or alternatively a 10 mg slow i.v. dose of~~ metoclopramide (Primperan®) *as per local label, administered before or after dosing.*

### 5.5 Additional ECG assessments added

Table 2-1 Flow chart

| Trial period | Screening | Treatment | | |
|---|---|---|---|---|
| Visit number | V1 | V2 | V3 | V4 |
| Trial day | -3 | 0 | 7 | 14 |
| Visit window (days) | -30 to - -3 | | ±1 | ±1 |
| **Patient related info/assessments** | | | | |
| Informed consent | X[1] | | | |
| Inclusion/exclusion criteria | X | X[2,3] | | |
| Demography | X | | | |
| Body measurements | X | | | |
| Medical history | X | | | |
| Concomitant illness | X | | | |
| Prior medications | X | | | |
| Concomitant medication | X | X | X | X |
| History of alcohol/drug abuse | X | | | |
| Randomization | | X | | |
| Withdrawal criteria | | X | X | X |
| Dosing day exclusion criteria | | X | X | X |
| **Safety assessments** | | | | |
| Physical examination | X | | | |
| Vital signs | X | X[4] | | X[4] |
| ECG | X | X[4,10] | X[10] | X[4,10] |

Footnotes

New text:

[10] *On dosing days Visit, 2, 3 and 4 ECG's are assessed pre-dose, and at 20, 35, 45 and 60 min post-dosing. The actual time for assessment should not deviate from the nominal time by more than ±5 min. Pre-dose is defined as within 5 min prior to dosing.*

Section 7.5.3 Procedures and assessments during the treatment period of the trial

Visit 2 and 4:

Updated text:

- ECG (pre-dose, and at *20, 35, 45* ~~30~~ and ~~90~~ *60* min post-dosing)

Visit 3:

New text:

- *ECG (pre-dose, and at 20, 35, 45 and 60 min post-dosing)*

Section 7.6.4.2 Safety examinations

Updated text:

ECG *is assessed at Screening (Visit 1) Visits 2, 3, and 4…*

### 5.6 Monitoring of patients' electrolyte levels

Section 2. Trial synopsis, Exclusion criteria

Updated text:

16. Aspartate aminotransferase (AST) or alanine aminotransferase (ALT) >2.5 X the upper limit of normal (ULN), bilirubin >1.5 X ULN, estimated glomerular filtration rate (eGFR) <30 mL/min/1.73m$^2$ according to the Modification of Diet in Renal Disease (MDRD) Study definition. *Altered electrolytes values of clinical relevance for cardiac conduction, as judged by the investigator.*

Section 7.3.2 Exclusion criteria

Updated text:

16. Aspartate aminotransferase (AST) or alanine aminotransferase (ALT) >2.5 X the upper limit of normal (ULN), bilirubin >1.5 X ULN, estimated glomerular filtration rate (eGFR) <30 mL/min/1.73m$^2$ according to the Modification of Diet in Renal Disease (MDRD) Study definition. *Altered electrolytes values of clinical relevance for cardiac conduction, as judged by the investigator.*

Section 7.4.2 Treatments administered

New text:

Patients will not be discharged until they are considered stable and with a blood glucose level in the range of 70-180 mg/dL. Before discharge, the investigator will provide instructions to the patients on management of their blood glucose levels. Each trial medication will be administered s.c. 3 times in total, with 7 days between dosing (i.e. dosing occurs at Days 0, 7, and 14; Visits 2, 3, and 4). *Should the patient experience vomiting following the first or second dasiglucagon or GlucaGen administration, the investigator must ensure normal electrolytes prior to the next dosing day. An electrolyte imbalance can be corrected by administration of an electrolyte supplement or by any other treatment modality considered appropriate by the investigator.*

### 5.7 Clinical event of interest added

Table 2-1 Flow chart

| Trial period | Screening | Treatment | | |
|---|---|---|---|---|
| Visit number | V1 | V2 | V3 | V4 |
| Trial day | -3 | 0 | 7 | 14 |
| Visit window (days) | -30 to - -3 | | ±1 | ±1 |
| **Patient related info/assessments** | | | | |
| Informed consent | X[1] | | | |
| Inclusion/exclusion criteria | X | X[2,3] | | |
| Demography | X | | | |
| Body measurements | X | | | |
| Medical history | X | | | |
| Concomitant illness | X | | | |
| Prior medications | X | | | |
| Concomitant medication | X | X | X | X |
| History of alcohol/drug abuse | X | | | |
| Randomization | | X | | |
| Withdrawal criteria | | X | X | X |
| Dosing day exclusion criteria | | X | X | X |
| **Safety assessments** | | | | |
| Physical examination | X | | | |
| Vital signs | X | X[124] | X[12] | X[124] |

Footnotes

New text:

*[12] On dosing days Visit 2, 3, and 4 vital signs are collected pre-dose and at 30, 90 and 120 min post-dosing. The actual time of assessment should not deviate from the nominal time by more than ±5 min. Pre-dose is defined as within 5 min prior to dosing.*

Section 5.1 Background of the trial, Safety of Dasiglucagon

Updated text:

*Glucagon has been described to exert positive inotropic and chronotropic effects and may therefore cause tachycardia and hypertension. In the phase 1 clinical trials, but not the phase 2 trial, temporary but clinically significant decreases in blood pressure were observed in a few healthy volunteers receiving investigational medicinal product (IMP) doses of at least 1.0 mg (4 with dasiglucagon and 1 with GlucaGen). This is not considered a safety concern, however hemodynamic changes post dosing will be considered a clinical event of interest.*

~~Therefore~~The *The* phase 1 and 2 results and the safety profile described to date do not give rise to specific safety concerns.

Section 7.5.3 Procedures and assessments during the treatment period of the trial

Visit 2 and 4:

Updated text:

- Vital signs (pre-dose, and at 30*,* ~~and~~ 90 *and 120* min post-dosing)

Visit 3:

New text:

- *Vital signs (pre-dose, and at 30, 90 and 120 min post-dosing)*

Section 7.6.4.2 Safety examinations

Updated text:

ECG is assessed at Screening (Visit 1) Visits 2, 3, and 4 and vital signs are assessed at Screening (Visit 1) Visits 2, *3,* 4, 5, and 7.

Section 8.1 Definitions

New text:

***Clinical event of interest***

*A clinical event of interest is an event which, in the evaluation of safety, has a special focus (e.g. required by health authorities).*

*In this trial hemodynamic changes, as defined below, are considered clinical event of interest:*

- *Post-dose clinical signs, or measured vital signs, indicating a clinical significant drop in blood pressure including signs of orthostatic hypotension, vasovagal responses or bradycardia*
- *Post-dose change in pulse or blood pressure considered an event of hypo- or hypertension as judged by the investigator.*

Section 8.2 Collection, recording and reporting of adverse events

Updated text:

All AEs must be recorded by the Investigator. One single Adverse Event Form must be used per AE from start to resolution. For SAEs, the Serious Adverse Event Form must also be completed. *For clinical events of Interest, the Clinical Event of Interest Form must also be completed.*

AE information should include the following:

- Date and time of onset and resolution
- Date and time of Investigator's first information on the (S)AE
- Seriousness
- Severity
- Causal relationship with IMP
- Interruption or withdrawal of treatment with IMP and other measures taken
- Outcome

All AEs are coded; details are described in the trial specific Data Management Plan.

*If an event classifies as a clinical event of interest, the Investigator must tick the Clinical Event of Interest box on the AE form and complete the Clinical Event of Interest Form. The Clinical Event of Interest Form will capture if the event was associated with any signs or symptoms and capture the highest/lowest blood pressure and pulse measured during the event.*

### 5.8 Treatment of patients experiencing hypo- or hyperglycemia prior dosing

Table 2-1 Flow chart, footnotes

Updated text:

[9] Prior to administration of trial medication patients must reach a target plasma glucose level of 70-150 mg/dL. Plasma glucose levels may be adjusted by ~~subcutaneous (s.c.)~~ administration of a fast-acting insulin analog or by glucose ingestion *at the discretion of the investigator*.

Section 7.4.2 Treatments administered

Updated text:

Prior to administration of trial medication at all dosing visits patients must reach a target plasma glucose level of 70-150 mg/dL. *If the patient presents with plasma glucose > 150 mg/dL, corrective amounts of fast acting insulin will be administered at the investigator's discretion. Plasma glucose measured after 20 and after 40 minutes should ensure that the patient has reached the targeted 70-150 mg/dL. If the patient presents with plasma glucose < 70 mg/dL glucose ingestion will be provided in order to reach target plasma glucose of 70-150 mg/dL documented by plasma glucose measurements after 20 and 40 minutes, respectively.* ~~Plasma glucose levels may be adjuseted by s.c. administration of a fast acting insulin analog if blood glucose exceeds 150 mg/dL or by glucose ingestion if blood glucose is below 70 mg/dL.~~

Section 7.5.3

Visit 2 and 4:

Updated text:

- Prior to administration of trial medication at all dosing visits patients must reach a target plasma glucose level of 70-150 mg/dL. *If the patient presents with plasma glucose > 150 mg/dL, corrective amounts of fast acting insulin will be administered at the investigator's discretion. Plasma glucose measured after 20 and after 40 minutes should ensure that the patient has reached the targeted 70-150 mg/dL. If the patient present with plasma glucose < 70 mg/dL glucose ingestion will be provided in order to reach target plasma glucose of 70-150 mg/dL documented by plasma glucose measurements after 20 and 40 minutes, respectively.* ~~Plasma glucose levels may be adjuseted by s.c. administration of a fast acting insulin analog or by glucose ingestion.~~

Visit 3:

Updated text:

- Prior to administration of trial medication at all dosing visits patients must reach a target plasma glucose level of 70-150 mg/dL. *If the patient presents with plasma glucose > 150 mg/dL, corrective amounts of fast acting insulin will be administered at the investigator's discretion. Plasma glucose measured after 20 and after 40 minutes should ensure that the patient has reached the targeted 70-150 mg/dL. If the patient present with plasma glucose < 70 mg/dL glucose ingestion will be provided in order to reach target plasma glucose of 70-150 mg/dL documented by plasma glucose measurements after 20 and 40 minutes, respectively.* ~~Plasma glucose levels may be adjuseted by s.c. administration of a fast acting insulin analog or by glucose ingestion.~~

### 5.9 Monitoring of potential pregnancies

Table 2-1 Flow chart

| Trial period | Screening | Treatment | | |
|---|---|---|---|---|
| Visit number | V1 | V2 | V3 | V4 |
| Trial day | -3 | 0 | 7 | 14 |
| Visit window (days) | -30 to - -3 | | ±1 | ±1 |
| **Laboratory** | | | | |
| Hematology, biochemistry, coagulation | X[4] | X[4] | | X[4] |
| Pregnancy test ~~(women only)~~ | X[11] | X[11] | X[11] | X[11] |

Footnotes

New text:

[11] *Pregnancy test is only applicable for women of childbearing potential. At Visit 1 a serum pregnancy test should be performed. At Visit 2, 3 and 4 a pre-dose urine pregnancy test should be performed.*

Section 7.3.3.1 Possible reasons for patient discontinuation

New Text:

A patient will be discontinued if the following applies:

- *Pregnancy. If a female patient becomes pregnant in the time between the screening visit and any one of the dosing visits*

Section 7.5.1 Screening examination

Updated text:

- Pregnancy test (women *of childbearing potential* only)

Section 7.5.3 Procedures and assessments during the treatment period of the trial

Visit 2 and 4:

New text:

- *Urine pregnancy test (pre-dose) at trial site (women of childbearing potential only)*

Visit 3

New text:

- *Urine pregnancy test (pre-dose) at trial site (women of childbearing potential only)*

Section 7.6.4.1 Safety laboratory tests

Updated text:

A pregnancy test will be performed at screening (Visit 1*) and pre-dose at the three dosing visits (Visit 2, 3 and 4)* for women *of childbearing potential* only.

### 5.10 Additional visits required for patients discontinuing treatment prematurely

Section 7.3.3.1 Possible reasons for patient discontinuation

New text:

If discontinuation occurs following administration of any trial medication, the patient will be asked to return and participate in the complete follow-up visit*s* at trial Day *35, 60, and* 104.

Section 7.4.2 Treatments administered

New text:

Following the first (Visit 2) and third (Visit 4) dose administration, patients must be fasting for 90 min after dosing. Following the 90-min blood sample draw after the administration of the first (Visit 2) and third (Visit 4) dose administration, patients may be treated individually in order to alleviate any potential side effects in order to minimize *prematurely treatment discontinuation* ~~withdrawals~~ and consequently reduce the amount of missing data. This treatment can also be instituted immediately after the second dose administration (Visit 3), as pharmacodynamics will not be assessed at this visit.

*In order to minimize the number of patients discontinuing treatment prematurely and consequently reduce the amount of missing data,* the following treatment modalities may be used, as considered appropriate by the investigator:

### 5.11 Specification on time windows for assessments

Flowchart 2-1, footnotes

New text:

[1] *Informed consent can be obtained on the same day as screening, but prior to any trial-related procedures* ~~At least 1 day before the screening visit (Visit 1)~~

…

[4] Coagulation parameters are measured at screening visit only. On dosing days Visit 2 and 4, ~~vital signs, ECG's and~~ blood samples are collected pre-dose, and at 30 and 90 min post-dosing. *The actual time for sampling/assessment should not deviate from the nominal time by more than ±5 min. Pre-dose is defined as within 5 min prior to dosing.*

[5] Local tolerability assessed at 0.5 and 2 h post-dose. *The actual time for assessment should not deviate from the nominal time by more than ±10 min.*

Section 7.5.3 Procedures and assessments during the treatment period of the trial

Updated text:

At Visit 2 (Day 0) patient eligibility is rechecked (check of changes between the screening visit and Visit 2)*. Withdrawal criteria and dosing day exclusion criteria are also checked* and patients eligible to participate will be randomized to treatment with dasiglucagon or GlucaGen.

Additionally, during the treatment period of the trial, at Visits 2 and 4 (Days 0 and 14), the following assessments will take place:

- Concomitant medication
- Check of withdrawal criteria *(prior randomization at Visit 2)*
- Check of dosing day exclusion criteria *(prior randomization at Visit 2)*

### 5.12 Specification of the requirements at the dosing visits

Table 2-1 Flowchart, footnotes

Updated text:

[9] Prior to administration of trial medication patients must reach a target plasma glucose level of 70-150 mg/dL. Plasma glucose levels may be adjusted by administration if fast-acting insulin analog or by glucse ingestion. ~~Patients~~ *At visit 2 and 4 patients* must be fasting for 90 min after administration of trial medication ~~and will be treated individually to alleciate any potential side effects~~. *At all dosing visits* ~~P~~*p*atients *will be treated individually to alleviate any potential side effects and* will be observed for at least 5 h post-dose.

Section 7.4.2 Treatments administered

Updated text:

*At visit 2 and 4:*

- *90 min after dosing patients will be allowed to eat and drink moderately to make them feel comfortable*
- *90 min after dosing a moderate and individualized corrective dose of insulin to convert the induced hyperglycemia to euglycemia, after agreement with the investigator*
- *antiemetic treatment in the form of metoclopramide (Primperan®) as per local label, administered before or after dosing*

*At visit 3:*

- patients will be allowed to eat and drink moderately to make them feel comfortable
- a moderate and individualized corrective dose of insulin to convert the induced hyperglycemia to euglycemia, after agreement with the investigator
- antiemetic treatment ~~e.g.~~ in the form of ~~an 8 mg slow intravenous (i.v.) dose of ondansetron (Zofran®) or alternatively a 10 mg slow i.v. dose of~~ metoclopramide (Primperan®) *as per local label, administered before or after dosing.*

Section 7.5.2 Instructions to patients prior to dosing

Updated text:

On dosing days, patients are allowed to consume a small meal for breakfast with corresponding administration of rapid acting insulin to address the meal and in accordance with their normal management of blood glucose levels. *At visit 2 and visit 4 the patient should aim to have a similar sized breakfast and corresponding rapid acting insulin dose.*

### 5.13 Responsibility of unblinded trial personnel

Section 7.4.6 Blinding

New text:

This is a double-blind trial. Since dasiglucagon is available as a liquid formulation and GlucaGen is available as a powder for reconstitution, and they are therefore not identical in appearance, unblinded trial personnel will be responsible for handling, preparing *according to*

*the prescription from the IWRS*, ~~and~~ administering the trial medication *and keep the records strictly confidential and accessible only for unblinded staff until after database lock.*

### 5.14 Randomization of replacement patients

Section 7.3.4 Replacement of patients

Updated text:

Patients prematurely withdrawn from the trial will be replaced in order to reach 90 completed patients. ~~Replacement patients will receive the same treatment allocation (i.e. 3 doses of dasiglucaon or GlucaGen) as the patients they are replacing.~~

### 5.15 Clarification to the reporting of (Serious) Adverse Events

Section 8.2 Collection recording and reporting of adverse events

Updated text:

At each contact with the site (visit or telephone, excluding ~~safety~~ visits, where the patient is not seeing the Investigator or his staff (e.g. visits to the laboratory)) the patient must be asked about AEs.

…

The Investigator must report initial information in writing (fax or email) on all SAEs ~~to the responsible medical monitor of the Sponsor (Zealand) and~~ to the Sponsor's responsible pharmacovigilance unit (*Safety CRO*~~Pharmalex~~) immediately (within 24 hours) after obtaining knowledge about the event. *Please refer to Appendix 1 for contact details. The Safety CRO will inform the medical monitor and the sponsor about the reported SAEs.*

~~Name: [redacted]~~
~~Company: PharmaLex (formerly Lindeq)~~
~~Address: Agern Allé 24, DK 2970 Hørsholm, Denmark~~
~~Tel: [redacted] (8 a.m. to 4 p.m.)~~
~~[redacted] (outside 8 a.m. to 4 p.m.)~~
~~Fax: [redacted]~~
~~E-mail: drugsafety@lindeq.com~~

~~In addition, and meeting the same timeline, Investigators have to report all SAEs to Zealand by forwarding the SAE form electronically (e.g. in PDF format) within 24 hours to the representatives of Zealand.~~

~~Names: [redacted] and [redacted]~~
~~Address: Smedeland 36, DK 2600 Glostrup, Denmark~~
~~Tel: [redacted] / [redacted]~~
~~E-mails: [redacted] / [redacted]~~

It is the responsibility of ~~PharmaLex~~*the Safety CRO* to report all SUSARs that occur in this trial to the Competent Authorities and IRBs/IECs in accordance with the local requirements in force and ICH guideline for GCP. The trial monitor must be informed accordingly.

Section 8.7 Safety Committee

Updated text:

*As a minimum t*~~T~~he SC convenes every quarter to review relevant safety information, including AEs and laboratory data.

### 5.16 Clarification on case report forms

Section 9.1 Case report forms

Updated text:

All key pages will be signed or initialed by the investigator, signifying agreement with and responsibility for the recorded data. Key pages are the following: ~~end of visit form,~~ AE-reporting form, *trial* medication form, concomitant medication form and trial-closure form.

*Data directly captured in the eCRF (i.e., data assigned or calculated automatically by the EDC system) is called e-source*. ~~A trial specific list should be generated prior to the performance of the trial, which specifies which data fields in the Case Report Form will be used by the medical personnel for direct entry during the performance of the trial.~~ This means that these data fields in the CRF are source documents. For the other CRF data fields, *which are entered by medical personnel during the trial,* the source data will be found in other documents (such as patients' files, worksheets, etc.), ~~that is~~ *i.e.* for these CRF data fields the "other" documents are the source documents. ~~Should such a list be created, it should be submitted to the authorities together with the protocol.~~

### 5.17 Subgroup analysis added to statistical section

10.1.9 Subgroup analysis

Updated text:

*Subgroup analyses for the primariy endpoint (voerl ADA incidence) by gender, age, and race will be performed.* ~~No subgroup analysis is currently planned.~~

### 5.18 Administrative and editorial changes

Front page

New text:

Sponsor code: ZP4207-16136
SynteractHCR: ZEA-DNK-01711
EudraCT number: 2017-000062-30

*IND Number: 127866*

*Dasiglucagon SUB code: SUB181296*

Coordinating Investigator

Updated text:

~~Linda Morrow, MD~~
~~Prosciento~~
~~855 Third Avenue~~
~~91911 Chula Vista~~
~~CA, USA~~
*Thomas R. Pieber, MD*
*Medical University of Graz*
*Auenbruggerplatz 15*
*A-8036 Graz, Austria*

Section 1. Signatures and agreement with protocol
Updated text:
Coordinating Investigator: ~~Linda Morrow, MD~~
~~Prosciento~~
~~855 Third Avenue~~
~~91911 Chula Vista~~
~~CA, USA~~

*Thomas R. Pieber, MD*
*Medical University of Graz*
*Auenbruggerplatz 15*
*A-8036 Graz, Austria*

Sponsor's representative: [redacted] [redacted]~~, MSc (Pharm)~~
Clinical ~~Trial~~ Project Manager
Zealand Pharma A/S
Smedeland 36, 2600 Glostrup, Denmark

Section 2. Trial synopsis
Coordinating Investigator
Updated text:
~~Linda Morrow, MD, Prosciento, 855 Third Avenue, 91911 Chula Vista, CA, USA~~

*Thomas R. Pieber, MD, Medical University of Graz, Auenbruggerplatz 15, A-8036 Graz, Austria*

Planned trial period
Updated text:
First Patient First Visit: *May* ~~March~~ 2017
Last Patient First Visit: *November* ~~September~~ 2017

**APPENDIX 1:**

| | |
|---|---|
| Coordinating investigator | ~~Linda Morrow, MD~~<br>~~Prosciento~~<br>~~855 Third Avenue~~<br>~~91911 Chula Vista, CA, USA~~<br>*Thomas R. Pieber, MD*<br>*Medical University of Graz*<br>*Auenbruggerplatz 15*<br>*A-8036 Graz, Austria* |
| Trial Monitor | ~~To be decided~~<br>SynteractHCR Deutschland GmbH<br>Albrechtstr. 14<br>80636 Munich, Germany |
| Sponsor representative | [redacted] [redacted]<br>Clinical ~~Trial~~ Project Manager<br>Zealand Pharma A/S<br>Smedeland 36, 2600 Glostrup, Denmark<br>Tel: [redacted]<br>E-mail: [redacted] |
| Clinical laboratory | ~~To be determined~~<br>*MLM Medical Labs GmbH*<br>[redacted]<br>*Dohrweg 63, 41066 Mönchengladbach, Germany*<br>*Tel:* [redacted]<br>*E-mail:* [redacted] |

# CLINICAL TRIAL PROTOCOL AMENDMENT

**A phase 3, Randomized, Double-Blind, Parallel Group Safety Trial to Evaluate the Immunogenicity of Dasiglucagon and GlucaGen® Administered Subcutaneously in Patients with Type 1 Diabetes Mellitus (T1DM)**

**Sponsor code: ZP4207-16136**
**SynteractHCR: ZEA-DNK-01711**
**EudraCT number: 2017-000062-30**

| | |
|---|---|
| Sponsor: | Zealand Pharma A/S,<br>Smedeland 36,<br>2600 Glostrup,<br>DENMARK |
| Clinical Research Organization: | SynteractHCR Deutchland GmbH,<br>Albrechtstr. 14,<br>80636 Munich<br>GERMANY |
| Study Drug Name: | Dasiglucagon |
| Development Phase: | 3 |
| Amendment Number: | 01 |
| Amendment Date: | May 8th 2017 |
| Type of Amendment: | Substantial |
| Amendment to protocol: | Final version 1, dated January 18th 2017 |

The study will be conducted according to the protocol and in compliance with Good Clinical Practice (GCP), with the Declaration of Helsinki [1] and with other applicable regulatory requirements.

## SIGNATURE PAGE

Declaration of Sponsor or Responsible Medical Officer

**Title: A phase 3, Randomized, Double-Blind, Parallel Group Safety Trial to Evaluate the Immunogenicity of Dasiglucagon and GlucaGen® Administered Subcutaneously in Patients with Type 1 Diabetes Mellitus (T1DM)**

This trial protocol amendment number 1 was subjected to critical review. The information it contains is consistent with current knowledge of the risks and benefits of the investigational product, as well as with the moral, ethical and scientific principles governing clinical research as set out in the 2008 revision of the Declaration of Helsinki [1] and the guidelines on Good Clinical Practice (GCP) [2].

[redacted] _____ [redacted]
Date

Title: Clinical Project Manager
Institution: Zealand Pharma a/s
Smedeland 36, 2600 Glostrup, Denmark

[redacted] _____ [redacted]
Date

Title: Medical Director
Institution: Zealand Pharma a/s
Smedeland 36, 2600 Glostrup, Denmark

[redacted] _____ [redacted]
Date

Title: Head of Clinical [redacted]pment
Institution: Zealand Pharma a/s
Smedeland 36, 2600 Glostrup, Denmark

**Declaration of the Investigator**

**Title: A phase 3, Randomized, Double-Blind, Parallel Group Safety Trial to Evaluate the Immunogenicity of Dasiglucagon and GlucaGen® Administered Subcutaneously in Patients with Type 1 Diabetes Mellitus (T1DM)**

All documentation for this trial that is supplied to me and that has not been previously published will be kept in the strictest confidence. This documentation includes the protocol, and this trial protocol amendment number 1, Investigator's Brochure, Case Report Forms (CRFs), and other scientific data.

The trial will not be commenced without the prior written approval of a properly constituted Institutional Review Board (IRB) or Independent Ethics Committee (IEC). No changes will be made to the trial protocol without the prior written approval of the Sponsor and the IRB or IEC, except where necessary to eliminate an immediate hazard to the patients.

I have read and understood and agree to abide by all the conditions and instructions contained in this protocol amendment.

Responsible Investigator of the local trial centre

_____ _____

Signature Date

_____

Name (block letters)

_____

Title (block letters)

_____

Institution (block letters)

_____

Phone number

## Table of Contents

## 1 Amendment type

This is substantial amendment number 01 to trial protocol version 1, dated 18 January 2017.

## 2 Rationale for amendment

The amendment will add precision to the description of the overall rationale of the trial. In addition, details pertaining to safety assessment of the patients are revised. Furthermore, the amendment serves to correct changes, errors or inconsistencies in the description of the operational set up of the trial.

## 3 Summary of Amendment

With this substantial amendment number 01, the following changes are made to the original protocol:

- Clarification on the statistical method and anti-drug antibody (ADA) assays
- Update of exclusion criterion on blood pressure
- Update of exclusion criterion on alcohol/drug abuse
- Specification of prohibited concomitant medication
- Additional ECG assessment added
- Clinical event of interest added
- Treatment options for patients experiencing hypo- or hyperglycemia prior dosing
- Monitoring of patients' electrolyte levels
- Monitoring of potential pregnancies
- Additional visits required for patients discontinuing treatment prematurely
- Specification on time windows for assessments
- Specification of the requirements at the dosing visits
- Responsibility of unblinded trial personnel
- Randomization of replacement patients
- Clarification to the reporting of (Serious) Adverse Events
- Clarification on case report forms
- Subgroup analysis added to the statistical section
- Administrative changes

Section 4 of this amendment will describe the overall changes and rationales.

Section 5 of this amendment will list the actual changes in the protocol text reflecting both superseded and new wording.

# 4 Changes and rationale:

## 4.1 Clarification on the statistical method and anti-drug antibody (ADA) assays

The primary objective has been updated to clarify that GlucaGen is considered a reference product and the objective of the trial is to evaluate the immunogenicity of both dasiglucagon and GlucaGen. It has been specified that the ADA assays to be used in this trial are both validated, but specific for dasiglucagon and GlucaGen, respectively. Hence, the performance of the assays are not directly comparable and a formal statistical comparison or non-inferiority testing is not performed. The rationale for the sample size calculation has been adapted accordingly. These clarifications have been implemented throughout the protocol including the protocol title.

The rationale for the update is to clarify that the trial is not a non-inferiority trial and no formal statistical testing will be performed.

## 4.2 Update of exclusion criterion on blood pressure

Exclusion criterion number 10 has been adjusted to exclude patients with a systolic blood pressure ≥160 mmHg or diastolic blood pressure ≥90 mmHg at screening.

The rationale for narrowing the limit of allowed blood pressure values at screening is to only include patients of good health, apart from having type 1 diabetes, in the trial.

## 4.3 Update of exclusion criterion on alcohol/drug abuse

Exclusion criterion number 19 has been updated to exclude patients with a significant history of alcohol or drug abuse or a positive alcohol or drug test at screening. Further, the limits for current alcohol consumption has been added.

In addition, it has been specified in the protocol which drugs the drug test will screen for and that a positive drug test during the trial will be a withdrawal criteria.

The rationale of the update is to ensure patients with a history or current abuse of alcohol and/or drugs are not included in the trial.

## 4.4 Specification of prohibited concomitant medication

An additional exclusion criterion has been added to exclude patients using any non-prescription or prescription drugs known to cause QT prolongation. It has further been specified that metoclopramide (Primperan®) is the only allowed antiemetic treatment that can be used at the dosing visits; 2, 3, and 4.

The rationale for the addition is due to the sporadic QTcB changes post dasiglucagon/GlucaGen administration observed in the phase 2 trial.

## 4.5 Additional ECG assessment added

ECG will be measured at 20, 35, 45 and 60 minutes post dosing at all three dosing visits; 2, 3, and 4.

The rationale for the change is due to the sporadic QTcB changes post dasiglucagon/GlucaGen administration observed in the phase 2 trial. The updated time points for ECG assessments are to cover the range of $t_{max}$ for dasiglucagon and GlucaGen at all three dosing visits.

### 4.6 Monitoring of patients' electrolyte levels

It has been added to exclusion criterion 16 that patients with altered electrolytes values of clinical relevance for cardiac conduction will be excluded from the trial. Further, the investigator must ensure that patients are actively treated to correct any electrolyte imbalances following potential events of vomiting at the dosing visits.

The rationale for the change is to ensure that patients do not have any electrolyte imbalances that could potentially affect the cardiac conduction and the QT interval.

### 4.7 Clinical event of interest added

Changes in blood pressure and/or pulse after dosing will be considered a clinical event of interest. Additional vital sign assessments have been added.

The rationale for the addition is due to the nonclinical and clinical findings in healthy volunteers of hemodynamic changes with dasiglucagon. Due to these findings additional information on such events will be captured in a specific form in the case report form.

### 4.8 Treatment of patients experiencing hypo- or hyperglycemia prior dosing

It has been specified that at all three dosing visits; 2, 3, and 4, the investigator should administer corrective doses of insulin if a patient has a plasma glucose >150 mg/dL before dosing. Likewise if a patient has a plasma glucose <70 mg/dL before dosing glucose ingestion should be provided to the patient.

The rationale for this addition is to clarify how the investigator should ensure patients have a plasma glucose level on 70-150mg/dL before dosing.

### 4.9 Monitoring of potential pregnancies

For women of childbearing potential a urine stick pregnancy test will be performed at the site prior dosing at all three dosing visits; 2, 3, and 4.

If a female patient becomes pregnant in the period between the screening visit and the last dosing visit, she must be discontinued from trial treatment.

The rationale for the change is to ensure that patients who have become pregnant in the period following the screening visit are not exposed to trial medication.

### 4.10 Additional visits required for patients discontinuing treatment prematurely

A patient prematurely discontinuing treatment will be asked to attend all three follow up visits; 5, 6, and 7, to have all follow up assessments performed. Only in case a patient withdraw consent or is excluded before exposure to trial product the follow up visits can be omitted.

The rationale for this addition is to avoid missing data for the primary and key secondary endpoints.

### 4.11 Specification on time windows and order of assessments

Time window allowance has been added to collection time of safety blood samples, vital signs and local tolerability assessments. Further, the time for obtaining informed consent has been specified as well as the time of randomization.

The rationale for the addition is to clarify the time and timing for the assessments to take place.

### 4.12 Specification of the requirements at the dosing visits

The requirements for fasting at the dosing visits; 2, 3, and 4, have been specified. Further, the instructions to patients prior dosing have been made more clear.

The rationale for the addition is to clarify the procedures around the dosing visits.

### 4.13 Responsibility of unblinded trial personnel

The responsibility of the unblinded trial personnel has been clarified.

The rationale for the addition is to clarify the procedures to maintain the double blinding.

### 4.14 Randomization of replacement patients

Replacement patients will not necessarily be randomized to the same treatment as the patient they are replacing.

The rationale is that it is not technically possible nor needed to assign the replacement patients to the same treatment as the patient they are replacing.

### 4.15 Clarification to the reporting of (Serious) Adverse Events

It has been clarified that the investigator only needs to send the SAE form to the Pharmacovigilance unit (Safety CRO). Further, the text has been updated to make it more simple.

The rationale for the changes is to make the process for SAE reporting more simple and to make the text more clear.

### 4.16 Clarification on case report forms

Minor clarifications have been made to the section on case report forms. E.g. it has been specified that no end-of-visit form will be used in the trial.

The rational for the change is to correct minor errors and make the section more clear.

### 4.17 Subgroup analyses added to statistical section

It has been added that subgroup analyses by gender, age and race will be performed for the primary endpoint. Further details if needed will be provided in the statistical analysis plan.

The rationale for including this in the protocol is due to regulatory requirements.

### 4.18 Administrative changes

- IND and dasiglucagon SUB numbers have been added
- Coordinating investigator and other trial personnel have been updated (changes also applicable for Appendix 1)

## 5 Amended text

This section will list the actual changes in the protocol text reflecting both the original and the new wording.

New wording is marked in *underlined italics* and deleted wording is marked with ~~strikethrough~~.

### 5.1 Clarification on the statistical method and anti-drug antibody (ADA) assays

Front page:

Updated text:

A phase 3, Randomized, Double-Blind, Parallel Group Safety Trial to Evaluate the Immunogenicity of Dasiglucagon *and* ~~compared to~~ GlucaGen® Administered Subcutaneously in Patients with Type 1 Diabetes Mellitus (T1DM)

Section 1. Signatures and agreement with protocol

Updated text:

**Title:** A phase 3, Randomized, Double-Blind, Parallel Group Safety Trial to Evaluate the Immunogenicity of Dasiglucagon *and* ~~compared to~~ GlucaGen® Administered Subcutaneously in Patients with Type 1 Diabetes Mellitus (T1DM)

Section 2. Trial synopsis

Title of the trial

Updated text:

A phase 3, Randomized, Double-Blind, Parallel Group Safety Trial to Evaluate the Immunogenicity of Dasiglucagon *and* ~~compared to~~ GlucaGen® Administered Subcutaneously in Patients with Type 1 Diabetes Mellitus (T1DM)

Objectives

Updated text:

The primary objective is to evaluate the immunogenicity of repeated single doses of dasiglucagon *and GlucaGen* following subcutaneous (s.c.) administration ~~compared with s.c. GlucaGen~~ in T1DM patients.

Trial design

Updated text:

This is a randomized, double-blind, parallel group trial *evaluating* ~~comparing~~ the immunogenicity of either dasiglucagon or GlucaGen administered to euglycemic T1DM patients.

Statistical methods:

Updated text:

All statistical analysis will be descriptive *i.e. no formal statistical testing will be performed*. Continuous variables will be summarized with means, standard deviations, medians, minimums, and maximums, and valid cases.

Sample size calculation:

Updated text:

The purpose of the present trial is to generate data describing the immunogenic potential of dasiglucagon *and GlucaGen*~~, when used as a rescue therapy form severe hypoglycemia and with reference to the immunogenic potential of GlucaGen. The sample size is based on generating data to show that the 90% confidence of the ADA incidence is no worse than 15% as the predefined acceptability criterion and with a reference to similar data being generated for GlucaGen~~. *The ADA assays to be used in this trial are both validated, but specific for GlucaGen and dasiglucagon, respectively, and the performance of the assays are thus not directly comparable. As a consequence, a formal comparison or non-inferiority analysis will not be performed.*

Currently, no ADA incidences have been detected in the completed clinical trials, where up to 5 repeated doses of dasiglucagon have been administered to the same patients within a week. Across the two phase 1 clinical trials and a completed phase 2 pharmacokinetic/pharmacodynamic trial, a total of 141 subjects have been exposed to dasiglucagon and no incidences of ADA development have been observed. The obtained data indicate that dasiglucagon has a low risk for induction of ADAs in the investigated settings and as a consequence ~~the sample size is based on showing that the ADA incidence is no worse than the predefined margin of 15%.~~*a meaningful sample size to compare both treatments cannot be estimated.*

*The sample size is therefore based on a certain precision of the confidence interval for the overall ADA incidence if no events are observed, respectively to ensure a certain probability for observing one event.*

*When no events are observed, to obtain an upper bound of 0.050 on the 90.0% confidence interval for the probability of such a rare event, would require a sample size of 45. Respectively, accepting a chance of observing at least one event of 90% and an actual probability of the event of 5% leads to a sample size of 45 patients completing the trial. In order to account for drop-outs, It is expected that 112 patients in total will be randomized and treated.*

Section 5.2 Trial rationale

Updated text:

The present trial aims to evaluate that immunogenicity risk with an assessment of the occurrence of ADAs and neutralizing ADAs, and of cross-reactivity with native glucagon, following repeated single doses of dasiglucagon by s.c. administration in T1DM patients. The *reference product* ~~comparator~~ in this trial is GlucaGen, a recombinant human glucagon approved for the treatment of the severe hypoglycemic reactions that may occur in the management of insulin-treated children and adults with diabetes mellitus. *Given the differences in ADA assay for dasiglucagon and GlucaGen, a formal statistical comparison or non-inferiority testing is not performed.*

Section 6 Trial objectives

Updated text:

Primary objective

- To evaluate the immunogenicity of repeated single doses of dasiglucagon *and GlucaGen* following s.c. administration ~~compared with s.c. GlucaGen~~ in T1DM patients.

Section 7.2 Discussion of trial design and choice of control groups

Updated text:

Euglycemic patients with T1DM will be randomized 1:1 in order to evaluate the immunogenicity of dasiglucagon *and* ~~compared to~~ GlucaGen. The randomized, double-blind, parallel group design, with administration of 3 fixed consecutive doses of randomized trial medication (dasiglucagon or GlucaGen) to the same patient will allow characterization of immunogenic potential *for* ~~and a relative comparison of the immunogenicity between~~ the 2 products.

Section 7.3 Selection of trial population

Updated text:

Dasiglucagon is indicated for treatment of severe hypoglycemia in patients with T1DM. Given the role of the immune system in the pathogenesis of T1DM, the present trial aims to evaluate the immune response of patients with T1DM taking repeated single doses of s.c. dasiglucagon *and* ~~compared to~~ GlucaGen.

Section 10.1.1 General Considerations

New text:

*All statistical analysis will be descriptive i.e. no formal testing will be performed.*

Section 10.1.2 Classification of patients to subsets

Updated text:

The following definitions are applicable:

| | |
|---|---|
| **Safety analysis set (SAS)** | all patients who were randomized and received at least one dose of trial medication |
| **Full analysis set (FAS)** | all patients of the SAS with at least one measurement of the ADA titer *at baseline* |
| **Per Protocol set (PPS)** | all patients of the FAS for whom no relevant protocol deviations were documented |

Section 10.2 Determination of sample size

Updated text:

The purpose of the present trial is to generate data describing the immunogenic potential of dasiglucagon *and GlucaGen*.~~, when used as a rescue therapy form severe hypoglycemia and with reference to the immunogenic potential of GlucaGen. The sample size is based on generating data to show that the 90% confidence of the ADA incidence is no worse than 15% as the predefined acceptability criterion and with a reference to similar data being generated for GlucaGen~~.*The ADA assays to be used in this trial are both validated, but specific for GlucaGen*

*and dasiglucagon, respectively, and the performance of the assays are thus not directly comparable. As a consequence, a formal comparison or non-inferiority analysis will not be performed.*

Currently, no ADA incidences have been detected in the completed clinical trials, where up to 5 repeated doses of dasiglucagon have been administered to the same patients within a week. Across the two phase 1 clinical trials and a completed phase 2 pharmacokinetic/pharmacodynamic trial, a total of 141 subjects have been exposed to dasiglucagon and no incidences of ADA development have been observed. The obtained data indicate that dasiglucagon has a low risk for induction of ADAs in the investigated settings and as a consequence ~~the sample size is based on showing that the ADA incidence is no worse than the predefined margin of 15%.~~*a meaningful sample size to compare both treatments cannot be estimated.*

*The sample size is therefore based on a certain precision of the confidence interval for the overall ADA incidence if no events are observed, respectively to ensure a certain probability for observing one event.*

*When no events are observed, to obtain an upper bound of 0.050 on the 90.0% confidence interval for the probability of such a rare event, would require a sample size of 45. Respectively, accepting a chance of observing at least one event of 90% and an actual probability of the event of 5% leads to a sample size of 45 patients completing the trial. In order to account for drop-outs, It is expected that 112 patients in total will be randomized and treated.*

### 5.2 Update of exclusion criterion on blood pressure

Section 2. Trial synopsis, Exclusion criteria

Updated text:

10. Inadequately treated blood pressure as defined as systolic blood pressure ≥*160* ~~180~~ mmHg or diastolic blood pressure ≥*90* ~~110~~ mmHg at screening

*Section 7.3.2 Exclusion criteria*

Updated text:

10. Inadequately treated blood pressure as defined as systolic blood pressure ≥*160* ~~180~~ mmHg or diastolic blood pressure ≥*90* ~~110~~ mmHg at screening

### 5.3 Update of exclusion criteria on alcohol/drug abuse

Section 2. Trial synopsis, Exclusion criteria

Updated text:

19. ~~Active substance of alcohol abuse.~~ *A positive result in the alcohol and/or urine drug screen at the screening visit. Significant history of alcoholism or drug abuse as judged by the investigator or consuming more than 24 g alcohol per day for men, or more than 12 g alcohol per day for women.*

*Section 7.3.2 Exclusion criteria*

Updated text:

19. ~~Active substance of alcohol abuse.~~ *A positive result in the alcohol and/or urine drug screen at the screening visit. Significant history of alcoholism or drug abuse as judged by the*

*investigator or consuming more than 24 g alcohol per day for men, or more than 12 g alcohol per day for women.*

Section 7.3.3.1 Possible reasons for patient discontinuation

Updated text:

A patient will be discontinued if the following applies:

- If a protocol deviation occurs which, in the clinical judgment of the Investigator, can invalidate the assessment of ADA responses to dasiglucagon or glucagon, the patient will be withdrawn by the Investigator
- AEs that are considered unacceptable by the patient or the Investigator
- *Positive result from an urine drug screen test*

Section 7.6.4.1 Safety laboratory tests

Updated text:

Alcohol breath tests and urine drug screen *(amphetamine, cocaine, MDMA, methamphetamine, opiate/morphine, marihuana)* will be performed at Screening (Visit 1) and at Visits 2, 3, and 4.

### 5.4 Specification of prohibited concomitant medication

Section 2. Trial synopsis, Exclusion criteria

New text:

*23. Use of prescription or non-prescription medications known to cause QT prolongation*

Section 7.3.2. Exclusion criteria

New text:

*23. Use of prescription or non-prescription medications known to cause QT prolongation*

Section 7.4.2 Treatments administered

Updated text:

- antiemetic treatment ~~e.g.~~ in the form of ~~an 8 mg slow intravenous (i.v.) dose of ondansetron (Zofran®) or alternatively a 10 mg slow i.v. dose of~~ metoclopramide (Primperan®) *as per local label, administered before or after dosing.*

### 5.5 Additional ECG assessments added

Table 2-1 Flow chart

| Trial period | Screening | Treatment | | |
|---|---|---|---|---|
| Visit number | V1 | V2 | V3 | V4 |
| Trial day | -3 | 0 | 7 | 14 |
| Visit window (days) | -30 to - -3 | | ±1 | ±1 |
| **Patient related info/assessments** | | | | |
| Informed consent | X[1] | | | |
| Inclusion/exclusion criteria | X | X[2,3] | | |
| Demography | X | | | |
| Body measurements | X | | | |
| Medical history | X | | | |
| Concomitant illness | X | | | |
| Prior medications | X | | | |
| Concomitant medication | X | X | X | X |
| History of alcohol/drug abuse | X | | | |
| Randomization | | X | | |
| Withdrawal criteria | | X | X | X |
| Dosing day exclusion criteria | | X | X | X |
| **Safety assessments** | | | | |
| Physical examination | X | | | |
| Vital signs | X | X[4] | | X[4] |
| ECG | X | X[4,10] | X[10] | X[4,10] |

Footnotes

New text:

[10] *On dosing days Visit, 2, 3 and 4 ECG's are assessed pre-dose, and at 20, 35, 45 and 60 min post-dosing. The actual time for assessment should not deviate from the nominal time by more than ±5 min. Pre-dose is defined as within 5 min prior to dosing.*

Section 7.5.3 Procedures and assessments during the treatment period of the trial

Visit 2 and 4:

Updated text:

- ECG (pre-dose, and at *20, 35, 45* ~~30~~ and ~~90~~ *60* min post-dosing)

Visit 3:

New text:

- *ECG (pre-dose, and at 20, 35, 45 and 60 min post-dosing)*

Section 7.6.4.2 Safety examinations

Updated text:

ECG *is assessed at Screening (Visit 1) Visits 2, 3, and 4…*

### 5.6 Monitoring of patients' electrolyte levels

Section 2. Trial synopsis, Exclusion criteria

Updated text:

16. Aspartate aminotransferase (AST) or alanine aminotransferase (ALT) >2.5 X the upper limit of normal (ULN), bilirubin >1.5 X ULN, estimated glomerular filtration rate (eGFR) <30 mL/min/1.73m$^2$ according to the Modification of Diet in Renal Disease (MDRD) Study definition. *Altered electrolytes values of clinical relevance for cardiac conduction, as judged by the investigator.*

Section 7.3.2 Exclusion criteria

Updated text:

16. Aspartate aminotransferase (AST) or alanine aminotransferase (ALT) >2.5 X the upper limit of normal (ULN), bilirubin >1.5 X ULN, estimated glomerular filtration rate (eGFR) <30 mL/min/1.73m$^2$ according to the Modification of Diet in Renal Disease (MDRD) Study definition. *Altered electrolytes values of clinical relevance for cardiac conduction, as judged by the investigator.*

Section 7.4.2 Treatments administered

New text:

Patients will not be discharged until they are considered stable and with a blood glucose level in the range of 70-180 mg/dL. Before discharge, the investigator will provide instructions to the patients on management of their blood glucose levels. Each trial medication will be administered s.c. 3 times in total, with 7 days between dosing (i.e. dosing occurs at Days 0, 7, and 14; Visits 2, 3, and 4). *Should the patient experience vomiting following the first or second dasiglucagon or GlucaGen administration, the investigator must ensure normal electrolytes prior to the next dosing day. An electrolyte imbalance can be corrected by administration of an electrolyte supplement or by any other treatment modality considered appropriate by the investigator.*

### 5.7 Clinical event of interest added

Table 2-1 Flow chart

| Trial period | Screening | Treatment | | |
|---|---|---|---|---|
| Visit number | V1 | V2 | V3 | V4 |
| Trial day | -3 | 0 | 7 | 14 |
| Visit window (days) | -30 to - -3 | | ±1 | ±1 |
| **Patient related info/assessments** | | | | |
| Informed consent | X[1] | | | |
| Inclusion/exclusion criteria | X | X[2,3] | | |
| Demography | X | | | |
| Body measurements | X | | | |
| Medical history | X | | | |
| Concomitant illness | X | | | |
| Prior medications | X | | | |
| Concomitant medication | X | X | X | X |
| History of alcohol/drug abuse | X | | | |
| Randomization | | X | | |
| Withdrawal criteria | | X | X | X |
| Dosing day exclusion criteria | | X | X | X |
| **Safety assessments** | | | | |
| Physical examination | X | | | |
| Vital signs | X | X[124] | X[12] | X[124] |

Footnotes

New text:

*[12] On dosing days Visit 2, 3, and 4 vital signs are collected pre-dose and at 30, 90 and 120 min post-dosing. The actual time of assessment should not deviate from the nominal time by more than ±5 min. Pre-dose is defined as within 5 min prior to dosing.*

Section 5.1 Background of the trial, Safety of Dasiglucagon

Updated text:

*Glucagon has been described to exert positive inotropic and chronotropic effects and may therefore cause tachycardia and hypertension. In the phase 1 clinical trials, but not the phase 2 trial, temporary but clinically significant decreases in blood pressure were observed in a few healthy volunteers receiving investigational medicinal product (IMP) doses of at least 1.0 mg (4 with dasiglucagon and 1 with GlucaGen). This is not considered a safety concern, however hemodynamic changes post dosing will be considered a clinical event of interest.*

~~Therefore~~The *The* phase 1 and 2 results and the safety profile described to date do not give rise to specific safety concerns.

Section 7.5.3 Procedures and assessments during the treatment period of the trial

Visit 2 and 4:

Updated text:

- Vital signs (pre-dose, and at 30*,* ~~and~~ 90 *and 120* min post-dosing)

Visit 3:

New text:

- *Vital signs (pre-dose, and at 30, 90 and 120 min post-dosing)*

Section 7.6.4.2 Safety examinations

Updated text:

ECG is assessed at Screening (Visit 1) Visits 2, 3, and 4 and vital signs are assessed at Screening (Visit 1) Visits 2, *3,* 4, 5, and 7.

Section 8.1 Definitions

New text:

***Clinical event of interest***

*A clinical event of interest is an event which, in the evaluation of safety, has a special focus (e.g. required by health authorities).*

*In this trial hemodynamic changes, as defined below, are considered clinical event of interest:*

- *Post-dose clinical signs, or measured vital signs, indicating a clinical significant drop in blood pressure including signs of orthostatic hypotension, vasovagal responses or bradycardia*
- *Post-dose change in pulse or blood pressure considered an event of hypo- or hypertension as judged by the investigator.*

Section 8.2 Collection, recording and reporting of adverse events

Updated text:

All AEs must be recorded by the Investigator. One single Adverse Event Form must be used per AE from start to resolution. For SAEs, the Serious Adverse Event Form must also be completed. *For clinical events of Interest, the Clinical Event of Interest Form must also be completed.*

AE information should include the following:

- Date and time of onset and resolution
- Date and time of Investigator's first information on the (S)AE
- Seriousness
- Severity
- Causal relationship with IMP
- Interruption or withdrawal of treatment with IMP and other measures taken
- Outcome

All AEs are coded; details are described in the trial specific Data Management Plan.

*If an event classifies as a clinical event of interest, the Investigator must tick the Clinical Event of Interest box on the AE form and complete the Clinical Event of Interest Form. The Clinical Event of Interest Form will capture if the event was associated with any signs or symptoms and capture the highest/lowest blood pressure and pulse measured during the event.*

### 5.8 Treatment of patients experiencing hypo- or hyperglycemia prior dosing

Table 2-1 Flow chart, footnotes

Updated text:

[9] Prior to administration of trial medication patients must reach a target plasma glucose level of 70-150 mg/dL. Plasma glucose levels may be adjusted by ~~subcutaneous (s.c.)~~ administration of a fast-acting insulin analog or by glucose ingestion *at the discretion of the investigator*.

Section 7.4.2 Treatments administered

Updated text:

Prior to administration of trial medication at all dosing visits patients must reach a target plasma glucose level of 70-150 mg/dL. *If the patient presents with plasma glucose > 150 mg/dL, corrective amounts of fast acting insulin will be administered at the investigator's discretion. Plasma glucose measured after 20 and after 40 minutes should ensure that the patient has reached the targeted 70-150 mg/dL. If the patient presents with plasma glucose < 70 mg/dL glucose ingestion will be provided in order to reach target plasma glucose of 70-150 mg/dL documented by plasma glucose measurements after 20 and 40 minutes, respectively.* ~~Plasma glucose levels may be adjuseted by s.c. administration of a fast acting insulin analog if blood glucose exceeds 150 mg/dL or by glucose ingestion if blood glucose is below 70 mg/dL.~~

Section 7.5.3

Visit 2 and 4:

Updated text:

- Prior to administration of trial medication at all dosing visits patients must reach a target plasma glucose level of 70-150 mg/dL. *If the patient presents with plasma glucose > 150 mg/dL, corrective amounts of fast acting insulin will be administered at the investigator's discretion. Plasma glucose measured after 20 and after 40 minutes should ensure that the patient has reached the targeted 70-150 mg/dL. If the patient present with plasma glucose < 70 mg/dL glucose ingestion will be provided in order to reach target plasma glucose of 70-150 mg/dL documented by plasma glucose measurements after 20 and 40 minutes, respectively.* ~~Plasma glucose levels may be adjuseted by s.c. administration of a fast acting insulin analog or by glucose ingestion.~~

Visit 3:

Updated text:

- Prior to administration of trial medication at all dosing visits patients must reach a target plasma glucose level of 70-150 mg/dL. *If the patient presents with plasma glucose > 150 mg/dL, corrective amounts of fast acting insulin will be administered at the investigator's discretion. Plasma glucose measured after 20 and after 40 minutes should ensure that the patient has reached the targeted 70-150 mg/dL. If the patient present with plasma glucose < 70 mg/dL glucose ingestion will be provided in order to reach target plasma glucose of 70-150 mg/dL documented by plasma glucose measurements after 20 and 40 minutes, respectively.* ~~Plasma glucose levels may be adjuseted by s.c. administration of a fast acting insulin analog or by glucose ingestion.~~

### 5.9 Monitoring of potential pregnancies

Table 2-1 Flow chart

| Trial period | Screening | Treatment | | |
|---|---|---|---|---|
| Visit number | V1 | V2 | V3 | V4 |
| Trial day | -3 | 0 | 7 | 14 |
| Visit window (days) | -30 to - -3 | | ±1 | ±1 |
| **Laboratory** | | | | |
| Hematology, biochemistry, coagulation | X[4] | X[4] | | X[4] |
| Pregnancy test ~~(women only)~~ | X[11] | X[11] | X[11] | X[11] |

Footnotes

New text:

[11] *Pregnancy test is only applicable for women of childbearing potential. At Visit 1 a serum pregnancy test should be performed. At Visit 2, 3 and 4 a pre-dose urine pregnancy test should be performed.*

Section 7.3.3.1 Possible reasons for patient discontinuation

New Text:

A patient will be discontinued if the following applies:

- *Pregnancy. If a female patient becomes pregnant in the time between the screening visit and any one of the dosing visits*

Section 7.5.1 Screening examination

Updated text:

- Pregnancy test (women *of childbearing potential* only)

Section 7.5.3 Procedures and assessments during the treatment period of the trial

Visit 2 and 4:

New text:

- *Urine pregnancy test (pre-dose) at trial site (women of childbearing potential only)*

Visit 3

New text:

- *Urine pregnancy test (pre-dose) at trial site (women of childbearing potential only)*

Section 7.6.4.1 Safety laboratory tests

Updated text:

A pregnancy test will be performed at screening (Visit 1*) and pre-dose at the three dosing visits (Visit 2, 3 and 4)* for women *of childbearing potential* only.

### 5.10 Additional visits required for patients discontinuing treatment prematurely

Section 7.3.3.1 Possible reasons for patient discontinuation

New text:

If discontinuation occurs following administration of any trial medication, the patient will be asked to return and participate in the complete follow-up visit*s* at trial Day *35, 60, and* 104.

Section 7.4.2 Treatments administered

New text:

Following the first (Visit 2) and third (Visit 4) dose administration, patients must be fasting for 90 min after dosing. Following the 90-min blood sample draw after the administration of the first (Visit 2) and third (Visit 4) dose administration, patients may be treated individually in order to alleviate any potential side effects in order to minimize *prematurely treatment discontinuation* ~~withdrawals~~ and consequently reduce the amount of missing data. This treatment can also be instituted immediately after the second dose administration (Visit 3), as pharmacodynamics will not be assessed at this visit.

*In order to minimize the number of patients discontinuing treatment prematurely and consequently reduce the amount of missing data,* the following treatment modalities may be used, as considered appropriate by the investigator:

### 5.11 Specification on time windows for assessments

Flowchart 2-1, footnotes

New text:

[1] *Informed consent can be obtained on the same day as screening, but prior to any trial-related procedures* ~~At least 1 day before the screening visit (Visit 1)~~

…

[4] Coagulation parameters are measured at screening visit only. On dosing days Visit 2 and 4, ~~vital signs, ECG's and~~ blood samples are collected pre-dose, and at 30 and 90 min post-dosing. *The actual time for sampling/assessment should not deviate from the nominal time by more than ±5 min. Pre-dose is defined as within 5 min prior to dosing.*

[5] Local tolerability assessed at 0.5 and 2 h post-dose. *The actual time for assessment should not deviate from the nominal time by more than ±10 min.*

Section 7.5.3 Procedures and assessments during the treatment period of the trial

Updated text:

At Visit 2 (Day 0) patient eligibility is rechecked (check of changes between the screening visit and Visit 2)*. Withdrawal criteria and dosing day exclusion criteria are also checked* and patients eligible to participate will be randomized to treatment with dasiglucagon or GlucaGen.

Additionally, during the treatment period of the trial, at Visits 2 and 4 (Days 0 and 14), the following assessments will take place:

- Concomitant medication
- Check of withdrawal criteria *(prior randomization at Visit 2)*
- Check of dosing day exclusion criteria *(prior randomization at Visit 2)*

### 5.12 Specification of the requirements at the dosing visits

Table 2-1 Flowchart, footnotes

Updated text:

[9] Prior to administration of trial medication patients must reach a target plasma glucose level of 70-150 mg/dL. Plasma glucose levels may be adjusted by administration if fast-acting insulin analog or by glucse ingestion. ~~Patients~~ *At visit 2 and 4 patients* must be fasting for 90 min after administration of trial medication ~~and will be treated individually to alleciate any potential side effects~~. *At all dosing visits* ~~P~~*p*atients *will be treated individually to alleviate any potential side effects and* will be observed for at least 5 h post-dose.

Section 7.4.2 Treatments administered

Updated text:

*At visit 2 and 4:*

- *90 min after dosing patients will be allowed to eat and drink moderately to make them feel comfortable*
- *90 min after dosing a moderate and individualized corrective dose of insulin to convert the induced hyperglycemia to euglycemia, after agreement with the investigator*
- *antiemetic treatment in the form of metoclopramide (Primperan®) as per local label, administered before or after dosing*

*At visit 3:*

- patients will be allowed to eat and drink moderately to make them feel comfortable
- a moderate and individualized corrective dose of insulin to convert the induced hyperglycemia to euglycemia, after agreement with the investigator
- antiemetic treatment ~~e.g.~~ in the form of ~~an 8 mg slow intravenous (i.v.) dose of ondansetron (Zofran®) or alternatively a 10 mg slow i.v. dose of~~ metoclopramide (Primperan®) *as per local label, administered before or after dosing.*

Section 7.5.2 Instructions to patients prior to dosing

Updated text:

On dosing days, patients are allowed to consume a small meal for breakfast with corresponding administration of rapid acting insulin to address the meal and in accordance with their normal management of blood glucose levels. *At visit 2 and visit 4 the patient should aim to have a similar sized breakfast and corresponding rapid acting insulin dose.*

### 5.13 Responsibility of unblinded trial personnel

Section 7.4.6 Blinding

New text:

This is a double-blind trial. Since dasiglucagon is available as a liquid formulation and GlucaGen is available as a powder for reconstitution, and they are therefore not identical in appearance, unblinded trial personnel will be responsible for handling, preparing *according to*

*the prescription from the IWRS*, ~~and~~ administering the trial medication *and keep the records strictly confidential and accessible only for unblinded staff until after database lock.*

### 5.14 Randomization of replacement patients

Section 7.3.4 Replacement of patients

Updated text:

Patients prematurely withdrawn from the trial will be replaced in order to reach 90 completed patients. ~~Replacement patients will receive the same treatment allocation (i.e. 3 doses of dasiglucaon or GlucaGen) as the patients they are replacing.~~

### 5.15 Clarification to the reporting of (Serious) Adverse Events

Section 8.2 Collection recording and reporting of adverse events

Updated text:

At each contact with the site (visit or telephone, excluding ~~safety~~ visits, where the patient is not seeing the Investigator or his staff (e.g. visits to the laboratory)) the patient must be asked about AEs.

…

The Investigator must report initial information in writing (fax or email) on all SAEs ~~to the responsible medical monitor of the Sponsor (Zealand) and~~ to the Sponsor's responsible pharmacovigilance unit (*Safety CRO*~~Pharmalex~~) immediately (within 24 hours) after obtaining knowledge about the event. *Please refer to Appendix 1 for contact details. The Safety CRO will inform the medical monitor and the sponsor about the reported SAEs.*

~~Name: [redacted]~~
~~Company: PharmaLex (formerly Lindeq)~~
~~Address: Agern Allé 24, DK 2970 Hørsholm, Denmark~~
~~Tel: [redacted] (8 a.m. to 4 p.m.)~~
~~[redacted] (outside 8 a.m. to 4 p.m.)~~
~~Fax: [redacted]~~
~~E-mail: drugsafety@lindeq.com~~

~~In addition, and meeting the same timeline, Investigators have to report all SAEs to Zealand by forwarding the SAE form electronically (e.g. in PDF format) within 24 hours to the representatives of Zealand.~~

~~Names: [redacted] and [redacted]~~
~~Address: Smedeland 36, DK 2600 Glostrup, Denmark~~
~~Tel: [redacted] / [redacted]~~
~~E-mails: [redacted] / [redacted]~~

It is the responsibility of ~~PharmaLex~~*the Safety CRO* to report all SUSARs that occur in this trial to the Competent Authorities and IRBs/IECs in accordance with the local requirements in force and ICH guideline for GCP. The trial monitor must be informed accordingly.

Section 8.7 Safety Committee

Updated text:

*As a minimum t*~~T~~he SC convenes every quarter to review relevant safety information, including AEs and laboratory data.

### 5.16 Clarification on case report forms

Section 9.1 Case report forms

Updated text:

All key pages will be signed or initialed by the investigator, signifying agreement with and responsibility for the recorded data. Key pages are the following: ~~end of visit form,~~ AE-reporting form, *trial* medication form, concomitant medication form and trial-closure form.

*Data directly captured in the eCRF (i.e., data assigned or calculated automatically by the EDC system) is called e-source*. ~~A trial specific list should be generated prior to the performance of the trial, which specifies which data fields in the Case Report Form will be used by the medical personnel for direct entry during the performance of the trial.~~ This means that these data fields in the CRF are source documents. For the other CRF data fields, *which are entered by medical personnel during the trial,* the source data will be found in other documents (such as patients' files, worksheets, etc.), ~~that is~~ *i.e.* for these CRF data fields the "other" documents are the source documents. ~~Should such a list be created, it should be submitted to the authorities together with the protocol.~~

### 5.17 Subgroup analysis added to statistical section

10.1.9 Subgroup analysis

Updated text:

*Subgroup analyses for the primariy endpoint (voerl ADA incidence) by gender, age, and race will be performed.* ~~No subgroup analysis is currently planned.~~

### 5.18 Administrative and editorial changes

Front page

New text:

Sponsor code: ZP4207-16136
SynteractHCR: ZEA-DNK-01711
EudraCT number: 2017-000062-30

*IND Number: 127866*

*Dasiglucagon SUB code: SUB181296*

Coordinating Investigator

Updated text:

~~Linda Morrow, MD~~
~~Prosciento~~
~~855 Third Avenue~~
~~91911 Chula Vista~~
~~CA, USA~~
*Thomas R. Pieber, MD*
*Medical University of Graz*
*Auenbruggerplatz 15*
*A-8036 Graz, Austria*

Section 1. Signatures and agreement with protocol
Updated text:
Coordinating Investigator: ~~Linda Morrow, MD~~
~~Prosciento~~
~~855 Third Avenue~~
~~91911 Chula Vista~~
~~CA, USA~~

*Thomas R. Pieber, MD*
*Medical University of Graz*
*Auenbruggerplatz 15*
*A-8036 Graz, Austria*

Sponsor's representative: [redacted] [redacted]~~, MSc (Pharm)~~
Clinical ~~Trial~~ Project Manager
Zealand Pharma A/S
Smedeland 36, 2600 Glostrup, Denmark

Section 2. Trial synopsis
Coordinating Investigator
Updated text:
~~Linda Morrow, MD, Prosciento, 855 Third Avenue, 91911 Chula Vista, CA, USA~~

*Thomas R. Pieber, MD, Medical University of Graz, Auenbruggerplatz 15, A-8036 Graz, Austria*

Planned trial period
Updated text:
First Patient First Visit: *May* ~~March~~ 2017
Last Patient First Visit: *November* ~~September~~ 2017

**APPENDIX 1:**

| | |
|---|---|
| Coordinating investigator | ~~Linda Morrow, MD~~<br>~~Prosciento~~<br>~~855 Third Avenue~~<br>~~91911 Chula Vista, CA, USA~~<br>*Thomas R. Pieber, MD*<br>*Medical University of Graz*<br>*Auenbruggerplatz 15*<br>*A-8036 Graz, Austria* |
| Trial Monitor | ~~To be decided~~<br>SynteractHCR Deutschland GmbH<br>Albrechtstr. 14<br>80636 Munich, Germany |
| Sponsor representative | [redacted] [redacted]<br>Clinical ~~Trial~~ Project Manager<br>Zealand Pharma A/S<br>Smedeland 36, 2600 Glostrup, Denmark<br>Tel: [redacted]<br>E-mail: [redacted] |
| Clinical laboratory | ~~To be determined~~<br>*MLM Medical Labs GmbH*<br>[redacted]<br>*Dohrweg 63, 41066 Mönchengladbach, Germany*<br>*Tel:* [redacted]<br>*E-mail:* [redacted] |

# Clinical Trial Protocol

***A phase 3, Randomized, Double-Blind, Parallel Group Safety Trial to Evaluate the Immunogenicity of Dasiglucagon and GlucaGen® Administered Subcutaneously in Patients with Type 1 Diabetes Mellitus (T1DM)***

**Sponsor code: ZP4207-16136**
**SynteractHCR: ZEA-DNK-01711**
**EudraCT number: 2017-000062-30**
**IND Number: 127866**
**Dasiglucagon SUB code: SUB181296**

Coordinating investigator: Thomas R. Pieber, MD
Medical University of Graz
Auenbruggerplatz 15
A-8036 Graz, Austria

Sponsor: Zealand Pharma A/S
Smedeland 36
2600 Glostrup, Copenhagen
Denmark

Version: final version 2. This version includes Protocol version 1, dated 18 January 2017 and Protocol Amendment 1, dated 08 May 2017

Date: 08 May 2017

***GCP statement***
*This trial will be performed in compliance with Good Clinical Practice (GCP), the Declaration of Helsinki (with amendments) and local legal and regulatory requirements.*

## SIGNATURE PAGE

Declaration of Sponsor or Responsible Medical Officer

**Title: A phase 3, Randomized, Double-Blind, Parallel Group Safety Trial to Evaluate the Immunogenicity of Dasiglucagon and GlucaGen® Administered Subcutaneously in Patients with Type 1 Diabetes Mellitus (T1DM)**

This trial protocol amendment number 1 was subjected to critical review. The information it contains is consistent with current knowledge of the risks and benefits of the investigational product, as well as with the moral, ethical and scientific principles governing clinical research as set out in the 2008 revision of the Declaration of Helsinki [1] and the guidelines on Good Clinical Practice (GCP) [2].

[redacted] _____ [redacted]
Date

Title: Clinical Project Manager
Institution: Zealand Pharma a/s
Smedeland 36, 2600 Glostrup, Denmark

[redacted] _____ [redacted]
Date

Title: Medical Director
Institution: Zealand Pharma a/s
Smedeland 36, 2600 Glostrup, Denmark

[redacted] _____ [redacted]
Date

Title: Head of Clinical [redacted]pment
Institution: Zealand Pharma a/s
Smedeland 36, 2600 Glostrup, Denmark

**Title:** A phase 3, randomized, double-blind, parallel group safety trial to evaluate the immunogenicity of dasiglucagon and GlucaGen® administered subcutaneously in patients with type 1 diabetes mellitus (T1DM)

I agree to conduct this trial according to the Trial Protocol.

I agree that the trial will be carried out in accordance with Good Clinical Practice (GCP), with the Declaration of Helsinki (with amendments) and with the laws and regulations of the countries in which the trial takes place.

Investigator

| Date | Signature |
|---|---|

Name and address

## 2. Trial synopsis

| | |
|---|---|
| **Title of the trial:**<br>A phase 3, randomized, double-blind, parallel group safety trial to evaluate the immunogenicity of dasiglucagon* and GlucaGen® administered subcutaneously in patients with type 1 diabetes mellitus (T1DM)<br>* Dasiglucagon is the proposed international nonproprietary name | |
| **EudraCT number:**<br>2017-000062-30 | **Protocol codes:**<br>Sponsor: ZP4207-16136<br>SynteractHCR: ZEA-DNK-01711 |
| **Sponsor or sponsor's representative in the European Union:**<br>Zealand Pharma A/S, Smedeland 36, 2600 Glostrup (Copenhagen), Denmark | |
| **Coordinating investigator:**<br>Thomas R. Pieber, MD, Medical University of Graz, Auenbruggerplatz 15,A-8036 Graz, Austria | |
| **Trial center(s):**<br>2 centers in the EU, 2 centers in the US, and 3 centers in Canada | |
| **Planned trial period:**<br>First Patient First Visit: May 2017<br>Last Patient First Visit: November 2017 | **Phase of Development:**<br>Phase 3 |
| **Objectives:**<br>The primary objective is to evaluate the immunogenicity of repeated single doses of dasiglucagon and GlucaGen following subcutaneous (s.c.) administration in T1DM patients.<br>The secondary objective is to evaluate the safety and tolerability of repeated single doses of dasiglucagon following s.c. administration compared with s.c. GlucaGen in T1DM patients. | |
| **Trial design:**<br>This is a randomized, double-blind, parallel group trial evaluating the immunogenicity of either dasiglucagon or GlucaGen administered to euglycemic T1DM patients. Patients will be randomized 1:1 to receive 3 s.c. injections of dasiglucagon or GlucaGen with 1 week between doses. Patients will be followed for at least 3 months from the day of the first dose to assess any immune response. A total of 90 patients are expected to complete the trial. Handling, preparation and administration of trial medication will be done by unblinded trial personnel. All trial assessments will be done by blinded trial personnel. However, exposure assessments and anti-drug antibody (ADA) assessments will be performed by unblinded personnel at the specialty laboratories, to make sure that dasiglucagon or GlucaGen administration is matched with the applicable bioanalytical assay. | |
| **Planned number of patients:**<br>90 completed patients (45 completed patients per treatment group). Prematurely discontinued patients will be replaced in order to reach 90 completed patients. It is expected that 112 patients in total will be randomized and treated.<br>To qualify as completed, the patient must be dosed according to protocol and have blood drawn for the planned anti-drug antibody (ADA) analyses. | |
| **Medical condition or disease under investigation:**<br>Given the role of the immune system in the pathogenesis of T1DM, the present trial is conducted in patients with T1DM. There are no data indicating an altered immune response with varying blood glucose levels. Therefore, for the safety and well-being of the patients, they will not be brought into hypoglycemia prior to dosing. Patients with previous exogenic glucagon exposure will not be excluded from the trial, but the information on previous glucagon administration will be recorded. | |
| **Inclusion criteria:**<br>To be included in the trial, patients have to fulfill all of the following criteria:<br>1. Informed consent obtained before any trial-related activities (trial-related activities are any procedure that would not have been performed during normal management of the patient)<br>2. Availability for the entire trial period<br>3. Age between 18 and 70 years, both inclusive<br>4. Male or female patients with T1DM for at least 1 year. Diagnostic criteria as defined by the | |

American Diabetes Association

5. Hemoglobin A1c ($HbA_{1c}$) <10%
6. Stable anti-diabetic treatment for at least 1 month (e.g. within 10% insulin dose adjustment)
7. A female participant must meet one of the following criteria
   a. Participant is of childbearing potential and agrees to use one of the accepted contraceptive regimens throughout the entire duration of the trial from screening until last follow-up visit. An acceptable method of contraception includes at least one of the following:
      i. Abstinence from heterosexual intercourse
      ii. Systemic contraceptives (birth control pills, injectable/implant/ insertable hormonal birth control products, transdermal patch); if the participant is using systemic contraceptives, she must use an additional form of acceptable contraception (iii or iv, below)
      iii. Intrauterine device (with and without hormones)
      iv. Condom with spermicide

   or

   b. Participant is of non-childbearing potential, defined as surgically sterile (i.e. has undergone complete hysterectomy, bilateral oophorectomy, or tubal ligation), or in a menopausal state (at least 1 year without menses)
8. A male must be surgically sterilized or willing to refrain from sexual intercourse from screening and until last follow-up visit or, if sexually active, uses a condom and partner practices contraception during the trial from screening and until the last follow-up visit

**Exclusion criteria:**

Patients meeting any of the following criteria during screening evaluations will be excluded from trial participation:

1. Previous administration of dasiglucagon (previously referred to as ZP4207)
2. Known or suspected allergy to trial medication(s) or related products
3. History of anaphylaxis or symptoms of severe systemic allergy (such as angioedema)
4. Previous participation (randomization) in this trial
5. Females who are pregnant according to a positive pregnancy test, actively attempting to get pregnant, or are lactating
6. Patients on a closed loop artificial pancreas
7. Receipt of any investigational drug within 3 months prior to screening
8. Active malignancy within the last 5 years
9. Congestive heart failure, New York Heart Association class II-IV
10. Inadequately treated blood pressure as defined as systolic blood pressure ≥160 mmHg or diastolic blood pressure ≥90 mmHg at screening
11. Current bleeding disorder, including use of anticoagulant treatment
12. Known presence or history of pheochromocytoma (i.e. adrenal gland tumor) or insulinoma (i.e. insulin-secreting pancreas tumor)
13. Known or suspected HIV infection
14. Use of a systemic beta-blocker drug, indomethacin, warfarin or anticholinergic drugs in the previous 28 days before Day 1 of this trial
15. Use of systemic corticosteroids, anti-inflammatory biological agents, kinase inhibitors or other immune modulating agents within the last 3 months prior to screening
16. Aspartate aminotransferase (AST) or alanine aminotransferase (ALT) >2.5 X the upper limit of normal (ULN), bilirubin >1.5 X ULN, estimated glomerular filtration rate (eGFR) <30 mL/min/1.73$m^2$ according to the Modification of Diet in Renal Disease (MDRD) Study definition. Altered electrolytes values of clinical relevance for cardiac conduction, as judged by the investigator.
17. Clinically significant abnormal ECG at screening as evaluated by Investigator
18. Donation of blood or plasma in the past month, or in excess of 500 mL within 12 weeks prior to screening
19. A positive result in the alcohol and/or urine drug screen at the screening visit. Significant history of alcoholism or drug abuse as judged by the investigator or consuming more than 24 g alcohol per day for men, or more than 12 g alcohol per day for women.

20. Patients with mental incapacity or language barriers that preclude adequate understanding or cooperation, who are unwilling to participate in the trial, or who in the opinion of the Investigator should not participate in the trial
21. Surgery or trauma with significant blood loss within the last 2 months prior to screening
22. Any condition interfering with trial participation or trial endpoints or that could be hazardous to the patient
23. Use of prescription or non-prescription medications known to cause QT prolongation

**Test product, dose and mode of administration:**

Dasiglucagon: 0.6 mg; liquid formulation, 1 mg/mL in prefilled syringes containing 0.6 mL

**Reference product, dose and mode of administration:**

GlucaGen, 1 mg; powder and solvent for reconstitution as 1 mL solution for injection (recombinant glucagon hydrochloride, Novo Nordisk)

**Duration of treatment:**

Patients will receive 3 s.c. injections of trial medication (dasiglucagon or GlucaGen) with 1 week between each dosing.

Prior to administration of trial medication patients must reach a target plasma glucose level of 70-150 mg/dL. Plasma glucose levels may be adjusted by s.c. administration of a fast-acting insulin analog or by glucose ingestion.

**Criteria for evaluation:**

**Immunogenicity:**

**Primary endpoint:**

- Overall ADA incidence
  This will be calculated as a percentage of the combined results of treatment-induced ADA-positive patients and treatment-boosted ADA-positive patients and the total number of evaluable patients, excluding baseline-positive patients without any samples available after drug administration.

**Key secondary endpoints:**

- Treatment-induced ADA
  Incidence calculated as a percentage of the total number of evaluable patients that were ADA negative at baseline and ADA positive after drug administration and the total number of evaluable patients, excluding baseline positive patients without any samples available after drug administration.
- Treatment-boosted ADA
  Incidence calculated as percentage of baseline ADA-positive patients with significant increases (≥5-fold) in ADA titer after drug administration and the total number of evaluable patients, excluding baseline-positive patients without any samples available after drug administration.

**Secondary endpoints:**

Characterization of ADA response:

- Incidence and titer of neutralizing activity of ADA positive patients
- Incidence of cross-reactivity of ADA positive patients towards endogenous glucagon
- Kinetics of ADA:
  The timing and duration of detected ADA response

Safety:

- The incidence, type and severity of AEs
- Changes from baseline in clinical laboratory parameters
- Changes from baseline in vital signs
- Clinically meaningful changes from baseline in physical examination and electrocardiogram (ECG)

Exposure endpoints, after administration of first and third doses of trial medication:

- Plasma dasiglucagon and glucagon concentrations from 0-90 min after dosing will be evaluated based on the following endpoints: $AUC_{0\text{-}30min}$, $AUC_{0\text{-}90\ min}$, $C_{max}$, $t_{max}$

Pharmacodynamics, after administration of first and third doses of trial medication:

- Plasma glucose profiles over the period from 0-90 min after dosing will be evaluated based on the following endpoints: $AUE_{0\text{-}30min}$, $AUE_{0\text{-}90\ min}$, $CE_{max}$, $t_{max}$

**Statistical methods:**
All statistical analysis will be descriptive i.e. no formal statistical testing will be performed. Continuous variables will be summarized with means, standard deviations, medians, minimums, and maximums, and valid cases. Other summaries (e.g. quartiles, 95% confidence intervals) may be used as appropriate. Categorical variables will be summarized by counts and by percentage of patients in corresponding categories.

**Sample size calculation:**
The purpose of the present trial is to generate data describing the immunogenic potential of dasiglucagon and GlucaGen. The ADA assays to be used in this trial are both validated, but specific for GlucaGen and dasiglucagon, respectively, and the performance of the assays are thus not directly comparable. As a consequence, a formal comparison or non-inferiority analysis will not be performed.
Currently, no ADA incidences have been detected in the completed clinical trials, where up to 5 repeated doses of dasiglucagon have been administered to the same patients within a week. Across the two phase 1 clinical trials and a completed phase 2 pharmacokinetic/pharmacodynamic trial, a total of 141 subjects have been exposed to dasiglucagon and no incidences of ADA development have been observed. The obtained data indicate that dasiglucagon has a low risk for induction of ADAs in the investigated settings and as a consequence a meaningful sample size to compare both treatments cannot be estimated.
The sample size is therefore based on a certain precision of the confidence interval for the overall ADA incidence if no events are observed, respectively to ensure a certain probability for observing one event.
When no events are observed, to obtain an upper bound of 0.050 on the 90.0% confidence interval for the probability of such a rare event, would require a sample size of 45. Respectively, accepting a chance of observing at least one event of 90% and an actual probability of the event of 5% leads to a sample size of 45 patients completing the trial. In order to account for drop-outs, It is expected that 112 patients in total will be randomized and treated.

**Table 2-1: Flow chart**

| Trial period | Screening | Treatment | | | Follow-up | | |
|---|---|---|---|---|---|---|---|
| Visit number | V1 | V2 | V3 | V4 | V5 | V6 | V7 (EoT) |
| Trial day | -3 | 0 | 7 | 14 | 35 | 60 | 104 |
| Visit window (days) | -30 to - -3 | | ±1 | ±1 | ±2 | ±5 | ±10 |
| **Patient related info/assessments** | | | | | | | |
| Informed consent | X[1] | | | | | | |
| Inclusion/exclusion criteria | X | X[2,3] | | | | | |
| Demography | X | | | | | | |
| Body measurements | X | | | | | | |
| Medical history | X | | | | | | |
| Concomitant illness | X | | | | | | |
| Prior medications | X | | | | | | |
| Concomitant medication | X | X | X | X | X | X | X |
| History of alcohol/drug abuse | X | | | | | | |
| Randomization | | X | | | | | |
| Withdrawal criteria | | X | X | X | X | X | |
| Dosing day exclusion criteria | | X | X | X | | | |
| **Safety assessments** | | | | | | | |
| Physical examination | X | | | | | | X |
| Vital signs | X | X[12] | X[12] | X[12] | X | | X |
| ECG | X | X[10] | X[10] | X[10] | X | | X |
| Local tolerability | | X[5] | X[5] | X[5] | | | |
| Adverse events | X | X | X | X | X | X | X |

| Trial period | Screening | Treatment | | | Follow-up | | |
|---|---|---|---|---|---|---|---|
| Visit number | V1 | V2 | V3 | V4 | V5 | V6 | V7 (EoT) |
| Trial day | -3 | 0 | 7 | 14 | 35 | 60 | 104 |
| Visit window (days) | -30 to - -3 | | ±1 | ±1 | ±2 | ±5 | ±10 |
| **Laboratory** | | | | | | | |
| Hematology, biochemistry, coagulation | X[4] | X[4] | | X[4] | X | | X |
| Pregnancy test | X[11] | X[11] | X[11] | X[11] | | | |
| Urinalysis | X | X[2] | | X[2] | | | X |
| Urine drug screen | X[6] | X[2,6] | X[2,6] | X[2,6] | | | |
| Alcohol breath test | X | X[2] | X[2] | X[2] | | | |
| **Exposure and pharmacodynamics (PD)** | | | | | | | |
| Dasiglucagon /glucagon | | X[7] | | X[7] | | | |
| Plasma glucose | | X[8] | | X[8] | | | |
| **Other assessments** | | | | | | | |
| Antibodies against dasiglucagon /glucagon | | X[2] | X[2] | X[2] | X | X | X |
| **Trial material** | | | | | | | |
| Administration of trial medication | | X[9] | X[9] | X[9] | | | |

ECG = electrocardiogram; EoT = End of Trial; ; PD = pharmacodynamics; V = visit

[1] Informed consent can be obtained on the same day as screening, but prior to any trial-related procedures [2] Pre-dose

[3] Only check of changes between the screening visit and V2.

[4] Coagulation parameters are measured at screening visit only. On dosing days Visit 2 and 4, blood samples are collected pre-dose, and at 30 and 90 min post-dosing. The actual time for sampling should not deviate from the nominal time by more than ±5 min. Pre-dose is defined as within 5 min prior to dosing.

[5] Local tolerability assessed at 0.5 and 2 h post-dose. The actual time for assessment should not deviate from the nominal time by more than ±10 min.

[6] Urine drug screen will be performed at trial site for visits 1-4

[7] Pre-dose, 5, 10, 30, 60, and 90 min post-dosing. The actual time of blood sampling for exposure should not deviate from the nominal time by more than ±1 min. Pre-dose is defined as within 5 min prior to dosing.

[8] Pre-dose, 5, 10, 30, 60, and 90 min post-dosing. The actual time for blood sampling for plasma glucose should not deviate from the nominal time by more than ±1 min. Pre-dose is defined as within 5 min prior to dosing.

[9] Prior to administration of trial medication patients must reach a target plasma glucose level of 70-150 mg/dL. Plasma glucose levels may be adjusted by administration of a fast-acting insulin analog or by glucose ingestion at the discretion of the investigator. At visit 2 and 4 patients must be fasting for 90 min after administration of trial medication. At all dosing visits, patients will be treated individually to alleviate any potential side effects and will be observed for at least 5 h post-dose.

[10] On dosing days Visit 2, 3 and 4 ECG's are assessed pre-dose, and at 20, 35, 45 and 60 min post-dosing. The actual time of assessment should not deviate from the nominal time by more than ±5 min. Pre-dose is defined as within 5 min prior to dosing.

[11] Pregnancy test is only applicable for women of childbearing potential. At Visit 1 a serum pregnancy test should be performed. At Visit 2, 3 and 4 a pre-dose urine pregnancy test should be performed.

[12] On dosing days Visit 2, 3, and 4 vitals signs are collected pre-dose and at 30, 90 and 120 min post-dosing. The actual time of assessment should not deviate from the nominal time by more than ±5 min. Pre-dose is defined as within 5 min prior to dosing.

## 3. Table of contents

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# 4. List of abbreviations and definition of terms

## 4.1 Abbreviations

| | |
|---|---|
| ADA | Anti-drug Antibody |
| AE | Adverse Event |
| ALT (SGPT) | Alanine Aminotransferase (Serum Glutamic Pyruvic Transaminase) |
| AST (SGOT) | Aspartate Aminotransferase (Serum Glutamic Oxaloacetic Transaminase) |
| BG | Blood Glucose |
| CA | Competent Authority (Directive 2001/20/EC) |
| CFR | Code of Federal Regulations |
| CRF/eCRF | Case Report Form/Electronic Case Report Form |
| CI | Confidence Interval |
| CRO | Contract Research Organization |
| CSII | Continuous Subcutaneous Insulin Infusion |
| CTA | Clinical Trial Authorization (Directive 2001/20/EC) |
| EC | Ethics Committee |
| ECG | Electrocardiogram |
| EDC | Electronic Data Capture |
| eGFR | Estimated Glomerular Filtration Rate |
| EoT | End of Trial |
| EudraCT | European Clinical Trials Database |
| FAS | Full Analysis Set |
| FDA | Food and Drug Administration |
| gamma-GT | gamma-Glutamyltransferase |
| GCP | Good Clinical Practice |
| $HbA_{1c}$ | Hemoglobin $A_{1c}$ |
| HEENT | Head, Ears, Eyes, Nose, Throat |
| HIV | Human Immunodeficiency Virus |
| ICF | Informed Consent Form |
| ICH | International Conference on Harmonization |
| IEC | Independent Ethics Committee |
| IM | Intramuscular(ly) |
| IMP | Investigational Medicinal Product |
| IND | Investigational New Drug |
| INR | International Normalized Ratio |
| IRB | Institutional Review Board |
| i.v. | Intravenous(ly) |
| IWRS | Interactive Web Response System |
| LOCF | Last Observation Carried Forward |
| MDRD | Modification of Diet in Renal Disease |
| MedDRA | Medical Dictionary for Regulatory Activities |
| Nab | Neutralizing Antibody |
| PG | Plasma Glucose |
| ODM | Operational Data Model |
| PD | Pharmacodynamic(s) |

| | |
|---|---|
| PK | Pharmacokinetic(s) |
| PPS | Per Protocol Set |
| PT | Preferred Term |
| SAP | Statistical Analysis Plan |
| SAS | Safety Analysis Set |
| SAE | Serious Adverse Event |
| SC | Safety Committee |
| s.c. | Subcutaneous(ly) |
| SOC | System Organ Class |
| SOP | Standard Operating Procedure |
| T1DM | Type 1 Diabetes Mellitus |
| T2DM | Type 2 Diabetes Mellitus |
| ULN | Upper Limit of Normal |
| V | Visit |
| ZP4207 | Dasiglucagon |

**Plasma concentrations of dasiglucagon/GlucaGen**

| | |
|---|---|
| $AUC_{0\text{-}30min}$ | Area under the plasma concentration curve from administration to observed concentration 30 min |
| $AUC_{0\text{-}90\ min}$ | Area under the plasma concentration curve from administration to observed concentration at 90 min |
| $C_{max}$ | Maximum plasma concentration |
| $t_{max}$ | Time until $C_{max}$ is reached |

**Plasma glucose concentrations**

| | |
|---|---|
| $AUE_{0\text{-}30min}$ | Area under the effect curve from administration to 30 min |
| $AUE_{0\text{-}90\ min}$ | Area under the effect curve from administration to 90 min |
| $CE_{max}$ | Change from baseline plasma glucose to maximum plasma glucose measured post dose |
| $t_{max}$ | Time to maximum effect |

## 4.2 Definitions of terms

Definition of the end of the trial: The trial ends with the last visit of the last patient participating in the trial.

# 5. Introduction

## 5.1 Background of the trial

### Hypoglycemia

Hypoglycemia in patients with diabetes is defined as episodes of an abnormally low plasma glucose concentration.[1] This is a common, unpredictable, and potentially dangerous side effect of treatment of diabetes mellitus with especially insulin or sulfonylureas. It is more frequent in patients with profound endogenous insulin deficiency, such as occurs in type 1 diabetes mellitus (T1DM) and advanced type 2 diabetes mellitus (T2DM). Treatment of T2DM with insulin causes hypoglycemia progressively and more frequently over time, whereas in T1DM, hypoglycemia is experienced throughout the course of established disease.

Symptoms and signs of hypoglycemia are not specific. Patients undergoing a hypoglycemic episode experience unpleasant symptoms such as anxiety, sweating, hunger, tremors, palpitations, paresthesia, nausea and pallor. Depending on its severity, the hypoglycemia may lead to mild confusion, behavioral changes, loss of consciousness, seizures, coma, and death.[2]

The incidence of hypoglycemic events or even the fear of hypoglycemia influences patients' adherence to prescribed treatment regimens for diabetes mellitus. This leads to inadequate glycemic control, which in turn may lead to an increased risk of diabetic complications.

### Glucagon

Glucagon is a naturally occurring hormone, secreted from the alpha cells of the pancreatic islets. Glucagon plays a central role in the regulation of glucose homeostasis and is the counterpart of insulin for controlling blood glucose levels (i.e. it acts in opposition to insulin in terms of effects on blood glucose levels). Glucagon stimulates hepatic glycogenolysis and gluconeogenesis in hypoglycemic states, thereby restoring glucose homeostasis. Glucagon receptor agonism has also been shown to exert effects on lipid metabolism, energy balance, body adipose tissue mass and food intake. Insulin decreases blood glucose levels and cases of hypoglycemia can be reversed by glucagon. Therefore, glucagon is indicated for the treatment of severe hypoglycemia.

Antibodies against therapeutic peptides like glucagon and analogues hereof may develop when injected subcutaneously. Although important, glucagon is not considered to have a critical endogenous function since other counter regulatory hormones are also induced during hypoglycemia (e.g. growth hormone, cortisol, and epinephrine). In addition, results from 3 mouse models defective in various pathways of the glucagon signaling have confirmed that glucagon action is dispensable for their development and survival. Also, in non-clinical toxicity studies performed with dasiglucagon (see below), no consequences of ADA formation have currently been observed. In summary, glucagon appears to have a partly redundant endogenous function. These non-clinical data are of importance when evaluating the consequences of ADA formation.

### Dasiglucagon

Dasiglucagon (ZP4207) is a stable peptide analog of human glucagon, available in a ready-to-use liquid formulation and is in development for the treatment of severe hypoglycemia in insulin dependent patients with diabetes mellitus. Dasiglucagon is a specific and full glucagon receptor agonist designed to mimic the effects of glucagon, having a fast absorption and elimination (minutes). It is a peptide of 29 amino acids, with 7 amino acid substitutions compared to native glucagon. The main purpose of the substitutions is to increase the physical and chemical stability of the glucagon analog compared to marketed glucagon products such as Lilly Glucagon or GlucaGen®. Dasiglucagon exhibits improved physical and chemical stability and is available in an aqueous solution at neutral pH.[3]

Three clinical trials have been completed with dasiglucagon, a first human dose trial (ZP4207-14013), a multiple-dose dose-escalation trial (ZP4207-15007) to evaluate the safety, tolerability, pharmacokinetics (PK) and pharmacodynamics (PD) of dasiglucagon, and a phase 2 crossover trial to assess the pharmacokinetics and pharmacodynamics of a single dose of an optimized formulation of dasiglucagon administered subcutaneously (s.c.) in patients with T1DM (ZP4207-15126).[3]

*Pharmacokinetics and pharmacodynamics of dasiglucagon*

The results of the phase 1 and 2 clinical trials confirm dose-proportionality for dasiglucagon pharmacokinetics, which is characterized by a fast absorption with a peak plasma concentration obtained after 35 minutes. Thereafter, the plasma concentration rapidly declines with an average half-life of 28 minutes. The median time to the maximum plasma concentration ($C_{max}$) was later for dasiglucagon than for GlucaGen (35 versus 20 minutes). Doses of 0.3 mg dasiglucagon and 0.5 mg GlucaGen and also 0.6 mg dasiglucagon and 1.0 mg GlucaGen were similar with regard to $C_{max}$. For $C_{max}$, the results indicated that 0.3 mg dasiglucagon was comparable to 0.5 mg GlucaGen (90% confidence interval (CI): 0.8167; 1.0068) and 0.6 mg dasiglucagon was comparable to 1.0 mg GlucaGen (90% CI: 0.8850; 1.1991).[3] At these dose levels, the total exposures ($AUC_{0\text{-}inf}$) were higher for dasiglucagon compared to GlucaGen.

At all dose levels in the phase 2 trial, all patients achieved a plasma glucose level of at least 70 mg/dL as well as an increase in plasma glucose by at least 20 mg/dL within 30 min post-dose. The maximal observed time to reach the 20 mg/dL plasma glucose increase ranged from 15 to 27 minutes across doses and decreased as the dose increased. The pharmacodynamic responses of 0.6 mg of dasiglucagon and 1.0 mg of GlucaGen were comparable.[3]

*Safety of dasiglucagon*

The safety data for dasiglucagon did not give rise to any relevant safety concerns for dasiglucagon beyond those related to the pharmacological effect of glucagon agonism. All adverse events (AEs) recorded in the trials were of mild or moderate severity. The most frequently reported systemic AE was nausea, which is a known side effect following administration of glucagon. Headache was the next most frequently reported event, occurring in all dose groups in the phase 2 trial. Injection site reactions were observed only sporadically after administration with either dasiglucagon or GlucaGen® and all were mild and transient. The most frequent injection site reaction was erythema, occurring in all treatment groups, including the placebo group, irrespective of dose. Glucagon has been described to exert positive inotropic and chronotropic effects and may therefore cause tachycardia and hypertension. In the phase 1 clinical trials, but not the phase 2 trial, temporary but clinically significant decreases in blood pressure were observed in a few healthy volunteers receiving investigational medicinal product (IMP) doses of at least 1.0 mg (4 with dasiglucagon and 1 with GlucaGen). This is not considered a safety concern, however hemodynamic changes post dosing will be considered a clinical event of interest.

The phase 1 and 2 results and the safety profile described to date do not give rise to specific safety concerns. For further information, please refer to the Investigator Brochure.[3]

*Immunogenicity of dasiglucagon*

To date, in the 3 clinical trials performed with dasiglucagon (described above), there have not been any anti-drug antibody (ADA) occurrences in a total of 141 subjects exposed to 1 or more doses. Data from the non-clinical toxicology program (7 non-clinical toxicity studies) showed that ADAs were detected in mice, rats, and dogs, and were most frequent in animals in the highest dasiglucagon dose groups. A fraction of the ADAs from rats and dogs were able to cross-react with native glucagon. However, the ADAs did not appear to be associated with changes in the safety or toxicity profiles compared to ADA-negative animals. In rats, the average exposure (AUC) was increased following 13 and 26 weeks of treatment in dose groups with higher ADA frequency (≥8 mg/kg/day). The ADA frequency and titers of consistently ADA-positive rats were reduced from 13 to 26 weeks of treatment, indicating a transient response in most animals. It

therefore appears that dasiglucagon has a low risk for induction of ADAs. A fraction of the antibodies detected in the non-clinical toxicity studies was found to cross-react with glucagon without advert clinical or toxicity findings. These data suggest that dasiglucagon and glucagon share epitopes and potential ADAs induced by dasiglucagon in humans may have the ability to cross-react with glucagon.[3]

Although glucagon is an important hormone for controlling blood glucose levels it is considered to have a partially redundant endogenous function since hypoglycemia can also be corrected by other means. The overall immunogenicity risk of dasiglucagon in a clinical context is therefore considered to be low and the potential effects of induced ADAs judged to be of limited clinical consequence.

As dasiglucagon contains 7 amino acid substitutions compared to native glucagon and historic data indicate that the immune system's tolerance to glucagon can be impaired in the intended target population, there is an inherent risk for the induction of an ADA response against dasiglucagon. However, other product specific characteristics, i.e. dasiglucagon being a chemical synthetized product without host cell contaminants, a reduced potential for aggregation, a physiological compatible formulation, and a high bioavailability with a short half-life, are all in favor of reducing the risk of dasiglucagon to induce an unwanted immune response. Considering the intended indication, in which dasiglucagon is administered as a single-dose rescue treatment on an infrequent basis (0.21 to 1.6 episodes per patient per year),[4,5] the induction of a high titer ADA response with effects on clinical safety seems unlikely. Overall, the risk of dasiglucagon to induce an ADA response is considered low. The present trial aims to evaluate that immunogenicity risk in patients with T1DM administered multiple s.c. doses of dasiglucagon.

### 5.2 Trial rationale

Dasiglucagon is in clinical development as a rescue treatment for severe hypoglycemia in patients with insulin-dependent diabetes mellitus. Immune responses to therapeutic peptides and proteins may develop after subcutaneous administration and could potentially adversely affect their safety and efficacy. The potential formation of high titer ADAs to dasiglucagon, although considered unlikely in a rescue indication, could influence the efficacy of dasiglucagon, either indirectly by interacting with the pharmacokinetics of dasiglucagon or directly by neutralizing the glucagon response.

In recent years, regulatory agencies have outlined and recommended the use of a risk-based approach in the evaluation and mitigation of immune responses to therapeutic compounds that may adversely affect their safety and/or efficacy. Both transient and persistent antibody response should be combined to determine the overall immunogenicity of a product. Persistent antibodies are of importance since patients with persistent antibodies could experience clinical adverse reactions affecting safety and efficacy, while a transient antibody response can resolve without further consequence.

The present trial aims to evaluate that immunogenicity risk with an assessment of the occurrence of ADAs and neutralizing ADAs, and of cross-reactivity with native glucagon, following repeated single doses of dasiglucagon by s.c. administration in T1DM patients. The reference product in this trial is GlucaGen, a recombinant human glucagon approved for the treatment of the severe hypoglycemic reactions that may occur in the management of insulin-treated children and adults with diabetes mellitus. Given the differences in ADA assay for dasiglucagon and GlucaGen, a formal statistical comparison or non-inferiority testing is not performed.

### 5.3 Assessment of anticipated benefits and risks

As glucagon and its analogs belong to a well-known drug class with a known mode of action, dasiglucagon is not expected to be a high-risk molecule.

Treatment with an investigational medicinal product may result in undesired effects or complaints. Undesired effects and complaints such as nausea, vomiting, and diarrhea are known AEs occurring with glucagon administration. Similar AEs have also been observed to a limited degree in the 3 clinical studies conducted with dasiglucagon. As with every novel drug substance, new and as yet unknown side effects also may occur.

There are limited data available to assess the immunogenic potential of glucagon products available on the market, but the data available indicate that marketed glucagon only has a small immunogenic potential. Based on the 3 clinical studies conducted with dasiglucagon to date (see Section 5.2), no anti-dasiglucagon or anti-glucagon antibodies have been detected.

In relation to diabetes therapy, development of insulin-antibodies is documented more substantially and the route of administration has been shown to influence the immunogenic potential of insulins. However, antibodies against insulin do not generally have an impact on insulin action and are thus not clinically relevant. In terms of consequence, development of high titer antibodies against dasiglucagon could, in theory reduce the activity of endogenous glucagon, which, in theory, could influence hypoglycemic episodes. Limited suppression of glucagon would, however, not be considered critical, since low glucose levels can also be corrected by other means, including oral intake of glucose and the action of other endogenous hormones such as oxyntomodulin and epinephrine.

Overall, dasiglucagon is judged to be a low-risk molecule, based upon the available clinical data as well as the outcome of the risk-based approach to the immunogenicity assessment. In line with the primary objective of this trial to assess the immunogenicity of dasiglucagon, sampling for measurement of antibodies against dasiglucagon will take place prior to first dosing (Visit 2), pre-dose at subsequent visits during the treatment period (Visits 3 and 4), and at all Follow-up visits (at 35, 60, and 104 days after the first dose of trial medication; i.e. at Visits 5, 6, and 7 (End of Trial [EoT] visit), respectively).

Administration of dasiglucagon may be associated with a risk of allergic reactions similar to those observed for other therapeutic peptides or proteins. Patients with known or suspected allergies to the trial medications or related products will be excluded from the trial. Mild or moderate allergic reactions may include symptoms of rash, fever, flu-like symptoms, nausea, headache, and myalgia. Acute generalized hypersensitivity reactions are usually very rare but may include symptoms of flushing, sweating, dizziness, change in blood pressure and difficulties in breathing. No severe acute hypersensitivity reactions have been observed in the 3 clinical trials conducted with dasiglucagon. Direct access to resuscitation equipment is ensured at the clinical trial sites.

With the exception of medical examinations, a patient participating in this trial is not likely to derive any personal health-related benefits. The results of the trial may contribute to the future use of dasiglucagon in patients with diabetes mellitus experiencing severe hypoglycemic reactions.

Overall, the benefit to risk ratio for patients entering the ZP4207-16136 trial is considered acceptable.

## 6. Trial objectives

**Primary objective**

- To evaluate the immunogenicity of repeated single doses of dasiglucagon and GlucaGen following s.c. administration in T1DM patients.

**Secondary objective**

- To evaluate the safety and tolerability of repeated single doses of dasiglucagon following s.c. administration compared with s.c. GlucaGen in T1DM patients.

**Primary endpoint:**

- Overall ADA incidence
  This will be calculated as a percentage of the combined results of treatment-induced ADA-positive patients and treatment-boosted ADA-positive patients and the total number of evaluable patients, excluding baseline-positive patients without any samples available after drug administration.

**Key secondary endpoints:**

- Treatment-induced ADA
  Incidence calculated as a percentage of the total number of evaluable patients that were ADA negative at baseline and ADA positive after drug administration and the total number of evaluable patients, excluding baseline positive patients without any samples available after drug administration.
- Treatment-boosted ADA
  Incidence calculated as percentage of baseline ADA-positive patients with significant increases (≥5-fold) in ADA titer after drug administration and the total number of evaluable patients, excluding baseline-positive patients without any samples available after drug administration.

**Secondary endpoints:**

Characterization of ADA response:

- Incidence and titer of neutralizing activity of ADA positive patients
- Incidence of cross-reactivity of ADA positive patients towards endogenous glucagon
- Kinetics of ADA:
  The timing and duration of detected ADA response

Safety:

- The incidence, type and severity of AEs
- Changes from baseline in clinical laboratory parameters
- Changes from baseline in vital signs
- Clinically meaningful changes from baseline in physical examination and electrocardiogram (ECG)

Exposure endpoints, after administration of first and third doses of trial medication:

- Plasma dasiglucagon and glucagon concentrations from 0-90 min after dosing will be evaluated based on the following endpoints: $AUC_{0\text{-}30min}$, $AUC_{0\text{-}90\ min}$, $C_{max}$, $t_{max}$

Pharmacodynamics after administration of first and third doses of trial medication:

- Plasma glucose profiles over the period from 0-90 min after dosing will be evaluated based on the following endpoints: $AUE_{0\text{-}30min}$, $AUE_{0\text{-}90\ min}$, $CE_{max}$, $t_{max}$

# 7. Investigational plan

## 7.1 Overall trial design and plan

This is a randomized, double-blind, parallel group trial comparing the immunogenicity of 3 fixed doses of either dasiglucagon or GlucaGen administered to euglycemic T1DM patients.

Patients with T1DM will be randomized 1:1 to receive 3 s.c. injections of either dasiglucagon (0.6 mg) or GlucaGen (1 mg), with 1 week between each dose. Patients will be followed for at least 3 months from the day of the first dose to assess any immune response. Patients with previous exogenic glucagon exposure will not be excluded from the trial, but the information on previous glucagon administration will be recorded to enable subgroup analyses. A total of 90 patients are expected to participate in and complete the trial (45 in each treatment arm). To qualify as completed, the patient must be dosed according to protocol and have blood drawn for the ADA analyses (as scheduled in Table 2-1). Prematurely discontinued patients will be replaced in order to reach 90 completed patients. It is expected 112 patients in total will be randomized and treated.

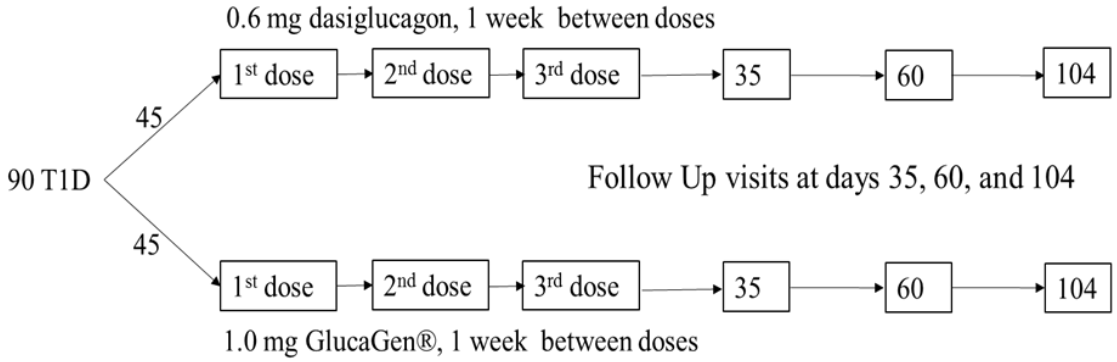
For the safety and well-being of the patients, they will not be brought into hypoglycemia prior to dosing. Prior to administration of trial product patients must reach a target plasma glucose level of 70-150 mg/dL.

The trial will include the following periods (as illustrated in Figure 7-1, below).

- A screening period from Day -30 to Day -3
- A treatment period, from Day 0 (day of randomization) to Day 14 (day of third and final dosing with trial medication), with s.c. trial medication administered on Day 0, Day 7, and Day 14. Handling, preparation and administration of trial medication will be done by unblinded trial personnel. All trial assessments will be done by blinded trial personnel.
- A follow-up period, from the end of the Treatment Period, with follow-up visits at Day 35, Day 60, and Day 104 (the EoT visit)

Time windows for each trial visit are given in Table 2-1.

**Figure 7-1 Overview of the trial design**




An overview on the trial procedures is given in the flow chart (Table 2-1). Patients should be seen for all visits on the designated day or as close to it as possible.

## 7.2 Discussion of trial design and choice of control groups

The trial will be randomized and double-blind to increase trial validity and to reduce bias during evaluation of assessments with the two treatments. Since the 2 trial medications are not identical in appearance (dasiglucagon is a liquid formulation and GlucaGen is available as a

powder for reconstitution), the handling, preparation and administration of trial medication will be done by unblinded trial personnel who will not be involved in other trial procedures and assessments. All trial assessments performed at the trial site will be done by blinded trial personnel. However, exposure assessments and ADA assessments will be performed by unblinded personnel at the specialty laboratories, to make sure that dasiglucagon or GlucaGen administration is matched with the applicable bioanalytical assay.

Euglycemic patients with T1DM will be randomized 1:1 in order to evaluate the immunogenicity of dasiglucagon and GlucaGen. The randomized, double-blind, parallel group design, with administration of 3 fixed consecutive doses of randomized trial medication (dasiglucagon or GlucaGen) to the same patient will allow characterization of immunogenic potential for the 2 products. Treatment with the 3 repeated doses (each separated by 1 week), with follow-up visits at Days 35 (where potential immune responses are known to be most pronounced), and at 60 and 104 days after the first dose (following the patient for 2-3 antibody half-lives after expected peak titer), is deemed relevant and sufficient for evaluating any immunogenic response to treatment. Patients that test positive for ADA will be monitored until the ADA levels return to baseline, and samples from the ADA positive patients will be tested for neutralizing potential in an Nab (neutralizing antibody) assay.

Dasiglucagon and GlucaGen will be administered at fixed doses independent of body weight because this is the intended therapeutic dosing regimen in the emergency treatment of hypoglycemia. The selected dose of 1 mg GlucaGen is the recommended dose for treatment of severe hypoglycemia. Based on pre-clinical and clinical studies, it has been demonstrated that 0.6 mg of dasiglucagon results in an initial pharmacodynamic response (i.e. acute glucose mobilization) comparable to 1 mg GlucaGen (see also Section 5.1).

For the safety and well-being of the patients, they will not be brought into hypoglycemia prior to dosing. However, very high blood glucose levels at dosing will potentially impact the reporting of nausea and other associated AE's. Therefore, to enable a more precise safety assessment, patients are required to be dosed while at a normal blood glucose level, and a pre-treatment plasma glucose level of 70-150 mg/dL will be targeted. Plasma glucose levels may be adjusted by s.c. administration of a fast-acting insulin analog or by glucose ingestion. Even with this precaution, it cannot be excluded that a higher frequency of nausea may be anticipated, if this AE is caused by hyperglycaemia.

The safety profile described to date does not give rise to specific safety concerns. In previous studies, dasiglucagon was associated with the AE's nausea, a known side effect following administration of glucagon, headache, and injection site reactions (erythema).

### 7.3 Selection of trial population

Dasiglucagon is indicated for treatment of severe hypoglycemia in patients with T1DM. Given the role of the immune system in the pathogenesis of T1DM, the present trial aims to evaluate the immune response of patients with T1DM taking repeated single doses of s.c. dasiglucagon and GlucaGen.

There are no data indicating an altered immune response with varying blood glucose levels, therefore, for the safety and well-being of the patients, they will not be brought into a hypoglycemic state prior to dosing. Patients with previous exogenic glucagon exposure will not be excluded from the trial, but the information on previous glucagon administration will be recorded to enable subgroup analyses.

The trial will enroll patients in centers in the EU, in the US, and in Canada.

### 7.3.1 Inclusion criteria

To be included in the trial, patients have to fulfill all of the following criteria:

(1) Informed consent obtained before any trial-related activities (trial-related activities are any procedure that would not have been performed during normal management of the patient)
(2) Availability for the entire trial period
(3) Age between 18 and 70 years, both inclusive
(4) Male or female patients with T1DM for at least 1 year. Diagnostic criteria as defined by the American Diabetes Association
(5) Hemoglobin $A_{1c}$ ($HbA_{1c}$) <10%
(6) Stable antidiabetic treatment for at least 1 month (e.g. within 10% insulin dose adjustment)
(7) A female participant must meet 1 of the following criteria

   a. Participant is of childbearing potential and agrees to use one of the accepted contraceptive regimens throughout the entire duration of the trial from screening until last follow-up visit. An acceptable method of contraception includes at least one of the following:

      i. Abstinence from heterosexual intercourse

      ii. Systemic contraceptives (birth control pills, injectable/implant/ insertable hormonal birth control products, transdermal patch); if the participant is using systemic contraceptives, she must use an additional form of acceptable contraception (iii or iv, below)

      iii. Intrauterine device (with and without hormones)

      iv. condom with spermicide

   or

   b. Participant is of non-childbearing potential, defined as surgically sterile (i.e. has undergone complete hysterectomy, bilateral oophorectomy, or tubal ligation), or in a menopausal state (at least 1 year without menses).

(8) A male must be surgically sterilized or willing to refrain from sexual intercourse from screening and until last follow-up visit or, if sexually active, uses a condom and partner practices contraception during the trial from screening and until the last follow-up visit.

### 7.3.2 Exclusion criteria

Patients meeting any of the following criteria during screening evaluations will be excluded from trial participation:

(1) Previous administration of dasiglucagon (previously referred to as ZP4207).
(2) Known or suspected allergy to trial medication(s) or related products
(3) History of anaphylaxis or symptoms of severe systemic allergy (such as angioedema)
(4) Previous participation (randomization) in this trial
(5) Females who are pregnant according to a positive pregnancy test, actively attempting to get pregnant, or are lactating
(6) Patients on a closed loop artificial pancreas
(7) Receipt of any investigational drug within 3 months prior to screening
(8) Active malignancy within the last 5 years
(9) Congestive heart failure, New York Heart Association class II-IV

(10) Inadequately treated blood pressure as defined as systolic blood pressure ≥160 mmHg or diastolic blood pressure ≥90 mmHg at screening.
(11) Current bleeding disorder, including use of anticoagulant treatment
(12) Known presence or history of pheochromocytoma (i.e. adrenal gland tumor) or insulinoma (i.e. insulin-secreting pancreas tumor)
(13) Known or suspected HIV infection
(14) Use of a systemic beta-blocker drug, indomethacin, warfarin or anticholinergic drugs in the previous 28 days before Day 1 of this trial)
(15) Use of systemic corticosteroids, anti-inflammatory biological agents, kinase inhibitors or other immune modulating agents within the last 3 months prior to screening
(16) Aspartate aminotransferase (AST) or alanine aminotransferase (ALT) >2.5 X the upper limit of normal (ULN), bilirubin >1.5 X ULN, estimated glomerular filtration rate (eGFR) <30 mL/min/1.73m$^2$ according to the Modification of Diet in Renal Disease (MDRD) Study definition. Altered electrolytes values of clinical relevance for cardiac conduction, as judged by the investigator.
(17) Clinically significant abnormal ECG at screening, as evaluated by Investigator
(18) Donation of blood or plasma in the past month, or in excess of 500 mL within 12 weeks prior to screening
(19) A positive result in the alcohol and/or urine drug screen at the screening visit. Significant history of alcoholism or drug abuse as judged by the investigator or consuming more than 24 g alcohol per day for men, or more than 12 g alcohol per day for women.
(20) Patients with mental incapacity or language barriers that preclude adequate understanding or cooperation, who are unwilling to participate in the trial, or who in the opinion of the Investigator should not participate in the trial
(21) Surgery or trauma with significant blood loss within the last 2 months prior to screening
(22) Any condition interfering with trial participation or trial endpoints or that could be hazardous to the patient
(23) Use of prescription or non-prescription medications known to cause QT prolongation

### 7.3.3 Premature removal from trial

Participation in the trial is strictly voluntary. A patient has the right to withdraw from the trial at any time and for any reason. If he/she chooses to withdraw, the investigator must be informed immediately. The investigator has the right to terminate participation of any patient at any time if the investigator deems it in the patient's best interest. The reason and circumstances for premature discontinuation will be documented in the electronic Case Report Form (eCRF).

#### 7.3.3.1 Possible reasons for patient discontinuation

A patient will be discontinued if the following applies:

- If a protocol deviation occurs which, in the clinical judgment of the Investigator, can invalidate the assessment of ADA responses to dasiglucagon or glucagon, the patient will be withdrawn by the Investigator
- AEs that are considered unacceptable by the patient or the Investigator
- Positive result from an urine drug screen test
- Pregnancy. If a female patient becomes pregnant in the time between the screening visit and any one of the dosing visits

If discontinuation occurs following administration of any trial medication, the patient will be asked to return and participate in the complete follow-up visits at trial Day 35, 60, and 104.

If trial participation is terminated due to an AE possibly related to the trial medication (including reference product) or trial examinations, the patient must be followed up by additional examinations according to the medical judgment of the investigator until the abnormal condition is resolved or the investigator deems further observations or examinations to be no longer medically indicated.

Patients, who meet one or more of the following dosing day exclusion criteria at a dosing visit, will be excluded from the dosing visit, but can be rescheduled to one of the following days (maximum 3 days postponement). Each dosing visit can only be rescheduled once.

- Strenuous exercise within 4 days prior to dosing, as judged by the Investigator. Strenuous exercise is not allowed during the treatment period of the trial
- Clinically significant illness that may interfere with trial objectives or impose a risk to patients, as judged by the Investigator
- Consumption of alcohol within 24 h prior to dosing visit, or positive results from an alcohol breath test
- Changes in medical history or concomitant medication resulting in fulfillment of clinical exclusion criteria, as judged by the Investigator

A total of 90 patients must complete the trial. To qualify as completed, the patient must be dosed according to protocol and have blood drawn for the ADA analyses described in the protocol. Discontinued patients will be replaced in order to reach 90 completed patients.

#### 7.3.3.2 Center discontinuation

The center can be closed and the trial terminated for the following reasons:

- The center is unlikely to be able to recruit sufficient patients within the agreed time frame
- The center does not respond to trial management requests
- Repeat protocol violations

#### 7.3.3.3 Trial termination

The sponsor reserves the right to modify or terminate the trial at any time. Possible reasons for termination are:

- Safety reasons – the incidence of AEs in this or any other trial using the same trial medication indicates a potential health risk for the patients.
- New scientific knowledge becomes known that makes the objectives of the trial no longer feasible/valid
- Unsatisfactory enrolment of patients

### 7.3.4 Replacement of patients

Patients prematurely withdrawn from the trial will be replaced in order to reach 90 completed patients.

## 7.4 Investigational medicinal product(s)

### 7.4.1 Identity of investigational medicinal product(s)

The identity of the investigation products is summarized in Table 7-1.

**Table 7-1: Identity of investigational products**

| | **Test product** | **Reference product** |
|---|---|---|
| Name | Dasiglucagon | GlucaGen® |
| Active substance | ZP4207 | Recombinant glucagon hydrochloride |
| Formulation | Liquid formulation, 0.6 mL | Powder and solvent for reconstitution as 1 mL solution for injection |
| Strength | 1 mg/mL | 1 mg |
| Container | Single use pre-filled syringe | Powder and solvent for reconstitution packed together in a plastic box. A “hypo-kit” |
| Manufacturer | Zealand Pharma A/S, Glostrup (Copenhagen), Denmark | Novo Nordisk A/S, Bagsværd, Denmark |
| Storage requirements | Store between 2 and 8°C | Store between 2 and 8°C |

Handling, preparation and administration of trial medication will be done by unblinded trial personnel. All trial assessments on the trial site will be done by blinded trial personnel. However, exposure assessments and ADA assessments will be performed by unblinded personnel at the specialty laboratories, to make sure that dasiglucagon or GlucaGen administration is matched with the applicable bioanalytical assay.

The test product will be packed by the sponsor. The information on the labels will be in the local language. The label on the product will be compliant with local laws and regulations.

### 7.4.2 Treatments administered

Dasiglucagon is a stable peptide analog of human glucagon in a ready-to-use liquid formulation indicated for treatment of severe hypoglycemia in insulin dependent patients with diabetes mellitus. Dasiglucagon is in clinical development and has no marketing authorization as yet. GlucaGen is approved in the EU and US and is indicated for treatment of severe hypoglycemic reactions, which may occur in the management of insulin-treated children and adults with diabetes mellitus.

Patients in this trial have not previously been treated with dasiglucagon (ZP4207) and will be randomly assigned (1:1) to receive 1 of the following trial medications:

- 0.6 mg dasiglucagon
- 1 mg GlucaGen

Prior to administration of trial medication at all dosing visits patients must reach a target plasma glucose level of 70-150 mg/dL. If the patient presents with plasma glucose > 150 mg/dL, corrective amounts of fast acting insulin will be administered at the investigator’s discretion. Plasma glucose measured after 20 and after 40 minutes should ensure that the patient has reached the targeted 70-150 mg/dL. If the patient presents with plasma glucose < 70 mg/dL glucose ingestion will be provided in order to reach target plasma glucose of 70-150 mg/dL documented by plasma glucose measurements after 20 and 40 minutes, respectively.

Following the first (Visit 2) and third (Visit 4) dose administration, patients must be fasting for 90 min after dosing. Following the 90-min blood sample draw after the administration of the first (Visit 2) and third (Visit 4) dose administration, patients may be treated individually in order to alleviate any potential side effects in order to minimize prematurely treatment discontinuation and consequently reduce the amount of missing data. This treatment can also be instituted immediately after the second dose administration (Visit 3), as pharmacodynamics will not be assessed at this visit.

In order to minimize the number of patients discontinuing treatment prematurely and consequently reduce the amount of missing data, the following treatment modalities may be used, as considered appropriate by the investigator:

At visit 2 and 4:

- 90 min after dosing patients will be allowed to eat and drink moderately to make them feel comfortable
- 90 min after dosing a moderate and individualized corrective dose of insulin to convert the induced hyperglycemia to euglycemia, after agreement with the investigator
- antiemetic treatment in the form of metoclopramide (Primperan®) as per local label, administered before or after dosing

At visit 3:

- patients will be allowed to eat and drink moderately to make them feel comfortable
- a moderate and individualized corrective dose of insulin to convert the induced hyperglycemia to euglycemia, after agreement with the investigator
- antiemetic treatment in the form of metoclopramide (Primperan®) as per local label, administered before or after dosing

Patients must be monitored for at least 5 h after dosing at the clinical site for safety observations, including blood glucose monitoring.

Patients will not be discharged until they are considered stable and with a blood glucose level in the range of 70-180 mg/dL. Before discharge, the investigator will provide instructions to the patients on management of their blood glucose levels. Each trial medication will be administered s.c. 3 times in total, with 7 days between dosing (i.e. dosing occurs at Days 0, 7, and 14; Visits 2, 3, and 4). Should the patient experience vomiting following the first or second dasiglucagon or GlucaGen administration, the investigator must ensure normal electrolytes prior to the next dosing day. An electrolyte imbalance can be corrected by administration of an electrolyte supplement or by any other treatment modality considered appropriate by the investigator.

### 7.4.3 Selection of doses in the trial

The selected dose of 1 mg GlucaGen is the recommended dose for treatment of severe hypoglycemia. Based on pre-clinical and clinical studies, it has been demonstrated that 0.6 mg of dasiglucagon results in an initial pharmacodynamic response (i.e. acute glucose mobilization) comparable to 1 mg GlucaGen.

### 7.4.4 Treatment compliance

All trial medications will be prepared and administered by unblinded trial personnel.

#### 7.4.5 Method of assigning patients to treatments or treatment sequences

Patients who meet all inclusion and none of the exclusion criteria and have given written informed consent will be randomized in a 1:1 ratio to either dasiglucagon or GlucaGen via an Interactive Web Response System (IWRS) that will assign a kit number to one of the 2 aforementioned treatment arms.

Patients with previous exogenic glucagon exposure will not be excluded from the trial, but the information on previous glucagon administration will be recorded, to enable subgroup analyses.

In the event of an emergency, e.g., when it becomes necessary for the investigator to know which treatment the patient is taking, the code for that patient can be broken by the investigator, preferably after consultation with the medical monitor. Emergency code breaks can be performed using the IWRS.

#### 7.4.6 Blinding

This is a double-blind trial. Since dasiglucagon is available as a liquid formulation and GlucaGen is available as a powder for reconstitution, and they are therefore not identical in appearance, unblinded trial personnel will be responsible for handling, preparing according to the prescription from the IWRS, administering the trial medication and keep the records strictly confidential and accessible only for unblinded staff until after database lock. To maintain double-blind conditions, all trial assessments at the trial site will be done by blinded trial personnel not involved in the administration of trial medications. However, exposure assessments and ADA assessments will be performed by unblinded personnel at the specialty laboratories, to make sure that dasiglucagon or GlucaGen administration is matched with the applicable bioanalytical assay.

#### 7.4.7 Drug accountability and disposal

Handling, preparation and administration of trial medication will be done by unblinded trial personnel. Each center will keep accurate records of the trial supplies received, stored, and dispensed, using appropriate forms. The trial supplies will be stored under appropriate conditions, locked and with restricted access.

All unused supplies and all empty and partially empty containers of trial medication will be stored until the trial closure visit has been performed and then sent to the sponsor.

#### 7.4.8 Prior and concomitant therapy

Prior glucagon exposure will be recorded in the eCRF at screening. All concomitant medications will be recorded in the eCRF at each visit.

Patients using any new concomitant medication resulting in fulfillment of a dosing day exclusion criterion will be excluded from the dosing visit, but can be rescheduled to one of the following days. Each dosing visit can only be rescheduled once. See Section 7.3.3.1 for possible reasons for patient discontinuation.

#### 7.4.9 Treatment after end of trial

Not applicable in this trial.

### 7.5 Assessments and schedule of measurements (overview)

The following assessments and measurements will be carried out at the times specified in the trial flow chart (Table 2-1).

Informed consent will be obtained prior to any trial-related procedures; see Section 11.3.

### 7.5.1 Screening examination

At screening (Visit 1), the following assessments will take place:

- Informed consent
- Check of patient eligibility
- Demographics
- Body measurement
- Medical history
- History of alcohol/drug abuse
- Concomitant illnesses
- Prior medications
- Concomitant medications
- Physical examination
- Vital signs
- ECG
- AEs
- Hematology, biochemistry, coagulation
- Pregnancy test (women of childbearing potential only)
- Urinalysis
- Urine drug screen (trial site testing)
- Alcohol breath test

### 7.5.2 Instructions to patients prior to dosing

On dosing days, patients are allowed to consume a small meal for breakfast with corresponding administration of rapid acting insulin to address the meal and in accordance with their normal management of blood glucose levels. At visit 2 and visit 4 the patient should aim to have a similar sized breakfast and corresponding rapid acting insulin dose.

Use of long-acting insulin and rate of basal continuous subcutaneous insulin infusion (CSII) will be continued according to normal glycemic management of the individual patients during dosing days.

### 7.5.3 Procedures and assessments during the treatment period of the trial

At Visit 2 (Day 0) patient eligibility is rechecked (check of changes between the screening visit and Visit 2) and patients eligible to participate will be randomized to treatment with dasiglucagon or GlucaGen.

Additionally, during the treatment period of the trial, at Visits 2 and 4 (Days 0 and 14), the following assessments will take place:

- Concomitant medication
- Check of withdrawal criteria
- Check of dosing day exclusion criteria
- Vital signs (pre-dose, and at 30, 90 and 120 min post-dosing)
- ECG (pre-dose, and at 20, 35, 45 and 60 min post-dosing)
- Local tolerability (at 0.5 and 2 h post-dosing)
- AEs
- Hematology and biochemistry (pre-dose, and at 30 and 90 min post-dosing)
- Urinalysis (pre-dose)

- Urine drug screen (pre-dose) at trial site
- Urine pregnancy test (pre-dose) at trial site (women of childbearing potential only)
- Alcohol breath test (pre-dose)
- Dasiglucagon/glucagon plasma concentrations
  (Pre-dose, 5, 10, 30, 60, and 90 min post-dosing. The actual time of blood sampling for exposure should not deviate from the nominal time by more than ±1 min. Pre-dose is defined as within 5 min prior to dosing.)
- Plasma glucose concentration
  (Pre-dose, 5, 10, 30, 60, and 90 min post-dosing. The actual time for blood sampling for plasma glucose should not deviate from the nominal time by more than ±1 min. Pre-dose is defined as within 5 min prior to dosing.)
- Antibodies against dasiglucagon/glucagon (pre-dose)
- Administration of trial medication
  Prior to administration of trial medication patients must reach a target plasma glucose level of 70-150 mg/dL. If the patient presents with plasma glucose > 150 mg/dL, corrective amounts of fast acting insulin will be administered at the investigator's discretion. Plasma glucose measured after 20 and after 40 minutes should ensure that the patient has reached the targeted 70-150 mg/dL. If the patient presents with plasma glucose < 70 mg/dL glucose ingestion will be provided in order to reach target plasma glucose of 70-150 mg/dL documented by plasma glucose measurements after 20 and 40 minutes, respectively . Patients must be fasting for 90 min after administration of trial medication, and can be treated individually to alleviate any potential side effects. See section 7.4.2. Patients will be observed for at least 5 h post-dose.

At Visit 3 of the treatment period (Day 7), only the following assessments will take place

- Concomitant medication
- Check of withdrawal criteria
- Check of dosing day exclusion criteria
- Vital signs (pre-dose, and at 30, 90 and 120 min post-dosing)
- ECG (pre-dose, and at 20, 35, 45 and 60 min post-dosing)
- Local tolerability (at 0.5 and 2 h post-dosing)
- AEs
- Urine drug screen (pre-dose) at trial site
- Urine pregnancy test (pre-dose) at trial site (women of childbearing potential only)Alcohol breath test (pre-dose)
- Antibodies against dasiglucagon/glucagon (pre-dose)
- Administration of trial medication
  Prior to administration of trial medication patients must reach a target plasma glucose level of 70-150 mg/dL. If the patient presents with plasma glucose > 150 mg/dL, corrective amounts of fast acting insulin will be administered at the investigator's discretion. Plasma glucose measured after 20 and after 40 minutes should ensure that the patient has reached the targeted 70-150 mg/dL. If the patient presents with plasma glucose < 70 mg/dL glucose ingestion will be provided in order to reach target plasma glucose of 70-150 mg/dL documented by plasma glucose measurements after 20 and 40 minutes, respectively. Patients are NOT required to be fasting after administration of trial medication, and can be treated individually to alleviate any potential side effects immediately following dosing. See section 7.4.2. Patients will be observed for at least 5 h post-dose.

### 7.5.4 Follow-up period of the trial

After the final dose of trial medication (at Visit 4, Day 14, described above), patients will be followed up until Day 104 (Visit 7). During the follow-up period, visits take place at Days 35, 60, and 104 (Visits 5, 6, and 7).

At Visits 5 and 6 during the follow-up period, the following assessments will take place:

- Concomitant medication
- Check of withdrawal criteria
- AEs
- Antibodies against dasiglucagon/glucagon

Additionally, the following assessments will take place at Visit 5 only:

- Vital signs
- ECG
- Hematology and biochemistry

At Visit 7, the final visit of the follow-up period and of the trial (EoT visit), the following assessments will take place:

- Concomitant medication
- Physical examination
- Vital signs
- ECG
- AEs
- Hematology and biochemistry
- Urinalysis
- Antibodies against dasiglucagon/glucagon

### 7.5.5 Final examination at the end of the trial

The final visit of the trial is Visit 7 (Day 104 of the follow-up period; EoT visit). See Section 7.5.4 for further details.

### 7.5.6 Additional (safety) examinations

If there are any unclear symptoms or observations the responsible physician in charge may perform further medical examinations, other than outlined in this protocol, including further clinical laboratory tests, in order to clarify the relevance or to diagnose symptoms.

### 7.5.7 Safety laboratory tests

Samples obtained will be prepared and transferred to the appropriate laboratory by SynteractHCR according to Standard Operating Procedures. The parameters listed in Section 7.6.4.1 will be determined using standard methods.

The total volume of blood sampled per patient for safety analyses is 68 mL.

## 7.6 Immunogenicity, pharmacokinetic, pharmacodynamic, tolerability and safety measurements

Details of sampling for immunogenicity testing, plasma glucose concentrations, plasma trial medication concentrations, and safety laboratory testing are provided in a laboratory manual (see the Investigator site file).

### 7.6.1 Immunogenicity measurements

Antibodies against dasiglucagon/glucagon will be measured at all visits after screening. During the treatment period (Visits 2, 3, and 4) samples will be collected pre-dose.

The clinical ADA assays have been validated in accordance with existing guidelines and recommendations.[6,7,8,9,10]

Confirmed positive anti- dasiglucagon antibody samples, (treatment-induced or treatment-boosted) from anti-dasiglucagon antibody-positive patients will be evaluated for binding titer neutralizing potential and titer as well as cross-reactivity towards endogenous glucagon.

No further serum sampling will be needed as the ADA samples can be used for Nab analysis.

The *in vitro* neutralizing effect of antibodies will be measured using an assay[9,12] based on glucagon receptor transfected human embryonic kidney cells. The calculated sensitivity in previous studies was about 51.8 ng/mL. The assay was also validated for recombinant glucagon with similar results.[11,12] In case of a positive result in the Nab assay, a titer estimation will be performed. The cell-based Nab analyses will be performed by a special laboratory, BioAgilytix, Durham, NC, USA.

*In vivo* neutralizing effect will be evaluated by comparing PK and PD endpoints between ADA positive and ADA negative patients, further investigation may be performed by correlating ADA titer with PK and PD endpoints.

Residual and additional antibody serum samples may be stored until approval of market authorization by the health authorities. Further characterization of the antibody response may be requested by the health authorities.

### 7.6.2 Plasma concentrations of dasiglucagon and GlucaGen

The exposure to trial medication (dasiglucagon or GlucaGen) will be assessed based on plasma concentration data ($AUC_{0\text{-}30min}$, $AUC_{0\text{-}90\ min}$, $C_{max}$, $t_{max}$) from samples collected at Visits 2 and 4 (after administration of the first and third doses of trial medication).

Samples will be collected pre-dose, and at 5, 10, 30, 60, and 90 min post-dosing. The actual time of blood sampling for exposure should not deviate from the nominal time by more than ±1 min. Pre-dose is defined as within 5 min prior to dosing.

### 7.6.3 Pharmacodynamic measurements

The plasma glucose profile will be assessed based on plasma concentration data ($AUE_{0\text{-}30min}$, $AUE_{0\text{-}90\ min}$, $CE_{max}$, $t_{max}$) from samples collected at dosing Visits 2 and 4 (first and third dosing visit).

Samples will be collected pre-dose, and at 5, 10, 30, 60, and 90 min post-dosing. The actual time of blood sampling for evaluation of plasma glucose should not deviate from the nominal time by more than ±1 min. Pre-dose is defined as within 5 min prior to dosing.

### 7.6.4 Safety and tolerability measurements

#### 7.6.4.1 Safety laboratory tests

Routine safety laboratory tests will be performed centrally. Samples for safety laboratory parameters will be collected at Visits 1, 2, 4, 5, and 7. Samples for urinalysis will be collected at Visits 1, 2, 4, and 7. The following parameters will be determined:

- Clinical chemistry: sodium, potassium, calcium, glucose, urea, creatinine, total bilirubin, AST (SGOT), ALT (SGPT), gamma-GT, alkaline phosphatase, total protein, C-reactive protein, $HbA_{1c}$, C-peptide

- Hematology: hemoglobin, red blood cell count (erythrocytes), hematocrit, platelet count (thrombocytes), total white blood cell count (leucocytes)
- Coagulation: international normalized ratio (INR), fibrinogen (at screening visit only)
- Urinalysis: pH, blood (leukocytes and erythrocytes), protein, glucose, ketones, nitrite

A pregnancy test will be performed at screening (Visit 1) and pre-dose at the three dosing visits (Visit 2, 3 and 4) for women of childbearing potential only.

Alcohol breath tests and urine drug screen (amphetamine, cocaine, MDMA, methamphetamine, opiate/morphine, marihuana) will be performed at Screening (Visit 1) and at Visits 2, 3, and 4.

For further details, please refer to the laboratory manual.

#### 7.6.4.2 Safety examinations

Physical examination is performed at Screening (Visit 1) and EoT (Visit 7).

AEs are assessed at all visits. Local tolerability is assessed at all dosing visits (Visits 2, 3, and 4). ECG is assessed at Screening (Visit 1) Visits 2, 3, and 4 and vital signs are assessed at Screening (Visit 1) Visits 2, 3, 4, 5, and 7.

- Physical examination includes examination of the following body systems: head, ears, eyes, nose, throat (HEENT), including the thyroid gland; heart, lung, chest; abdomen; skin; musculoskeletal system; nervous system; lymph node
- Vital signs include: pulse rate and blood pressure in a sitting position after 5 min, body temperature
- Local tolerability: skin reactions will be assessed at the injection site at 0.5 and 2 h post-dosing. Skin reactions will be reported as AEs (see Section 8).
- 12-lead ECG. Details from ECG assessments will be recorded, including PR, QRS and QT intervals.

## 8. Adverse events

### 8.1 Definitions

**Adverse Event (AE)**

An AE is any untoward medical occurrence in a trial patient administered an investigational medicinal product (IMP) and which does not necessarily have a causal relationship with the treatment. An AE can therefore be any unfavorable and unintended sign (for example, an abnormal laboratory finding), symptom, or disease temporally associated with the use of an IMP, whether or not considered related to the IMP.

Note: This includes events from the first trial related activity after the patient has signed the informed consent.

AEs include:

- A clinically significant worsening of a concomitant illness.
- A clinical laboratory abnormality which is clinically significant, i.e. any abnormality that suggests a disease and/or organ toxicity and is of a severity that requires active management. Active management includes active treatment or further investigations, for example change of medicine dose or more frequent follow-up due to the abnormality.
- Hypoglycemic episodes (see Section 8.4)
- Injection site reactions

The following should not be recorded as AEs, if recorded at screening (on Screening Form or CRF):

- Pre-planned procedure, unless the condition for which the procedure was planned has worsened from the first trial related activity after the patient has signed the informed consent.
- Pre-existing conditions found as a result of screening procedures (pre-existing conditions should be reported as medical history or concomitant illness).

**Serious adverse event (SAE)**

A SAE is any untoward medical occurrence that at any dose:

- results in death
- is life-threatening
- requires inpatient hospitalization or prolongation of existing hospitalization
- results in persistent or significant disability or incapacity
- is a congenital anomaly or birth defect
- is medically important
  Medical judgement must be exercised in deciding whether an AE is believed to be 'medically important'. Medically important event may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above.

**Suspected unexpected serious adverse reactions (SUSAR)**

An AE, fulfilling one of the criteria of seriousness and being assessed as related to IMP application, the nature or severity of which is not consistent with the applicable reference document (e.g. ZP4207 IB[3] or package leaflet/SmPC for an approved product such as GlucaGen[13]).

**Clinical event of interest**

A clinical event of interest is an event which, in the evaluation of safety, has a special focus (e.g. required by health authorities).

In this trial hemodynamic changes, as defined below, are considered clinical events of interest:

- Post-dose clinical signs or measured vital signs indicating a clinical significant drop in blood pressure including signs of orthostatic hypotension, vasovagal responses or bradycardia.
- Post-dose change in pulse or blood pressure considered an event of hypo- or hypertension as judged by the investigator.

**Intensity of an adverse event**

The maximum intensity (severity) of all AEs must be assessed by the Investigator and documented. Severity should be graded when the AE outcome is known:

Mild: A type of adverse event that is usually transient and may require only minimal treatment or therapeutic intervention. The event does not generally interfere with usual activities of daily living.

Moderate: A type of adverse event that is usually alleviated with additional specific therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort but poses no significant or permanent risk of harm to the research participant.

Severe: A type of adverse event that interrupts usual activities of daily living, or significantly affects clinical status, or may require intensive therapeutic intervention.

A ‘severe’ reaction does not necessarily deem the AE as ‘serious’ and a SAE may not be ‘severe’ in nature.

**Causality relationship to trial medication**

The causality of each AE should be assessed by the Investigator according to the following classification:

Probable: Good reason and sufficient documentation to assume a causal relationship.

Possible: A causal relationship is conceivable and cannot be dismissed.

Unlikely: The event is most likely related to etiology other than the trial product.

Not related: No relationship to trial product

**Outcome of an adverse event**

The outcome of all AEs must be assessed by the Investigator and documented by his/her staff. The following definitions should be used:

| | |
|---|---|
| Recovered/resolved: | The patient has fully recovered, or by medical or surgical treatment the condition has returned to the level observed at the first trial-related activity after the patient signed the informed consent. |
| Recovering/resolving: | The condition is improving and the patient is expected to recover from the event. This term is only applicable if the patient has completed the trial. |
| Recovered/resolved with sequelae: | The patient has recovered from the condition, but with lasting effect due to a disease, injury, treatment or procedure. If a sequela meets an SAE criterion, the AE must be reported as an SAE. |
| Not recovered/ not resolved: | The condition of the patient has not improved and the symptoms are unchanged. |
| Fatal: | This term is only applicable if the patient died from a condition related to the reported AE. Outcomes of other reported AEs in a patient before he/she died should be assessed as "recovered/resolved", "recovering/resolving", "recovered/ resolved with sequelae" or "not recovered/not resolved". An AE with fatal outcome must be reported as an SAE. |
| Unknown: | This term is only applicable if the patient is lost to follow-up. |

## 8.2 Collection, recording and reporting of adverse events

All events meeting the definition of an AE must be collected and reported from the first trial related activity after the patient has signed the informed consent until the end of the post-treatment follow-up period. At each contact with the site (visit or telephone, excluding visits, where the patient is not seeing the Investigator or his staff (e.g. visits to the laboratory)) the patient must be asked about AEs. All AEs, either observed by the Investigator or reported by the patient, must be recorded by the Investigator and evaluated.

The Investigator should record the diagnosis, if possible. If no diagnosis can be made the Investigator should record each sign and symptom as individual AEs.

All AEs must be recorded by the Investigator. One single Adverse Event Form must be used per AE from start to resolution. For SAEs, the Serious Adverse Event Form must also be completed. For clinical events of interest, the Clinical Event of Interest Form must also be completed.

AE information should include the following:

- Date and time of onset and resolution
- Date and time of Investigator's first information on the (S)AE
- Seriousness
- Severity
- Causal relationship with IMP
- Interruption or withdrawal of treatment with IMP and other measures taken
- Outcome

All AEs are coded; details are described in the trial specific Data Management Plan.

If an event classifies as a clinical event of interest, the Investigator must tick the Clinical Event of Interest box on the AE form and complete the Clinical Event of Interest Form. The Clinical Event of Interest form will capture if the event was associated with any signs or symptoms and capture the highest/lowest blood pressure and pulse measured during the event.

The Investigator must report initial information in writing (fax or email) on all SAEs to the Sponsor's responsible pharmacovigilance unit (Safety CRO) immediately (within 24 hours) after obtaining knowledge about the event. Please refer to Appendix 1 for contact details. The Safety CRO will inform the medical monitor and the sponsor about the reported SAEs.

It is the responsibility of the Safety CRO to report all SUSARs that occur in this trial to the Competent Authorities and IRBs/IECs in accordance with the local requirements in force and ICH guideline for GCP. The trial monitor must be informed accordingly.

### 8.3 Follow-up of adverse events

All AEs that are ongoing at the end of the patient's participation in the study will be followed-up until the event is resolved or reaches a satisfactory outcome as deemed by the Investigator.

Follow-up actions for all SAEs will be performed according to appropriate clinical care practices and may depend on the nature of the event. These will be determined after internal review and/or sponsor review.

The follow up information should only include new (updated and/or additional) information that reflects the situation at the time of the Investigator's signature.

Follow-up information on (S)AEs will be updated using the (S)AE Form. If a non-serious event becomes serious during the follow-up the AE Form and SAE Form have to be used and reporting timelines follow those of a SAE.

The Investigator must forward follow-up information on SAEs and if previously non-serious AEs become SAEs to the Sponsor immediately (within 24 hours) after obtaining knowledge about the new information.

### 8.4 Hypoglycemia

Hypoglycemia will be regarded as an AE and recorded and documented on an AE Form.

Hypoglycemia is defined as a fall of plasma glucose below 3.9 mmol/L (70 mg/dL).

During the dosing visits, mild to moderate symptoms of hypoglycemia, or a blood glucose (BG) value less than a threshold of <2.8 mmol/L (50 mg/dL), corresponding to a plasma glucose (PG) value of <3.1 mmol/L (56 mg/dL), will be treated by i.v. glucose solution at the investigator's discretion according to best available medical practice. Treatment is to be repeated until BG

value stabilized above the threshold limit again. BG measurements will only be done due to safety concerns. Insulin-induced hypoglycemia should be recorded as an AE.

### 8.5 Pregnancy

Female patients must be instructed to notify the Investigator immediately if they become pregnant or if they suspect to be pregnant during the trial. The Sponsor has a responsibility to monitor the outcome of all pregnancies reported during the clinical trial.

Male patients must be instructed to notify the Investigator immediately if their partner becomes pregnant or suspects to be pregnant. The Sponsor has a responsibility to monitor the outcome of all pregnancies reported during the clinical trial. During an information meeting at the trial site, the patient's partner will be fully informed by a physician of her participation in capturing the outcome data, and will be asked to provide her written consent.

The Investigator must report all information on pregnancies on the initial pregnancy form. The completed initial pregnancy form must be forwarded to the Sponsor according to the procedure stated in Section 8.2. Any (S)AEs in the mother, as well as other pregnancy complications (ectopic pregnancy) fulfilling the criteria of an (S)AE, must be reported on the (S)AE form.

The following must be collected in the initial pregnancy form:

- Medical history of the mother
- Family history
- Course of the pregnancy, including expected delivery date

The Investigator must follow the pregnancy until the pregnancy outcome and the newborn infant(s) until the age of 1 month. The Investigator must collect information on the pregnancy and pregnancy complications as well as the pregnancy outcome including the health of the newborn infant(s) on the pregnancy outcome form. The completed pregnancy outcome form must be forwarded to the Sponsor according to the procedure stated in Section 8.2. Any (S)AEs in the newborn must be reported on the (S)AE form.

The SAEs that must be reported including abnormal outcome - such as congenital anomalies, fetal death and termination of pregnancy (spontaneous or elective abortion), including any anomalies of the fetus observed at gross examination or during autopsy.

The following must be collected in the pregnancy outcome form:

- Course of the pregnancy
- Outcome of the pregnancy
- Condition of the newborn
- Any AEs in the newborn infant must be followed till the age of 1 month

### 8.6 Precautions

Normal precautions taken for a human trial, including the provision of emergency equipment, will be taken during this trial. Qualified and well trained physicians and medical staff will instruct the patients. During a patient's participation in the trial, the Investigator should ensure that adequate medical care is provided to the patients for any AEs, including clinically significant laboratory values related to the trial. The Investigator should inform the patient when medical care is needed for intercurrent illnesses of which the Investigator becomes aware.

For further information on safety precautions for ZP4207 and GlucaGen, please refer to the current version of the Investigator's Brochure[3] and the SmPC for GlucaGen[13], respectively.

### 8.7 Safety Committee

The internal Zealand Pharma Safety Committee (SC) is constituted to perform ongoing safety surveillance of clinical trials with dasiglucagon, including this trial.

If safety signals are observed, either based on reported SAEs, periodic review of laboratory parameters, review of all AEs reported between the SC meetings, or on notification of significant findings, the SC will take appropriate measures to safeguard the patients.

As a minimum the SC convenes every quarter to review relevant safety information, including AEs and laboratory data.

# 9. Data management and quality control

## 9.1 Case report forms

All the information collected during the trial will be recorded in the eCRFs, which are identified by patient number. Suitable eCRFs will be designed by SynteractHCR. The investigator will ensure that the eCRFs are correctly completed. All key pages will be signed or initialed by the investigator, signifying agreement with and responsibility for the recorded data. Key pages are the following: AE-reporting form, trial medication form, concomitant medication form and trial-closure form.

Data directly captured in the eCRF (i.e., data assigned or calculated automatically by the EDC system) is called e-source. This means that these data fields in the CRF are source documents. For the other CRF data fields, which are entered by medical personnel during the trial, the source data will be found in other documents (such as patients' files, worksheets, etc.), i.e. for these CRF data fields the "other" documents are the source documents.

## 9.2 Quality control

The investigator will permit trial-related monitoring, IEC review, and regulatory inspections, providing direct access to source data /documents. Sponsor-authorized quality assurance personnel may carry out audits for which the investigator must provide support.

The trial will be supervised by a monitor from SynteractHCR. The trial monitor will contact the investigator regularly to discuss the progress of the trial and to check the trial documents including the informed consent forms for completeness and consistency.

The trial monitor or a representative of the sponsor will cross-check the data entered in the eCRFs with the source data at the trial site and observe the trial procedures in order to verify adherence to the trial protocol.

The eCRFs will be checked for completeness and correctness by the monitor and data management department of SynteractHCR according to the SynteractHCR SOPs and any queries will be resolved by the investigator.

All of the clinical data will be captured via electronic data capture (EDC) using a web-based tool. The software Marvin from the company XClinical (www.xclinical.com/) is the preferred EDC software. Marvin is compliant with all legislation relevant to electronic data capture (FDA 21 CFR Part 11, GCP).

The investigator site staff will enter and edit the data via a secure network, with secure access features (username and password). A complete electronic audit trail will be maintained. The investigator will approve the data using an electronic signature (Ref: 21 CFR Part 11), and this approval is used to confirm the accuracy of the data recorded.

eCRFs will be used for all patients. The investigator's data will be accessible from the investigator's site throughout the trial. The electronic CRFs must be kept current to reflect patient status at each phase during the course of the trial. The electronic CRF will not capture personalized data. The investigator must make a separate confidential record of personalized details (name and initials) on the patient identification and enrollment log. All changes to data are done by the investigator through the EDC system.

It is the responsibility of the Principal Investigator of the respective site to ensure that all patient discontinuations or changes in trial or other medications entered on the patient's eCRF are also made on the patient's medical records.

The eCRFs for any patient leaving the trial should be completed at the time of the final visit or shortly thereafter.

### 9.3 Data management

Data management will be performed according to SynteractHCR SOPs.

# 10. Statistical methods and determination of sample size

## 10.1 Statistical and analysis plan

A separate Statistical Analysis Plan (SAP) will be finalized that details the planned statistical analysis and may include necessary adaptations to the planned statistical analysis before unblinding of the data.

Any deviations from the planned analyses will be described and justified in the final integrated trial report.

### 10.1.1 General considerations

All data obtained in this trial and documented in the eCRFs will be listed and summarized with statistics or frequency tables as appropriate. In case of termination of the trial, all data collected up to that time point will be included into the analysis.

Raw data listings and summary tables will be generated using the software SAS© Version 9 or higher.

All statistical analysis will be descriptive i.e. no formal testing will be performed.

Continuous variables will be summarized with means, standard deviations, medians, minimums, and maximums and valid cases.

Other summaries (e.g. quartiles, 95% confidence intervals) may be used as appropriate. Categorical efficacy and safety variables will be summarized by counts and by percentage of patients in corresponding categories.

### 10.1.2 Classification of patients to subsets

For the statistical analysis the randomized patients will be divided up into the following datasets:

The following definitions are applicable:

| | |
|---|---|
| **Safety analysis set (SAS)** | all patients who were randomized and received at least one dose of trial medication |
| **Full analysis set (FAS)** | all patients of the SAS with at least one measurement of the ADA titer at baseline |
| **Per Protocol set (PPS)** | all patients of the FAS for whom no relevant protocol deviations were documented |

The analysis of the primary endpoint will be based on the FAS. A secondary analysis of the primary endpoint will be based on the PPS. Safety analysis will be based on the SAS.

The decision whether a protocol deviation is relevant or not for the exclusion of patients from the PPS set will be made case-by-case in a data review meeting.

### 10.1.3 Immunogenicity data

All immunogenicity parameters will be analyzed descriptively per treatment group. No statistical tests are planned. Baseline ADA-positive patients will be calculated as a percentage of the total number of patients whose baseline samples were tested for ADA. Titer will be reported as median and interquartile range.

95% confidence intervals for incidence rates and for rate differences will be given.

Secondary immunogenicity parameters will be described with appropriate descriptive statistics for dichotomous, categorical or continuous variables.

#### 10.1.4 Exposure and PD endpoints

Plasma dasiglucagon and glucagon concentrations 0-90 min from dosing: $AUC_{0\text{-}30min}$, $AUC_{0\text{-}90\ min}$, $C_{max}$, and $t_{max}$ will be summarized with descriptive statistics.

Plasma glucose profiles 0-90 min from dosing: $AUE_{0\text{-}30min}$, $AUE_{0\text{-}90\ min}$, $CE_{max}$, and $t_{max}$ will be summarized with descriptive statistics.

#### 10.1.5 Safety data

**Clinical laboratory data**

Clinical laboratory test results will be marked whether the result is below, within or above the respective reference range. The number of values outside of the reference range will be counted.

**Adverse events**

Adverse events will be tabulated by system organ class (SOC) and preferred term (PT) after medical coding using the Medical Dictionary for Regulatory Activities (MedDRA). AE summary tables will include counts and percentages of patients who experienced adverse events summarized by system organ class (SOC) and preferred term (PT).

**Other safety data**

Vital signs, physical examination, ECG and local tolerability data will be summarized with descriptive statistics.

#### 10.1.6 Further data

Baseline and demographic data will be summarized using descriptive statistics. Baseline ADA-positive patients will be calculated as a percentage of the total number of patients whose baseline samples were tested for ADA.

All other data obtained in this trial and documented in the eCRF will be listed.

#### 10.1.7 Withdrawals, drop-outs and missing data

In the case of drop-outs, no imputation of values for immunogenicity measurements will be done. Analysis is done on valid cases only i.e. no imputation technique like LOCF (last observation carried forward) will be applied.

#### 10.1.8 Baseline and center comparisons

Demographic and other baseline characteristics will be compared.

#### 10.1.9 Subgroup analysis

Subgroup analyses for the primariy endpoint (voerl ADA incidence) by gender, age, and race will be performed.

#### 10.1.10 Interim analysis

No interim analysis is currently planned.

### 10.2 Determination of sample size

The purpose of the present trial is to generate data describing the immunogenic potential of dasiglucagon and GlucaGen. The ADA assays to be used in this trial are both validated, but specific for GlucaGen and dasiglucagon, respectively, and the performance of the assays are thus not directly comparable. As a consequence, a formal comparison or non-inferiority analysis will not be performed

Currently, no ADA incidences have been detected in the completed clinical trials, where up to 5 repeated doses of dasiglucagon have been administered to the same patients within a week. Across the two phase 1 clinical trials and a completed phase 2 PK/PD trial, a total of 141 subjects have been exposed to dasiglucagon and no incidences of ADA development have been observed. The obtained data indicate that dasiglucagon has a low risk for induction of ADAs in the investigated settings and as a consequence a meaningful sample size to compare both treatments cannot be estimated.
The sample size is therefore based on a certain precision of the confidence interval for the overall ADA incidence if no events are observed, respectively to ensure a certain probability for observing one event.

When no events are observed, to obtain an upper bound of 0.050 on the 90.0% confidence interval for the probability of such a rare event, would require a sample size of 45. Respectively, accepting a chance of observing at least one event of 90% and an actual probability of the event of 5% leads to a sample size of 45 patients completing the trial. In order to account for drop-outs, It is expected that 112 patients in total will be randomized and treated.

# 11. Ethics and regulations

## 11.1 Independent ethics committees and competent authorities

The clinical trial authorization (CTA) granted by the competent authority (CA) and a favorable opinion from the relevant independent ethics committee(s) (IEC(s)) /Institutional Review Board (IRB) will be obtained prior to the start of the trial. The local authorities will be notified about the trial as required by law.

The CA and the EC/IRB will be notified about the end of the trial and a report summarizing the trial results will be sent to the CA and the EC within one year after the end of the trial. If the trial is terminated early, the CA and the EC will be notified within 15 days.

The IECs and/or IRBs met the requirements of the International Conference on Harmonisation (ICH) Harmonised Tripartite Guideline for Good Clinical Practice (GCP) and local legislation. They also met the requirements of 21 CFR 312.3.

## 11.2 Ethical conduct of the trial

The trial will be conducted in accordance with the ethical principles set forth in the Declaration of Helsinki (including amendments).

## 11.3 Patient information and consent

Written informed consent will be obtained from all patients prior to entry into the trial. The investigator will explain to each patient orally and in writing (patient information sheet) the nature, significance, risks and implications of the trial before inclusion. In particular, the patients will be informed about the following:

- the possibility of withdrawing from the clinical trial at any time by revoking the consent and without any resulting disadvantage
- how personal and health-related data will be collected and used during the trial
- the patient must be informed that his/ her medical records may be examined by authorized monitors or Clinical Quality Assurance auditors appointed by the Sponsor, by appropriate IEC members, and by inspectors from regulatory authorities.

All patients will receive a copy of the patient information sheet and a copy of their signed and dated informed consent form.

All patients will be insured against injury caused by their participation in the trial according to legal requirements. They will be informed about the insurance and the resulting obligations on their part.

## 11.4 Legal and regulatory requirements

This trial will be carried out in accordance with:

- ICH guidelines for GCP, United States investigational new drug (IND) regulations (21 CFR 312), the regulations on electronic records and electronic signature (21 CFR 11), the most recent guidelines of the Declaration of Helsinki, and the relevant laws and regulations of the country in which the trial takes place.
- Standard operating procedures (SOPs for clinical investigation and documentation in force at SynteractHCR)

# 12. Trial administration

## 12.1 Responsibilities

Zealand A/S is the sponsor of this trial. SynteractHCR, a contract research organization (CRO), will organize the performance of this trial.

A list with the names and addresses of the responsible institutions and persons is provided in Appendix 1 of this protocol.

## 12.2 Protocol deviations

The investigator agrees to conduct the trial in compliance with the protocol. Prospective protocol deviations or waivers will not be granted for this trial.

Any deviation from the clinical trial protocol in the conduct of the clinical trial will be notified to the Monitor on an ongoing basis.

## 12.3 Protocol changes

Amendments to this trial protocol may be made following the procedures specified by local laws and regulations. Substantial amendments to this trial protocol may be implemented only if the approval of the CA(s) and a favorable opinion of the ethics committee(s) have been obtained.

Substantial amendments to the conduct of the clinical trial may arise from changes to the protocol or from new information relating to the scientific documents in support of the trial. Amendments to the trial are regarded as “substantial” where they are likely to have a significant impact on:

- the safety, physical health and mental integrity of the patients;
- the scientific value of the trial;
- the conduct or management of the trial; or
- the quality or safety of any investigational medicinal product used in the trial.

If a new event occurs related to the conduct of the trial or the development of the investigational product, which may affect the safety of the patients, the sponsor and the investigator will take appropriate safety measures to protect the patients against any immediate hazard. The sponsor will immediately inform the CA(s) and ethics committee(s) of the new events and the measures taken.

## 12.4 Publication of results

The original eCRFs and the data generated from the eCRFs or otherwise obtained during the trial under this trial protocol will become the property of the sponsor. Publication of the results of this trial by SynteractHCR or the investigator is possible only after written consent has been obtained from the sponsor. Any material intended for publication will be given to the sponsor at least 4 weeks before submission for publication. The sponsor will have the right to comment on the intended publication and to take any reasonable measures for patent protection.

## 12.5 Clinical trial report

After completion of the trial, the results will be tabulated, evaluated and issued as a complete final clinical trial report according to the ICH-E3 Note for guidance on structure and content of clinical trial reports.

The sponsor will send a summary of this clinical trial report to the EC and CA within one year after the end of the trial.

### 12.6 Retention of trial records

Records and documents pertaining to the conduct of the trial and the distribution of the investigational product (e.g. ICFs, laboratory slips, medication inventory records, and other pertinent information) must be retained by the Investigator according to local requirements.

To meet regulatory requirements, the eCRF data will be electronically stored at sites. The CDISC ODM (see http://www.cdisc.org/ for details) will be used to store and archive all electronic data at the sites. Since CDISC ODM is also the source for the EDC-web-based system, no transcription of data is necessary. CDISC ODM is a platform-independent standardized data format including the complete trial metadata and audit trail. The data can be reviewed at a later stage using off-the-shelf tools. CDISC provides a complete CDISC ODM Viewer for these purposes. If needed, PDF-files can be created from the ODM file.

## 13. References


1. Seaquist ER, Anderson J., Childs B, Cryer P, Dagogo-Jack S, Fish L, et al. Hypoglycemia and diabetes: a report of a workgroup of the American Diabetes Association and the Endocrine Society. Diabetes Care 2013; 36(5):1384-95. doi:10.2337/dc12-2480.
2. American Diabetes Association Position Statement Standards of medical care in diabetes - 2016. Diabetes Care 2016; 39(Suppl. 1):S1-S112.
3. Dasiglucagon Investigator Brochure, Edition 3, 12 Dec 2016. Zealand Pharma A/S.
4. Kedia N. Treatment of severe diabetic hypoglycemia with glucagon: an underutilized therapeutic approach. Diabetes Metab Syndr Obes 2011;4:337-46.
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8. Shankar G, Devanaryan V, Amaravadi L, Barrett YCBowsher R, Finco-Kent D, et al. Recommendations for the validation of immunoassays used for detection of host antibodies against biotechnology products. J Pharm Biomed Anal 2008; 48(5):1267-81.
9. Commissioning and validation of a method for the validation of anti-ZP4207 antibodies in human serum. Zealand Pharma Report, Study 14-173
10. Commissioning and validation of a method for the validation of anti-glucagon antibodies in human serum. Zealand Pharma Report, Study 14-174
11. Bioagilytix Labs. Validation of an Assay to detect neutralizing Antibodies to ZP4207 by Measuring Cyclic Adenosine Monophosphate (cAMP) in Glucagon Receptor Transected Cell Line, study no. BAL-14-206-004-REP. Zealand Pharma, Study 15-039. 09 Aug 2016.
12. Bioagilytix Labs. Validation of an Assay to Detect Neutralizing Antibodies to Glucagon by Measuring Cyclic Adenosine Monophosphate (cAMP) in a Glucagon Receptor Transfected Cell Line, BAL-14-206-005. Zealand Pharma, Study 15-040. 04 Jul 2016.
13. SPC Novo Nordisk GlucaGen® HypoKit 1 mg powder and solvent for solution for injection. http://www.medicines.org.uk/emc/medicine/4258/SPC/GlucaGen+Hypokit+1+mg

# APPENDIX 1:

## List of names and addresses

| | |
|---|---|
| Sponsor | Zealand Pharma A/S<br>Smedeland 36<br>2600 Glostrup, Copenhagen<br>Denmark |
| Contract Research Organization (CRO) | SynteractHCR Deutschland GmbH<br>Albrechtstr. 14<br>80636 Munich, Germany |
| Coordinating investigator | Thomas R. Pieber, MD<br>Medical University of Graz<br>Auenbruggerplatz 15<br>A-8036 Graz, Austria |
| Trial monitor | SynteractHCR Deutschland GmbH<br>Albrechtstr. 14<br>80636 Munich, Germany |
| Statistician | [redacted]<br>Senior Director Biometrics EMEA<br>SynteractHCR Deutschland GmbH<br>Albrechtstr. 14<br>80636 Munich, Germany |
| Sponsor representative | [redacted]<br>Clinical Project Manager<br>Zealand Pharma A/S<br>Smedeland 36, 2600 Glostrup, Denmark<br>Tel: [redacted]<br>E-mail: [redacted] |
| Sponsor's medical expert | [redacted]<br>Medical Director<br>Zealand Pharma A/S<br>Smedeland 36, 2600 Glostrup, Denmark<br>Tel: [redacted]<br>E-mail: [redacted] |
| Sponsor representative for pharmacovigilance | [redacted]<br>PharmaLex A/S Agern Allé 24, 2970 Hørsholm, Denmark<br>Tel: [redacted] (8 a.m. to 4 p.m.)<br>[redacted] (outside 8 a.m. to 4 p.m.)<br>Fax: [redacted]<br>E-mail: drugsafety@lindeq.com |
| Clinical laboratory | MLM Medical Labs Gmbh |

| | |
|---|---|
| | [redacted]<br>Dohrweg 63, 41066 Mönchengladbach, Germany<br>Tel: [redacted]<br>E-mail: [redacted] |
| Analytical laboratory<br>(ZP4207 PK, ZP4207 ADA,<br>Glucagon ADA) | York Bioanalytical Solutions (YBS)<br>[redacted] (ZP4207 Bioanalysis)<br>[redacted] (ADA analyses)<br>Cedar House<br>Northminster Business Park<br>Northfield Lane<br>York, YO26 6QR<br>United Kingdom<br>E-mail: [redacted]<br>E-mail: [redacted] |
| (ZP4207 and Glucagon Nab) | [redacted]<br>BioAgilytix Labs<br>2300 Englert Drive<br>Durham, NC 27713<br>USA<br>[redacted] |

# CLINICAL TRIAL PROTOCOL AMENDMENT

**A phase 3, Randomized, Double-Blind, Parallel Group Safety Trial to Evaluate the Immunogenicity of Dasiglucagon and GlucaGen® Administered Subcutaneously in Patients with Type 1 Diabetes Mellitus (T1DM)**

**Sponsor code: ZP4207-16136**
**SynteractHCR: ZEA-DNK-01711**
**EudraCT number: 2017-000062-30**

| | |
|---|---|
| Sponsor: | Zealand Pharma A/S,<br>Smedeland 36,<br>2600 Glostrup,<br>DENMARK |
| Clinical Research Organization: | SynteractHCR Deutchland GmbH,<br>Albrechtstr. 14,<br>80636 Munich<br>GERMANY |
| Study Drug Name: | Dasiglucagon |
| Development Phase: | 3 |
| Amendment Number: | 02 |
| Amendment Date: | 21 August 2017 |
| Type of Amendment: | Substantial |
| Amendment to protocol: | Final version 1, dated January 18th 2017 |

The study will be conducted according to the protocol and in compliance with Good Clinical Practice (GCP), with the Declaration of Helsinki [1] and with other applicable regulatory requirements.

## SIGNATURE PAGE

Declaration of Sponsor or Responsible Medical Officer

**Title: A phase 3, Randomized, Double-Blind, Parallel Group Safety Trial to Evaluate the Immunogenicity of Dasiglucagon and GlucaGen® Administered Subcutaneously in Patients with Type 1 Diabetes Mellitus (T1DM)**

This trial protocol amendment number 2 was subjected to critical review. The information it contains is consistent with current knowledge of the risks and benefits of the investigational product, as well as with the moral, ethical and scientific principles governing clinical research as set out in the 2008 revision of the Declaration of Helsinki [1] and the guidelines on Good Clinical Practice (GCP) [2].

[redacted] [redacted]

Date

Title: Clinical Project Manager
Institution: Zealand Pharma a/s
Smedeland 36, 2600 Glostrup, Denmark

[redacted] [redacted]

Date

Title: Medical Officer
Institution: Zealand Pharma a/s
Smedeland 36, 2600 Glostrup, Denmark

## Declaration of the Investigator

**Title: A phase 3, Randomized, Double-Blind, Parallel Group Safety Trial to Evaluate the Immunogenicity of Dasiglucagon and GlucaGen® Administered Subcutaneously in Patients with Type 1 Diabetes Mellitus (T1DM)**

All documentation for this trial that is supplied to me and that has not been previously published will be kept in the strictest confidence. This documentation includes the protocol, and this trial protocol amendment number 2, Investigator's Brochure, Case Report Forms (CRFs), and other scientific data.

The trial will not be commenced without the prior written approval of a properly constituted Institutional Review Board (IRB) or Independent Ethics Committee (IEC). No changes will be made to the trial protocol without the prior written approval of the Sponsor and the IRB or IEC, except where necessary to eliminate an immediate hazard to the patients.

I have read and understood and agree to abide by all the conditions and instructions contained in this protocol amendment.

Responsible Investigator of the local trial centre

_____ _____

Signature Date

_____

Name (block letters)

_____

Title (block letters)

_____

Institution (block letters)

_____

Phone number

## Table of Contents

## 1 Amendment type

This is substantial amendment number 02 to trial protocol version 1, dated 18 January 2017.

## 2 Rationale for amendment

The amendment is prepared in order to investigate how pharmacodynamic and pharmacokinetic endpoints correlate with potential anti-drug antibody responses that may develop. In order to investigate this also in patients that may develop antibodies late during the course of the trial, an extra visit will be implemented for patients, who have developed anti-drug antibodies after trial drug administration. The amendment is further prepared to include an additional pharmacodynamic endpoint and specify that patients who discontinue the trial prematurely will not be replaced.

Section 3 of this amendment will describe the overall changes and rationales.
Section 4 of this amendment will list the actual changes in the protocol text reflecting both superseded and new wording.

## 3 Changes and rationale:

### 3.1 Characterization of the pharmacodynamic and pharmacokinetic responses in antidrug antibody (ADA) positive patients

Any patient with a treatment induced or treatment boosted ADA response at any time during the trial must be called in for an additional dosing visit. At this visit, the patient will undergo assessments identical to those at the scheduled third dosing visit, Visit 4, including trial drug administration and assessments for pharmacodynamic and pharmacokinetic responses.

The rationale for the change is to obtain clinical data in all patients that may develop ADA at any time during the trial after trial drug administration in order to optimally correlate the consequence of an antibody response, if any, to pharmacodynamic and pharmacokinetic endpoints.

### 3.2 Addition of pharmacodynamic endpoint

Achieving a plasma glucose increase of ≥20 mg/dL within 30 minutes post-treatment will be added as a secondary endpoint. Further it will be specified that the glucose samples will be analyzed using a sensitive and validated assay.

The rationale for the addition is to support the bridging of the trial results to the pivotal efficacy trial in the development program. The additional endpoint will be used to further evaluate the clinical efficacy.

As the assessment of pharmacodynamics endpoints (clinical efficacy) will rely on glucose measurements, a sensitive and validated assay will be used to ensure the glucose concentrations are measured in a robust manner.

### 3.3 Replacement patients

Patients who discontinue participation in the trial prematurely will not be replaced.
The rationale for the change is to preserve the integrity of the randomization.

### 3.4 Administrative changes

Sponsor's Medical Expert has been replaced with a new person (also applicable for Appendix 1)

## 4 Amended text

This section will list the actual changes in the protocol text reflecting both the original and the new wording.
New wording is marked in *underlined italics* and deleted wording is marked with ~~strikethrough~~.

### 4.1 Characterization of the pharmacodynamic and pharmacokinetic response in ADA positive patients

**5.2 Trial Rationale**

...

The present trial aims to evaluate that immunogenicity risk with an assessment of the occurrence of ADAs and neutralizing ADAs, and of cross-reactivity with native glucagon, following repeated single doses of dasiglucagon by s.c. administration in T1DM patients. *The trial further aims to evaluate the pharmacodynamic and pharmacokinetic responses and to correlate the consequence of an antibody response, if any, to pharmacodynamic and pharmacokinetic endpoints.*

**7.2 Discussion of trial design and choice of control groups**

...

Patients ~~that test positive for ADA~~ will be monitored until the ADA levels return to baseline, and samples from the ADA positive patients will be tested for neutralizing potential in a~~n Nab~~ *NAb* (neutralizing antibody) assay. *Further, a patient with treatment induced or treatment boosted ADA response will be called in for an additional (unscheduled) visit to evaluate the clinical effect of immunogenicity on the pharmacodynamic and pharmacokinetic responses.*

***7.5.6 Unscheduled visit for ADA positive patients***

*A patient with a treatment induced or treatment boosted ADA response at visit 3-7 must be called in for an unscheduled visit. The procedures of this visit will be identical to the procedures performed at Visit 4 including administration of trial medication and assessments of pharmacodynamic and pharmacokinetic responses.*

*The visit should be scheduled as soon as the investigator is informed about a positive ADA result. The investigator should instruct the patient of the same dosing day requirements as for the regular dosing visits (Section 7.5.2) and instruct the patient to aim at having a similar sized breakfast and corresponding rapid acting insulin dose as prior Visit 2 and Visit 4.*

*A patient with treatment induced or treatment boosted ADA response will be monitored until the ADA levels return to baseline.*

**7.6.1 Immunogenicity measurements**

Antibodies against dasiglucagon/glucagon will be measured at all visits after screening. During the treatment period (Visits 2, 3, and 4) samples will be collected pre-dose *as well as at unscheduled dosing visits for ADA positive patients*.
*The ADA samples will be analyzed at a special laboratory (refer to Appendix 1). Samples collected at the planned visits will be analyzed in batches at three occasions during the trial and any positive results will immediately be communicated to the investigator in a blinded manner.*

**9.1 Case report forms**

All the information collected during the trial will be recorded in the eCRFs, which are identified by patient number. Suitable eCRFs will be designed by SynteractHCR. The investigator will ensure that the eCRFs are correctly completed. All key pages will be signed or initialed by the investigator, signifying agreement with and responsibility for the recorded data. Key pages are the following: AE-reporting form, trial medication form, concomitant medication form and trial-closure form.
*Any information (e.g. results from the neutralizing antibody assay or additional unscheduled visits) may be reported separately pending availability of the results.*

## 4.2 Addition of pharmacodynamic endpoint

**2. Trial synopsis**

...

**Objectives:**

The primary objective is to evaluate the immunogenicity of repeated single doses of dasiglucagon and GlucaGen following subcutaneous (s.c.) administration in T1DM patients.
The secondary objective is to evaluate the safety*,* ~~and~~ tolerability *and pharmacodynamic response* of repeated single doses of dasiglucagon following s.c. administration compared with s.c. GlucaGen in T1DM patients.

**Secondary endpoints:**

...

Pharmacodynamics, after administration of first and third doses of trial medication:

- Plasma glucose profiles over the period from 0-90 min after dosing will be evaluated based on the following endpoints: $AUE_{0\text{-}30min}$, $AUE_{0\text{-}90\ min}$, $CE_{max}$, $t_{max}$
- *Achieving a plasma glucose increase of ≥20 mg/dL within 30 minutes after treatment*

**6. Trial objectives**

...

**Secondary objective**

- The secondary objective is to evaluate the safety*,* ~~and~~ tolerability *and pharmacodynamic responses* of repeated single doses of dasiglucagon following s.c. administration compared with s.c. GlucaGen in T1DM patients.

**Secondary endpoints:**

...

Pharmacodynamics, after administration of first and third doses of trial medication:

- Plasma glucose profiles over the period from 0-90 min after dosing will be evaluated based on the following endpoints: $AUE_{0\text{-}30min}$, $AUE_{0\text{-}90\ min}$, $CE_{max}$, $t_{max}$
- *Achieving a plasma glucose increase of ≥20 mg/dL within 30 minutes after treatment*

### 7.6.3 Pharmacodynamic measurements

...

*The samples will be sent to the clinical laboratory and analyzed using a sensitive and validated assay for glucose measurement.*

### 10.1.4 Exposure and PD endpoints

Plasma dasiglucagon and glucagon concentrations 0-90 min from dosing: AUC0-30min, AUC0-90 min, Cmax, and tmax will be summarized with descriptive statistics.

Plasma glucose profiles 0-90 min from dosing: AUE0-30min, AUE0-90 min, CEmax, and tmax will be summarized with descriptive statistics.

*Achieving a plasma glucose increase of ≥20 mg/dL within 30 minutes after treatment will be summarized with descriptive statistics.*

## 4.3 Replacement patients

### 2. Trial synopsis

**Planned number of patients:**

90 completed patients (45 completed patients per treatment group). ~~Prematurely discontinued patients will be replaced in order to reach 90 completed patients.~~ It is expected that 112 patients in total will be randomized and treated.

### 7.1 Overall trial design and plan

...

A total of 90 patients are expected to participate in and complete the trial (45 in each treatment arm). To qualify as completed, the patient must be dosed according to protocol and have blood drawn for the ADA analyses (as scheduled in Table 2 1Table 2 1). ~~Prematurely discontinued patients will be replaced in order to reach 90 completed patients.~~ It is expected 112 patients in total will be randomized and treated.

### 7.3.3.1 Possible reasons for patient discontinuation

...

A total of 90 patients must complete the trial. To qualify as completed, the patient must be dosed according to protocol and have blood drawn for the ADA analyses described in the protocol. D~~iscontinued patients will be replaced in order to reach 90 completed patients.~~

### 7.3.4 Replacement of patients

Patients prematurely withdrawn from the trial will *not be replaced.*~~be replaced in order to reach 90 completed patients.~~

### 4.4 Administrative changes

Section 1. Signatures and agreement with protocol

Sponsor's representative: [redacted] MD, PhD
*Medical Officer* ~~Medical Director~~
Zealand Pharma
Smedeland 36
2600 Glostrup, Denmark

**APPENDIX 1:**

Sponsor's medical expert [redacted]
*Medical Officer* ~~Medical Director~~
Zealand Pharma A/S
Smedeland 36, 2600 Glostrup, Denmark
Tel: [redacted]
E-mail: [redacted]

# Clinical Trial Protocol

***A phase 3, Randomized, Double-Blind, Parallel Group Safety Trial to Evaluate the Immunogenicity of Dasiglucagon and GlucaGen® Administered Subcutaneously in Patients with Type 1 Diabetes Mellitus (T1DM)***

**Sponsor code: ZP4207-16136**
**SynteractHCR: ZEA-DNK-01711**
**EudraCT number: 2017-000062-30**
**IND Number: 127866**
**Dasiglucagon SUB code: SUB181296**

Coordinating investigator: Thomas R. Pieber, MD
Medical University of Graz
Auenbruggerplatz 15
A-8036 Graz, Austria

Sponsor: Zealand Pharma A/S
Smedeland 36
2600 Glostrup, Copenhagen
Denmark

Version: final version 3. This version includes Protocol version 1, dated 18 January 2017, Protocol Amendment 1, dated 08 May 2017 and Protocol Amendment 2, dated 21 August 2017.

Date: 21 August 2017

***GCP statement***
*This trial will be performed in compliance with Good Clinical Practice (GCP), the Declaration of Helsinki (with amendments) and local legal and regulatory requirements.*

## 1. Signatures and agreement with protocol

**Title:** A phase 3, randomized, double-blind, parallel group safety trial to evaluate the immunogenicity of dasiglucagon and GlucaGen® administered subcutaneously in patients with type 1 diabetes mellitus (T1DM)

We, the undersigned, agree to conduct this trial according to the Trial Protocol.

We agree that the trial will be carried out in accordance with Good Clinical Practice (GCP), with the Declaration of Helsinki (with amendments) and with the laws and regulations of the countries in which the trial takes place.

| | | | |
|---|---|---|---|
| Coordinating investigator | Thomas R. Pieber, MD<br>Medical University of Graz<br>Auenbruggerplatz 15<br>A-8036 Graz, Austria | | |
| | | Date | Signature |
| Statistician | [redacted]<br>Senior Director Biometrics EMEA<br>SynteractHCR Deutschland GmbH<br>Albrechtstr. 14<br>80636 Munich, Germany | | |
| | | Date | Signature |
| Sponsor's representative | [redacted]<br>Clinical Project Manager<br>Zealand Pharma<br>Smedeland 36<br>2600 Glostrup, Denmark | | |
| | | Date | Signature |
| | [redacted], MD, PhD<br>Medical OfficerZealand Pharma<br>Smedeland 36<br>2600 Glostrup, Denmark | | |
| | | Date | Signature |

**Title:** A phase 3, randomized, double-blind, parallel group safety trial to evaluate the immunogenicity of dasiglucagon and GlucaGen® administered subcutaneously in patients with type 1 diabetes mellitus (T1DM)

I agree to conduct this trial according to the Trial Protocol.

I agree that the trial will be carried out in accordance with Good Clinical Practice (GCP), with the Declaration of Helsinki (with amendments) and with the laws and regulations of the countries in which the trial takes place.

Investigator

| Date | Signature |
|---|---|

Name and address

## 2. Trial synopsis

| | |
|---|---|
| **Title of the trial:**<br>A phase 3, randomized, double-blind, parallel group safety trial to evaluate the immunogenicity of dasiglucagon* and GlucaGen® administered subcutaneously in patients with type 1 diabetes mellitus (T1DM)<br>* Dasiglucagon is the proposed international nonproprietary name | |
| **EudraCT number:**<br>2017-000062-30 | **Protocol codes:**<br>Sponsor: ZP4207-16136<br>SynteractHCR: ZEA-DNK-01711 |
| **Sponsor or sponsor's representative in the European Union:**<br>Zealand Pharma A/S, Smedeland 36, 2600 Glostrup (Copenhagen), Denmark | |
| **Coordinating investigator:**<br>Thomas R. Pieber, MD, Medical University of Graz, Auenbruggerplatz 15,A-8036 Graz, Austria | |
| **Trial center(s):**<br>2 centers in the EU, 2 centers in the US, and 3 centers in Canada | |
| **Planned trial period:**<br>First Patient First Visit: May 2017<br>Last Patient First Visit: November 2017 | **Phase of Development:**<br>Phase 3 |
| **Objectives:**<br>The primary objective is to evaluate the immunogenicity of repeated single doses of dasiglucagon and GlucaGen following subcutaneous (s.c.) administration in T1DM patients.<br>The secondary objective is to evaluate the safety, tolerability and pharmacodynamic response of repeated single doses of dasiglucagon following s.c. administration compared with s.c. GlucaGen in T1DM patients. | |
| **Trial design:**<br>This is a randomized, double-blind, parallel group trial evaluating the immunogenicity of either dasiglucagon or GlucaGen administered to euglycemic T1DM patients. Patients will be randomized 1:1 to receive 3 s.c. injections of dasiglucagon or GlucaGen with 1 week between doses. Patients will be followed for at least 3 months from the day of the first dose to assess any immune response. A total of 90 patients are expected to complete the trial. Handling, preparation and administration of trial medication will be done by unblinded trial personnel. All trial assessments will be done by blinded trial personnel. However, exposure assessments and anti-drug antibody (ADA) assessments will be performed by unblinded personnel at the specialty laboratories, to make sure that dasiglucagon or GlucaGen administration is matched with the applicable bioanalytical assay. | |
| **Planned number of patients:**<br>90 completed patients (45 completed patients per treatment group). It is expected that 112 patients in total will be randomized and treated.<br>To qualify as completed, the patient must be dosed according to protocol and have blood drawn for the planned anti-drug antibody (ADA) analyses. | |
| **Medical condition or disease under investigation:**<br>Given the role of the immune system in the pathogenesis of T1DM, the present trial is conducted in patients with T1DM. There are no data indicating an altered immune response with varying blood glucose levels. Therefore, for the safety and well-being of the patients, they will not be brought into hypoglycemia prior to dosing. Patients with previous exogenic glucagon exposure will not be excluded from the trial, but the information on previous glucagon administration will be recorded. | |
| **Inclusion criteria:**<br>To be included in the trial, patients have to fulfill all of the following criteria:<br>1. Informed consent obtained before any trial-related activities (trial-related activities are any procedure that would not have been performed during normal management of the patient)<br>2. Availability for the entire trial period<br>3. Age between 18 and 70 years, both inclusive<br>4. Male or female patients with T1DM for at least 1 year. Diagnostic criteria as defined by the | |

American Diabetes Association
5. Hemoglobin A1c ($HbA_{1c}$) <10%
6. Stable anti-diabetic treatment for at least 1 month (e.g. within 10% insulin dose adjustment)
7. A female participant must meet one of the following criteria
   a. Participant is of childbearing potential and agrees to use one of the accepted contraceptive regimens throughout the entire duration of the trial from screening until last follow-up visit. An acceptable method of contraception includes at least one of the following:
      i. Abstinence from heterosexual intercourse
      ii. Systemic contraceptives (birth control pills, injectable/implant/ insertable hormonal birth control products, transdermal patch); if the participant is using systemic contraceptives, she must use an additional form of acceptable contraception (iii or iv, below)
      iii. Intrauterine device (with and without hormones)
      iv. Condom with spermicide

   or

   b. Participant is of non-childbearing potential, defined as surgically sterile (i.e. has undergone complete hysterectomy, bilateral oophorectomy, or tubal ligation), or in a menopausal state (at least 1 year without menses)
8. A male must be surgically sterilized or willing to refrain from sexual intercourse from screening and until last follow-up visit or, if sexually active, uses a condom and partner practices contraception during the trial from screening and until the last follow-up visit

**Exclusion criteria:**

Patients meeting any of the following criteria during screening evaluations will be excluded from trial participation:

1. Previous administration of dasiglucagon (previously referred to as ZP4207)
2. Known or suspected allergy to trial medication(s) or related products
3. History of anaphylaxis or symptoms of severe systemic allergy (such as angioedema)
4. Previous participation (randomization) in this trial
5. Females who are pregnant according to a positive pregnancy test, actively attempting to get pregnant, or are lactating
6. Patients on a closed loop artificial pancreas
7. Receipt of any investigational drug within 3 months prior to screening
8. Active malignancy within the last 5 years
9. Congestive heart failure, New York Heart Association class II-IV
10. Inadequately treated blood pressure as defined as systolic blood pressure ≥160 mmHg or diastolic blood pressure ≥90 mmHg at screening
11. Current bleeding disorder, including use of anticoagulant treatment
12. Known presence or history of pheochromocytoma (i.e. adrenal gland tumor) or insulinoma (i.e. insulin-secreting pancreas tumor)
13. Known or suspected HIV infection
14. Use of a systemic beta-blocker drug, indomethacin, warfarin or anticholinergic drugs in the previous 28 days before Day 1 of this trial
15. Use of systemic corticosteroids, anti-inflammatory biological agents, kinase inhibitors or other immune modulating agents within the last 3 months prior to screening
16. Aspartate aminotransferase (AST) or alanine aminotransferase (ALT) >2.5 X the upper limit of normal (ULN), bilirubin >1.5 X ULN, estimated glomerular filtration rate (eGFR) <30 mL/min/1.73$m^2$ according to the Modification of Diet in Renal Disease (MDRD) Study definition. Altered electrolytes values of clinical relevance for cardiac conduction, as judged by the investigator.
17. Clinically significant abnormal ECG at screening as evaluated by Investigator
18. Donation of blood or plasma in the past month, or in excess of 500 mL within 12 weeks prior to screening
19. A positive result in the alcohol and/or urine drug screen at the screening visit. Significant history of alcoholism or drug abuse as judged by the investigator or consuming more than 24 g alcohol per day for men, or more than 12 g alcohol per day for women.

20. Patients with mental incapacity or language barriers that preclude adequate understanding or cooperation, who are unwilling to participate in the trial, or who in the opinion of the Investigator should not participate in the trial
21. Surgery or trauma with significant blood loss within the last 2 months prior to screening
22. Any condition interfering with trial participation or trial endpoints or that could be hazardous to the patient
23. Use of prescription or non-prescription medications known to cause QT prolongation

**Test product, dose and mode of administration:**
Dasiglucagon: 0.6 mg; liquid formulation, 1 mg/mL in prefilled syringes containing 0.6 mL

**Reference product, dose and mode of administration:**
GlucaGen, 1 mg; powder and solvent for reconstitution as 1 mL solution for injection (recombinant glucagon hydrochloride, Novo Nordisk)

**Duration of treatment:**
Patients will receive 3 s.c. injections of trial medication (dasiglucagon or GlucaGen) with 1 week between each dosing.
Prior to administration of trial medication patients must reach a target plasma glucose level of 70-150 mg/dL. Plasma glucose levels may be adjusted by s.c. administration of a fast-acting insulin analog or by glucose ingestion.

**Criteria for evaluation:**

**Immunogenicity:**
**Primary endpoint:**

- Overall ADA incidence
  This will be calculated as a percentage of the combined results of treatment-induced ADA-positive patients and treatment-boosted ADA-positive patients and the total number of evaluable patients, excluding baseline-positive patients without any samples available after drug administration.

**Key secondary endpoints:**

- Treatment-induced ADA
  Incidence calculated as a percentage of the total number of evaluable patients that were ADA negative at baseline and ADA positive after drug administration and the total number of evaluable patients, excluding baseline positive patients without any samples available after drug administration.
- Treatment-boosted ADA
  Incidence calculated as percentage of baseline ADA-positive patients with significant increases (≥5-fold) in ADA titer after drug administration and the total number of evaluable patients, excluding baseline-positive patients without any samples available after drug administration.

**Secondary endpoints:**

Characterization of ADA response:

- Incidence and titer of neutralizing activity of ADA positive patients
- Incidence of cross-reactivity of ADA positive patients towards endogenous glucagon
- Kinetics of ADA:
  The timing and duration of detected ADA response

Safety:

- The incidence, type and severity of AEs
- Changes from baseline in clinical laboratory parameters
- Changes from baseline in vital signs
- Clinically meaningful changes from baseline in physical examination and electrocardiogram (ECG)

Exposure endpoints, after administration of first and third doses of trial medication:

- Plasma dasiglucagon and glucagon concentrations from 0-90 min after dosing will be evaluated based on the following endpoints: $AUC_{0\text{-}30min}$, $AUC_{0\text{-}90\ min}$, $C_{max}$, $t_{max}$

Pharmacodynamics, after administration of first and third doses of trial medication:

- Plasma glucose profiles over the period from 0-90 min after dosing will be evaluated based on the following endpoints: $AUE_{0\text{-}30min}$, $AUE_{0\text{-}90\ min}$, $CE_{max}$, $t_{max}$
- Achieving a plasma glucose increase of ≥20 mg/dL within 30 minutes after treatment

**Statistical methods:**
All statistical analysis will be descriptive i.e. no formal statistical testing will be performed. Continuous variables will be summarized with means, standard deviations, medians, minimums, and maximums, and valid cases. Other summaries (e.g. quartiles, 95% confidence intervals) may be used as appropriate. Categorical variables will be summarized by counts and by percentage of patients in corresponding categories.

**Sample size calculation:**
The purpose of the present trial is to generate data describing the immunogenic potential of dasiglucagon and GlucaGen. The ADA assays to be used in this trial are both validated, but specific for GlucaGen and dasiglucagon, respectively, and the performance of the assays are thus not directly comparable. As a consequence, a formal comparison or non-inferiority analysis will not be performed.
Currently, no ADA incidences have been detected in the completed clinical trials, where up to 5 repeated doses of dasiglucagon have been administered to the same patients within a week. Across the two phase 1 clinical trials and a completed phase 2 pharmacokinetic/pharmacodynamic trial, a total of 141 subjects have been exposed to dasiglucagon and no incidences of ADA development have been observed. The obtained data indicate that dasiglucagon has a low risk for induction of ADAs in the investigated settings and as a consequence a meaningful sample size to compare both treatments cannot be estimated.
The sample size is therefore based on a certain precision of the confidence interval for the overall ADA incidence if no events are observed, respectively to ensure a certain probability for observing one event.
When no events are observed, to obtain an upper bound of 0.050 on the 90.0% confidence interval for the probability of such a rare event, would require a sample size of 45. Respectively, accepting a chance of observing at least one event of 90% and an actual probability of the event of 5% leads to a sample size of 45 patients completing the trial. In order to account for drop-outs, It is expected that 112 patients in total will be randomized and treated.

**Table 2-1: Flow chart**

| Trial period | Screening | Treatment | | | Follow-up | | |
|---|---|---|---|---|---|---|---|
| Visit number | V1 | V2 | V3 | V4 | V5 | V6 | V7 (EoT) |
| Trial day | -3 | 0 | 7 | 14 | 35 | 60 | 104 |
| Visit window (days) | -30 to - -3 | | ±1 | ±1 | ±2 | ±5 | ±10 |
| **Patient related info/assessments** | | | | | | | |
| Informed consent | X[1] | | | | | | |
| Inclusion/exclusion criteria | X | X[2,3] | | | | | |
| Demography | X | | | | | | |
| Body measurements | X | | | | | | |
| Medical history | X | | | | | | |
| Concomitant illness | X | | | | | | |
| Prior medications | X | | | | | | |
| Concomitant medication | X | X | X | X | X | X | X |
| History of alcohol/drug abuse | X | | | | | | |
| Randomization | | X | | | | | |
| Withdrawal criteria | | X | X | X | X | X | |
| Dosing day exclusion criteria | | X | X | X | | | |
| **Safety assessments** | | | | | | | |
| Physical examination | X | | | | | | X |
| Vital signs | X | X[12] | X[12] | X[12] | X | | X |
| ECG | X | X[10] | X[10] | X[10] | X | | X |
| Local tolerability | | X[5] | X[5] | X[5] | | | |
| Adverse events | X | X | X | X | X | X | X |

| Trial period | Screening | Treatment | | | Follow-up | | |
|---|---|---|---|---|---|---|---|
| Visit number | V1 | V2 | V3 | V4 | V5 | V6 | V7 (EoT) |
| Trial day | -3 | 0 | 7 | 14 | 35 | 60 | 104 |
| Visit window (days) | -30 to - -3 | | ±1 | ±1 | ±2 | ±5 | ±10 |
| **Laboratory** | | | | | | | |
| Hematology, biochemistry, coagulation | X[4] | X[4] | | X[4] | X | | X |
| Pregnancy test | X[11] | X[11] | X[11] | X[11] | | | |
| Urinalysis | X | X[2] | | X[2] | | | X |
| Urine drug screen | X[6] | X[2,6] | X[2,6] | X[2,6] | | | |
| Alcohol breath test | X | X[2] | X[2] | X[2] | | | |
| **Exposure and pharmacodynamics (PD)** | | | | | | | |
| Dasiglucagon /glucagon | | X[7] | | X[7] | | | |
| Plasma glucose | | X[8] | | X[8] | | | |
| **Other assessments** | | | | | | | |
| Antibodies against dasiglucagon /glucagon | | X[2] | X[2] | X[2] | X | X | X |
| **Trial material** | | | | | | | |
| Administration of trial medication | | X[9] | X[9] | X[9] | | | |

ECG = electrocardiogram; EoT = End of Trial; ; PD = pharmacodynamics; V = visit

[1] Informed consent can be obtained on the same day as screening, but prior to any trial-related procedures [2] Pre-dose

[3] Only check of changes between the screening visit and V2.

[4] Coagulation parameters are measured at screening visit only. On dosing days Visit 2 and 4, blood samples are collected pre-dose, and at 30 and 90 min post-dosing. The actual time for sampling should not deviate from the nominal time by more than ±5 min. Pre-dose is defined as within 5 min prior to dosing.

[5] Local tolerability assessed at 0.5 and 2 h post-dose. The actual time for assessment should not deviate from the nominal time by more than ±10 min.

[6] Urine drug screen will be performed at trial site for visits 1-4

[7] Pre-dose, 5, 10, 30, 60, and 90 min post-dosing. The actual time of blood sampling for exposure should not deviate from the nominal time by more than ±1 min. Pre-dose is defined as within 5 min prior to dosing.

[8] Pre-dose, 5, 10, 30, 60, and 90 min post-dosing. The actual time for blood sampling for plasma glucose should not deviate from the nominal time by more than ±1 min. Pre-dose is defined as within 5 min prior to dosing.

[9] Prior to administration of trial medication patients must reach a target plasma glucose level of 70-150 mg/dL. Plasma glucose levels may be adjusted by administration of a fast-acting insulin analog or by glucose ingestion at the discretion of the investigator. At visit 2 and 4 patients must be fasting for 90 min after administration of trial medication. At all dosing visits, patients will be treated individually to alleviate any potential side effects and will be observed for at least 5 h post-dose.

[10] On dosing days Visit 2, 3 and 4 ECG's are assessed pre-dose, and at 20, 35, 45 and 60 min post-dosing. The actual time of assessment should not deviate from the nominal time by more than ±5 min. Pre-dose is defined as within 5 min prior to dosing.

[11] Pregnancy test is only applicable for women of childbearing potential. At Visit 1 a serum pregnancy test should be performed. At Visit 2, 3 and 4 a pre-dose urine pregnancy test should be performed.

[12] On dosing days Visit 2, 3, and 4 vitals signs are collected pre-dose and at 30, 90 and 120 min post-dosing. The actual time of assessment should not deviate from the nominal time by more than ±5 min. Pre-dose is defined as within 5 min prior to dosing.

## 3. Table of contents

**Appendices:**

(1) List of names and addresses

**Index of in-text tables**



**Index of in-text figures**

## 4. List of abbreviations and definition of terms

### 4.1 Abbreviations

| | |
|---|---|
| ADA | Anti-drug Antibody |
| AE | Adverse Event |
| ALT (SGPT) | Alanine Aminotransferase (Serum Glutamic Pyruvic Transaminase) |
| AST (SGOT) | Aspartate Aminotransferase (Serum Glutamic Oxaloacetic Transaminase) |
| BG | Blood Glucose |
| CA | Competent Authority (Directive 2001/20/EC) |
| CFR | Code of Federal Regulations |
| CRF/eCRF | Case Report Form/Electronic Case Report Form |
| CI | Confidence Interval |
| CRO | Contract Research Organization |
| CSII | Continuous Subcutaneous Insulin Infusion |
| CTA | Clinical Trial Authorization (Directive 2001/20/EC) |
| EC | Ethics Committee |
| ECG | Electrocardiogram |
| EDC | Electronic Data Capture |
| eGFR | Estimated Glomerular Filtration Rate |
| EoT | End of Trial |
| EudraCT | European Clinical Trials Database |
| FAS | Full Analysis Set |
| FDA | Food and Drug Administration |
| gamma-GT | gamma-Glutamyltransferase |
| GCP | Good Clinical Practice |
| $HbA_{1c}$ | Hemoglobin $A_{1c}$ |
| HEENT | Head, Ears, Eyes, Nose, Throat |
| HIV | Human Immunodeficiency Virus |
| ICF | Informed Consent Form |
| ICH | International Conference on Harmonization |
| IEC | Independent Ethics Committee |
| IM | Intramuscular(ly) |
| IMP | Investigational Medicinal Product |
| IND | Investigational New Drug |
| INR | International Normalized Ratio |
| IRB | Institutional Review Board |
| i.v. | Intravenous(ly) |
| IWRS | Interactive Web Response System |
| LOCF | Last Observation Carried Forward |
| MDRD | Modification of Diet in Renal Disease |
| MedDRA | Medical Dictionary for Regulatory Activities |
| Nab | Neutralizing Antibody |
| PG | Plasma Glucose |
| ODM | Operational Data Model |
| PD | Pharmacodynamic(s) |

| | |
|---|---|
| PK | Pharmacokinetic(s) |
| PPS | Per Protocol Set |
| PT | Preferred Term |
| SAP | Statistical Analysis Plan |
| SAS | Safety Analysis Set |
| SAE | Serious Adverse Event |
| SC | Safety Committee |
| s.c. | Subcutaneous(ly) |
| SOC | System Organ Class |
| SOP | Standard Operating Procedure |
| T1DM | Type 1 Diabetes Mellitus |
| T2DM | Type 2 Diabetes Mellitus |
| ULN | Upper Limit of Normal |
| V | Visit |
| ZP4207 | Dasiglucagon |

**Plasma concentrations of dasiglucagon/GlucaGen**

| | |
|---|---|
| $AUC_{0\text{-}30min}$ | Area under the plasma concentration curve from administration to observed concentration 30 min |
| $AUC_{0\text{-}90\ min}$ | Area under the plasma concentration curve from administration to observed concentration at 90 min |
| $C_{max}$ | Maximum plasma concentration |
| $t_{max}$ | Time until $C_{max}$ is reached |

**Plasma glucose concentrations**

| | |
|---|---|
| $AUE_{0\text{-}30min}$ | Area under the effect curve from administration to 30 min |
| $AUE_{0\text{-}90\ min}$ | Area under the effect curve from administration to 90 min |
| $CE_{max}$ | Change from baseline plasma glucose to maximum plasma glucose measured post dose |
| $t_{max}$ | Time to maximum effect |

## 4.2 Definitions of terms

Definition of the end of the trial: The trial ends with the last visit of the last patient participating in the trial.

# 5. Introduction

## 5.1 Background of the trial

### Hypoglycemia

Hypoglycemia in patients with diabetes is defined as episodes of an abnormally low plasma glucose concentration.[1] This is a common, unpredictable, and potentially dangerous side effect of treatment of diabetes mellitus with especially insulin or sulfonylureas. It is more frequent in patients with profound endogenous insulin deficiency, such as occurs in type 1 diabetes mellitus (T1DM) and advanced type 2 diabetes mellitus (T2DM). Treatment of T2DM with insulin causes hypoglycemia progressively and more frequently over time, whereas in T1DM, hypoglycemia is experienced throughout the course of established disease.

Symptoms and signs of hypoglycemia are not specific. Patients undergoing a hypoglycemic episode experience unpleasant symptoms such as anxiety, sweating, hunger, tremors, palpitations, paresthesia, nausea and pallor. Depending on its severity, the hypoglycemia may lead to mild confusion, behavioral changes, loss of consciousness, seizures, coma, and death.[2]

The incidence of hypoglycemic events or even the fear of hypoglycemia influences patients' adherence to prescribed treatment regimens for diabetes mellitus. This leads to inadequate glycemic control, which in turn may lead to an increased risk of diabetic complications.

### Glucagon

Glucagon is a naturally occurring hormone, secreted from the alpha cells of the pancreatic islets. Glucagon plays a central role in the regulation of glucose homeostasis and is the counterpart of insulin for controlling blood glucose levels (i.e. it acts in opposition to insulin in terms of effects on blood glucose levels). Glucagon stimulates hepatic glycogenolysis and gluconeogenesis in hypoglycemic states, thereby restoring glucose homeostasis. Glucagon receptor agonism has also been shown to exert effects on lipid metabolism, energy balance, body adipose tissue mass and food intake. Insulin decreases blood glucose levels and cases of hypoglycemia can be reversed by glucagon. Therefore, glucagon is indicated for the treatment of severe hypoglycemia.

Antibodies against therapeutic peptides like glucagon and analogues hereof may develop when injected subcutaneously. Although important, glucagon is not considered to have a critical endogenous function since other counter regulatory hormones are also induced during hypoglycemia (e.g. growth hormone, cortisol, and epinephrine). In addition, results from 3 mouse models defective in various pathways of the glucagon signaling have con rmed that glucagon action is dispensable for their development and survival. Also, in non-clinical toxicity studies performed with dasiglucagon (see below), no consequences of ADA formation have currently been observed. In summary, glucagon appears to have a partly redundant endogenous function. These non-clinical data are of importance when evaluating the consequences of ADA formation.

### Dasiglucagon

Dasiglucagon (ZP4207) is a stable peptide analog of human glucagon, available in a ready-to-use liquid formulation and is in development for the treatment of severe hypoglycemia in insulin dependent patients with diabetes mellitus. Dasiglucagon is a specific and full glucagon receptor agonist designed to mimic the effects of glucagon, having a fast absorption and elimination (minutes). It is a peptide of 29 amino acids, with 7 amino acid substitutions compared to native glucagon. The main purpose of the substitutions is to increase the physical and chemical stability of the glucagon analog compared to marketed glucagon products such as Lilly Glucagon or GlucaGen®. Dasiglucagon exhibits improved physical and chemical stability and is available in an aqueous solution at neutral pH.[3]

Three clinical trials have been completed with dasiglucagon, a first human dose trial (ZP4207-14013), a multiple-dose dose-escalation trial (ZP4207-15007) to evaluate the safety, tolerability, pharmacokinetics (PK) and pharmacodynamics (PD) of dasiglucagon, and a phase 2 crossover trial to assess the pharmacokinetics and pharmacodynamics of a single dose of an optimized formulation of dasiglucagon administered subcutaneously (s.c.) in patients with T1DM (ZP4207-15126).[3]

*Pharmacokinetics and pharmacodynamics of dasiglucagon*

The results of the phase 1 and 2 clinical trials confirm dose-proportionality for dasiglucagon pharmacokinetics, which is characterized by a fast absorption with a peak plasma concentration obtained after 35 minutes. Thereafter, the plasma concentration rapidly declines with an average half-life of 28 minutes. The median time to the maximum plasma concentration ($C_{max}$) was later for dasiglucagon than for GlucaGen (35 versus 20 minutes). Doses of 0.3 mg dasiglucagon and 0.5 mg GlucaGen and also 0.6 mg dasiglucagon and 1.0 mg GlucaGen were similar with regard to $C_{max}$. For $C_{max}$, the results indicated that 0.3 mg dasiglucagon was comparable to 0.5 mg GlucaGen (90% confidence interval (CI): 0.8167; 1.0068) and 0.6 mg dasiglucagon was comparable to 1.0 mg GlucaGen (90% CI: 0.8850; 1.1991).[3] At these dose levels, the total exposures ($AUC_{0-inf}$) were higher for dasiglucagon compared to GlucaGen.

At all dose levels in the phase 2 trial, all patients achieved a plasma glucose level of at least 70 mg/dL as well as an increase in plasma glucose by at least 20 mg/dL within 30 min post-dose. The maximal observed time to reach the 20 mg/dL plasma glucose increase ranged from 15 to 27 minutes across doses and decreased as the dose increased. The pharmacodynamic responses of 0.6 mg of dasiglucagon and 1.0 mg of GlucaGen were comparable.[3]

*Safety of dasiglucagon*

The safety data for dasiglucagon did not give rise to any relevant safety concerns for dasiglucagon beyond those related to the pharmacological effect of glucagon agonism. All adverse events (AEs) recorded in the trials were of mild or moderate severity. The most frequently reported systemic AE was nausea, which is a known side effect following administration of glucagon. Headache was the next most frequently reported event, occurring in all dose groups in the phase 2 trial. Injection site reactions were observed only sporadically after administration with either dasiglucagon or GlucaGen® and all were mild and transient. The most frequent injection site reaction was erythema, occurring in all treatment groups, including the placebo group, irrespective of dose. Glucagon has been described to exert positive inotropic and chronotropic effects and may therefore cause tachycardia and hypertension. In the phase 1 clinical trials, but not the phase 2 trial, temporary but clinically significant decreases in blood pressure were observed in a few healthy volunteers receiving investigational medicinal product (IMP) doses of at least 1.0 mg (4 with dasiglucagon and 1 with GlucaGen). This is not considered a safety concern, however hemodynamic changes post dosing will be considered a clinical event of interest.

The phase 1 and 2 results and the safety profile described to date do not give rise to specific safety concerns. For further information, please refer to the Investigator Brochure.[3]

*Immunogenicity of dasiglucagon*

To date, in the 3 clinical trials performed with dasiglucagon (described above), there have not been any anti-drug antibody (ADA) occurrences in a total of 141 subjects exposed to 1 or more doses. Data from the non-clinical toxicology program (7 non-clinical toxicity studies) showed that ADAs were detected in mice, rats, and dogs, and were most frequent in animals in the highest dasiglucagon dose groups. A fraction of the ADAs from rats and dogs were able to cross-react with native glucagon. However, the ADAs did not appear to be associated with changes in the safety or toxicity profiles compared to ADA-negative animals. In rats, the average exposure (AUC) was increased following 13 and 26 weeks of treatment in dose groups with higher ADA frequency (• 8 mg/kg/day). The ADA frequency and titers of consistently ADA-positive rats were reduced from 13 to 26 weeks of treatment, indicating a transient response in most animals. It

therefore appears that dasiglucagon has a low risk for induction of ADAs. A fraction of the antibodies detected in the non-clinical toxicity studies was found to cross-react with glucagon without advert clinical or toxicity findings. These data suggest that dasiglucagon and glucagon share epitopes and potential ADAs induced by dasiglucagon in humans may have the ability to cross-react with glucagon.[3]

Although glucagon is an important hormone for controlling blood glucose levels it is considered to have a partially redundant endogenous function since hypoglycemia can also be corrected by other means. The overall immunogenicity risk of dasiglucagon in a clinical context is therefore considered to be low and the potential effects of induced ADAs judged to be of limited clinical consequence.

As dasiglucagon contains 7 amino acid substitutions compared to native glucagon and historic data indicate that the immune system's tolerance to glucagon can be impaired in the intended target population, there is an inherent risk for the induction of an ADA response against dasiglucagon. However, other product specific characteristics, i.e. dasiglucagon being a chemical synthetized product without host cell contaminants, a reduced potential for aggregation, a physiological compatible formulation, and a high bioavailability with a short half-life, are all in favor of reducing the risk of dasiglucagon to induce an unwanted immune response. Considering the intended indication, in which dasiglucagon is administered as a single-dose rescue treatment on an infrequent basis (0.21 to 1.6 episodes per patient per year),[4,5] the induction of a high titer ADA response with effects on clinical safety seems unlikely. Overall, the risk of dasiglucagon to induce an ADA response is considered low. The present trial aims to evaluate that immunogenicity risk in patients with T1DM administered multiple s.c. doses of dasiglucagon.

### 5.2 Trial rationale

Dasiglucagon is in clinical development as a rescue treatment for severe hypoglycemia in patients with insulin-dependent diabetes mellitus. Immune responses to therapeutic peptides and proteins may develop after subcutaneous administration and could potentially adversely affect their safety and efficacy. The potential formation of high titer ADAs to dasiglucagon, although considered unlikely in a rescue indication, could influence the efficacy of dasiglucagon, either indirectly by interacting with the pharmacokinetics of dasiglucagon or directly by neutralizing the glucagon response.

In recent years, regulatory agencies have outlined and recommended the use of a risk-based approach in the evaluation and mitigation of immune responses to therapeutic compounds that may adversely affect their safety and/or efficacy. Both transient and persistent antibody response should be combined to determine the overall immunogenicity of a product. Persistent antibodies are of importance since patients with persistent antibodies could experience clinical adverse reactions affecting safety and efficacy, while a transient antibody response can resolve without further consequence.

The present trial aims to evaluate that immunogenicity risk with an assessment of the occurrence of ADAs and neutralizing ADAs, and of cross-reactivity with native glucagon, following repeated single doses of dasiglucagon by s.c. administration in T1DM patients. The trial further aims to evaluate the pharmacodynamics and pharmacokinetic responses and to correlate the consequence of an antibody response, if any, to pharmacodynamic and pharmacokinetic endpoints. The reference product in this trial is GlucaGen, a recombinant human glucagon approved for the treatment of the severe hypoglycemic reactions that may occur in the management of insulin-treated children and adults with diabetes mellitus. Given the differences in ADA assay for dasiglucagon and GlucaGen, a formal statistical comparison or non-inferiority testing is not performed.

### 5.3 Assessment of anticipated benefits and risks

As glucagon and its analogs belong to a well-known drug class with a known mode of action, dasiglucagon is not expected to be a high-risk molecule.

Treatment with an investigational medicinal product may result in undesired effects or complaints. Undesired effects and complaints such as nausea, vomiting, and diarrhea are known AEs occurring with glucagon administration. Similar AEs have also been observed to a limited degree in the 3 clinical studies conducted with dasiglucagon. As with every novel drug substance, new and as yet unknown side effects also may occur.

There are limited data available to assess the immunogenic potential of glucagon products available on the market, but the data available indicate that marketed glucagon only has a small immunogenic potential. Based on the 3 clinical studies conducted with dasiglucagon to date (see Section 5.2), no anti-dasiglucagon or anti-glucagon antibodies have been detected.

In relation to diabetes therapy, development of insulin-antibodies is documented more substantially and the route of administration has been shown to influence the immunogenic potential of insulins. However, antibodies against insulin do not generally have an impact on insulin action and are thus not clinically relevant. In terms of consequence, development of high titer antibodies against dasiglucagon could, in theory reduce the activity of endogenous glucagon, which, in theory, could influence hypoglycemic episodes. Limited suppression of glucagon would, however, not be considered critical, since low glucose levels can also be corrected by other means, including oral intake of glucose and the action of other endogenous hormones such as oxyntomodulin and epinephrine.

Overall, dasiglucagon is judged to be a low-risk molecule, based upon the available clinical data as well as the outcome of the risk-based approach to the immunogenicity assessment. In line with the primary objective of this trial to assess the immunogenicity of dasiglucagon, sampling for measurement of antibodies against dasiglucagon will take place prior to first dosing (Visit 2), pre-dose at subsequent visits during the treatment period (Visits 3 and 4), and at all Follow-up visits (at 35, 60, and 104 days after the first dose of trial medication; i.e. at Visits 5, 6, and 7 (End of Trial [EoT] visit), respectively).

Administration of dasiglucagon may be associated with a risk of allergic reactions similar to those observed for other therapeutic peptides or proteins. Patients with known or suspected allergies to the trial medications or related products will be excluded from the trial. Mild or moderate allergic reactions may include symptoms of rash, fever, flu-like symptoms, nausea, headache, and myalgia. Acute generalized hypersensitivity reactions are usually very rare but may include symptoms of flushing, sweating, dizziness, change in blood pressure and difficulties in breathing. No severe acute hypersensitivity reactions have been observed in the 3 clinical trials conducted with dasiglucagon. Direct access to resuscitation equipment is ensured at the clinical trial sites.

With the exception of medical examinations, a patient participating in this trial is not likely to derive any personal health-related benefits. The results of the trial may contribute to the future use of dasiglucagon in patients with diabetes mellitus experiencing severe hypoglycemic reactions.

Overall, the benefit to risk ratio for patients entering the ZP4207-16136 trial is considered acceptable.

## 6. Trial objectives

**Primary objective**

- To evaluate the immunogenicity of repeated single doses of dasiglucagon and GlucaGen following s.c. administration in T1DM patients.

**Secondary objective**

- To evaluate the safety, tolerability and pharmacodynamic response of repeated single doses of dasiglucagon following s.c. administration compared with s.c. GlucaGen in T1DM patients.

**Primary endpoint:**

- Overall ADA incidence
  This will be calculated as a percentage of the combined results of treatment-induced ADA-positive patients and treatment-boosted ADA-positive patients and the total number of evaluable patients, excluding baseline-positive patients without any samples available after drug administration.

**Key secondary endpoints:**

- Treatment-induced ADA
  Incidence calculated as a percentage of the total number of evaluable patients that were ADA negative at baseline and ADA positive after drug administration and the total number of evaluable patients, excluding baseline positive patients without any samples available after drug administration.
- Treatment-boosted ADA
  Incidence calculated as percentage of baseline ADA-positive patients with significant increases (≥5-fold) in ADA titer after drug administration and the total number of evaluable patients, excluding baseline-positive patients without any samples available after drug administration.

**Secondary endpoints:**

Characterization of ADA response:

- Incidence and titer of neutralizing activity of ADA positive patients
- Incidence of cross-reactivity of ADA positive patients towards endogenous glucagon
- Kinetics of ADA:
  The timing and duration of detected ADA response

Safety:

- The incidence, type and severity of AEs
- Changes from baseline in clinical laboratory parameters
- Changes from baseline in vital signs
- Clinically meaningful changes from baseline in physical examination and electrocardiogram (ECG)

Exposure endpoints, after administration of first and third doses of trial medication:

- Plasma dasiglucagon and glucagon concentrations from 0-90 min after dosing will be evaluated based on the following endpoints: $AUC_{0\text{-}30min}$, $AUC_{0\text{-}90\ min}$, $C_{max}$, $t_{max}$

Pharmacodynamics after administration of first and third doses of trial medication:

- Plasma glucose profiles over the period from 0-90 min after dosing will be evaluated based on the following endpoints: $AUE_{0\text{-}30min}$, $AUE_{0\text{-}90\ min}$, $CE_{max}$, $t_{max}$
- Achieving a plasma glucose increase of ≥20 mg/dL within 30 minutes after treatment

# 7. Investigational plan

## 7.1 Overall trial design and plan

This is a randomized, double-blind, parallel group trial comparing the immunogenicity of 3 fixed doses of either dasiglucagon or GlucaGen administered to euglycemic T1DM patients.

Patients with T1DM will be randomized 1:1 to receive 3 s.c. injections of either dasiglucagon (0.6 mg) or GlucaGen (1 mg), with 1 week between each dose. Patients will be followed for at least 3 months from the day of the first dose to assess any immune response. Patients with previous exogenic glucagon exposure will not be excluded from the trial, but the information on previous glucagon administration will be recorded to enable subgroup analyses. A total of 90 patients are expected to participate in and complete the trial (45 in each treatment arm). To qualify as completed, the patient must be dosed according to protocol and have blood drawn for the ADA analyses (as scheduled in Table 2-1). It is expected 112 patients in total will be randomized and treated.

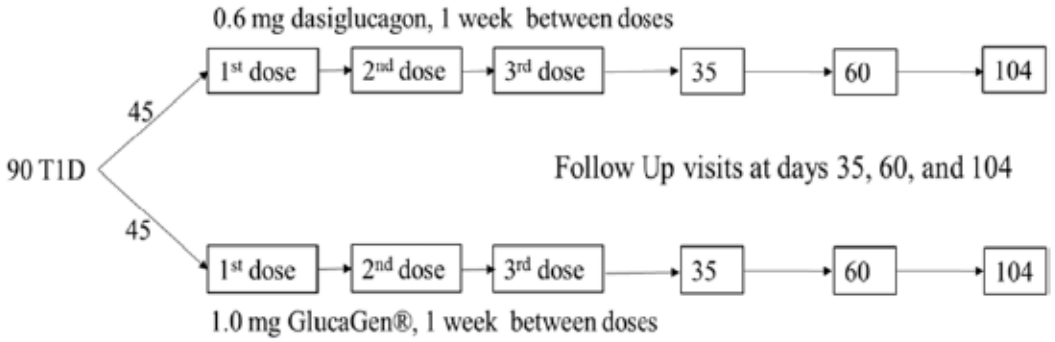
For the safety and well-being of the patients, they will not be brought into hypoglycemia prior to dosing. Prior to administration of trial product patients must reach a target plasma glucose level of 70-150 mg/dL.

The trial will include the following periods (as illustrated in Figure 7-1, below).

- A screening period from Day -30 to Day -3
- A treatment period, from Day 0 (day of randomization) to Day 14 (day of third and final dosing with trial medication), with s.c. trial medication administered on Day 0, Day 7, and Day 14. Handling, preparation and administration of trial medication will be done by unblinded trial personnel. All trial assessments will be done by blinded trial personnel.
- A follow-up period, from the end of the Treatment Period, with follow-up visits at Day 35, Day 60, and Day 104 (the EoT visit)

Time windows for each trial visit are given in Table 2-1.

**Figure 7-1 Overview of the trial design**




An overview on the trial procedures is given in the flow chart (Table 2-1). Patients should be seen for all visits on the designated day or as close to it as possible.

## 7.2 Discussion of trial design and choice of control groups

The trial will be randomized and double-blind to increase trial validity and to reduce bias during evaluation of assessments with the two treatments. Since the 2 trial medications are not identical in appearance (dasiglucagon is a liquid formulation and GlucaGen is available as a powder for reconstitution), the handling, preparation and administration of trial medication will be

done by unblinded trial personnel who will not be involved in other trial procedures and assessments. All trial assessments performed at the trial site will be done by blinded trial personnel. However, exposure assessments and ADA assessments will be performed by unblinded personnel at the specialty laboratories, to make sure that dasiglucagon or GlucaGen administration is matched with the applicable bioanalytical assay.

Euglycemic patients with T1DM will be randomized 1:1 in order to evaluate the immunogenicity of dasiglucagon and GlucaGen. The randomized, double-blind, parallel group design, with administration of 3 fixed consecutive doses of randomized trial medication (dasiglucagon or GlucaGen) to the same patient will allow characterization of immunogenic potential for the 2 products. Treatment with the 3 repeated doses (each separated by 1 week), with follow-up visits at Days 35 (where potential immune responses are known to be most pronounced), and at 60 and 104 days after the first dose (following the patient for 2-3 antibody half-lives after expected peak titer), is deemed relevant and sufficient for evaluating any immunogenic response to treatment. Patients will be monitored until the ADA levels return to baseline, and samples from the ADA positive patients will be tested for neutralizing potential in a NAb (neutralizing antibody) assay. Further, a patient with a treatment induced or treatment boosted ADA response will be called in for an additional (unscheduled) visit to evaluate the clinical effect of immunogenicity of the pharmacodynamic and pharmacokinetic reponses.

Dasiglucagon and GlucaGen will be administered at fixed doses independent of body weight because this is the intended therapeutic dosing regimen in the emergency treatment of hypoglycemia. The selected dose of 1 mg GlucaGen is the recommended dose for treatment of severe hypoglycemia. Based on pre-clinical and clinical studies, it has been demonstrated that 0.6 mg of dasiglucagon results in an initial pharmacodynamic response (i.e. acute glucose mobilization) comparable to 1 mg GlucaGen (see also Section 5.1).

For the safety and well-being of the patients, they will not be brought into hypoglycemia prior to dosing. However, very high blood glucose levels at dosing will potentially impact the reporting of nausea and other associated AE's. Therefore, to enable a more precise safety assessment, patients are required to be dosed while at a normal blood glucose level, and a pre-treatment plasma glucose level of 70-150 mg/dL will be targeted. Plasma glucose levels may be adjusted by s.c. administration of a fast-acting insulin analog or by glucose ingestion. Even with this precaution, it cannot be excluded that a higher frequency of nausea may be anticipated, if this AE is caused by hyperglycaemia.

The safety profile described to date does not give rise to specific safety concerns. In previous studies, dasiglucagon was associated with the AE's nausea, a known side effect following administration of glucagon, headache, and injection site reactions (erythema).

### 7.3 Selection of trial population

Dasiglucagon is indicated for treatment of severe hypoglycemia in patients with T1DM. Given the role of the immune system in the pathogenesis of T1DM, the present trial aims to evaluate the immune response of patients with T1DM taking repeated single doses of s.c. dasiglucagon and GlucaGen.

There are no data indicating an altered immune response with varying blood glucose levels, therefore, for the safety and well-being of the patients, they will not be brought into a hypoglycemic state prior to dosing. Patients with previous exogenic glucagon exposure will not be excluded from the trial, but the information on previous glucagon administration will be recorded to enable subgroup analyses.

The trial will enroll patients in centers in the EU, in the US, and in Canada.

### 7.3.1 Inclusion criteria

To be included in the trial, patients have to fulfill all of the following criteria:

(1) Informed consent obtained before any trial-related activities (trial-related activities are any procedure that would not have been performed during normal management of the patient)
(2) Availability for the entire trial period
(3) Age between 18 and 70 years, both inclusive
(4) Male or female patients with T1DM for at least 1 year. Diagnostic criteria as defined by the American Diabetes Association
(5) Hemoglobin $A_{1c}$ ($HbA_{1c}$) <10%
(6) Stable antidiabetic treatment for at least 1 month (e.g. within 10% insulin dose adjustment)
(7) A female participant must meet 1 of the following criteria

   a. Participant is of childbearing potential and agrees to use one of the accepted contraceptive regimens throughout the entire duration of the trial from screening until last follow-up visit. An acceptable method of contraception includes at least one of the following:

      i. Abstinence from heterosexual intercourse

      ii. Systemic contraceptives (birth control pills, injectable/implant/ insertable hormonal birth control products, transdermal patch); if the participant is using systemic contraceptives, she must use an additional form of acceptable contraception (iii or iv, below)

      iii. Intrauterine device (with and without hormones)

      iv. condom with spermicide

   or

   b. Participant is of non-childbearing potential, defined as surgically sterile (i.e. has undergone complete hysterectomy, bilateral oophorectomy, or tubal ligation), or in a menopausal state (at least 1 year without menses).

(8) A male must be surgically sterilized or willing to refrain from sexual intercourse from screening and until last follow-up visit or, if sexually active, uses a condom and partner practices contraception during the trial from screening and until the last follow-up visit.

### 7.3.2 Exclusion criteria

Patients meeting any of the following criteria during screening evaluations will be excluded from trial participation:

(1) Previous administration of dasiglucagon (previously referred to as ZP4207).
(2) Known or suspected allergy to trial medication(s) or related products
(3) History of anaphylaxis or symptoms of severe systemic allergy (such as angioedema)
(4) Previous participation (randomization) in this trial
(5) Females who are pregnant according to a positive pregnancy test, actively attempting to get pregnant, or are lactating
(6) Patients on a closed loop artificial pancreas
(7) Receipt of any investigational drug within 3 months prior to screening
(8) Active malignancy within the last 5 years
(9) Congestive heart failure, New York Heart Association class II-IV

(10) Inadequately treated blood pressure as defined as systolic blood pressure ≥160 mmHg or diastolic blood pressure ≥90 mmHg at screening.
(11) Current bleeding disorder, including use of anticoagulant treatment
(12) Known presence or history of pheochromocytoma (i.e. adrenal gland tumor) or insulinoma (i.e. insulin-secreting pancreas tumor)
(13) Known or suspected HIV infection
(14) Use of a systemic beta-blocker drug, indomethacin, warfarin or anticholinergic drugs in the previous 28 days before Day 1 of this trial)
(15) Use of systemic corticosteroids, anti-inflammatory biological agents, kinase inhibitors or other immune modulating agents within the last 3 months prior to screening
(16) Aspartate aminotransferase (AST) or alanine aminotransferase (ALT) >2.5 X the upper limit of normal (ULN), bilirubin >1.5 X ULN, estimated glomerular filtration rate (eGFR) <30 mL/min/1.73m$^2$ according to the Modification of Diet in Renal Disease (MDRD) Study definition. Altered electrolytes values of clinical relevance for cardiac conduction, as judged by the investigator.
(17) Clinically significant abnormal ECG at screening, as evaluated by Investigator
(18) Donation of blood or plasma in the past month, or in excess of 500 mL within 12 weeks prior to screening
(19) A positive result in the alcohol and/or urine drug screen at the screening visit. Significant history of alcoholism or drug abuse as judged by the investigator or consuming more than 24 g alcohol per day for men, or more than 12 g alcohol per day for women.
(20) Patients with mental incapacity or language barriers that preclude adequate understanding or cooperation, who are unwilling to participate in the trial, or who in the opinion of the Investigator should not participate in the trial
(21) Surgery or trauma with significant blood loss within the last 2 months prior to screening
(22) Any condition interfering with trial participation or trial endpoints or that could be hazardous to the patient
(23) Use of prescription or non-prescription medications known to cause QT prolongation

### 7.3.3 Premature removal from trial

Participation in the trial is strictly voluntary. A patient has the right to withdraw from the trial at any time and for any reason. If he/she chooses to withdraw, the investigator must be informed immediately. The investigator has the right to terminate participation of any patient at any time if the investigator deems it in the patient's best interest. The reason and circumstances for premature discontinuation will be documented in the electronic Case Report Form (eCRF).

#### 7.3.3.1 Possible reasons for patient discontinuation

A patient will be discontinued if the following applies:

- If a protocol deviation occurs which, in the clinical judgment of the Investigator, can invalidate the assessment of ADA responses to dasiglucagon or glucagon, the patient will be withdrawn by the Investigator
- AEs that are considered unacceptable by the patient or the Investigator
- Positive result from an urine drug screen test
- Pregnancy. If a female patient becomes pregnant in the time between the screening visit and any one of the dosing visits

If discontinuation occurs following administration of any trial medication, the patient will be asked to return and participate in the complete follow-up visits at trial Day 35, 60, and 104.

If trial participation is terminated due to an AE possibly related to the trial medication (including reference product) or trial examinations, the patient must be followed up by additional examinations according to the medical judgment of the investigator until the abnormal condition is resolved or the investigator deems further observations or examinations to be no longer medically indicated.

Patients, who meet one or more of the following dosing day exclusion criteria at a dosing visit, will be excluded from the dosing visit, but can be rescheduled to one of the following days (maximum 3 days postponement). Each dosing visit can only be rescheduled once.

- Strenuous exercise within 4 days prior to dosing, as judged by the Investigator. Strenuous exercise is not allowed during the treatment period of the trial
- Clinically significant illness that may interfere with trial objectives or impose a risk to patients, as judged by the Investigator
- Consumption of alcohol within 24 h prior to dosing visit, or positive results from an alcohol breath test
- Changes in medical history or concomitant medication resulting in fulfillment of clinical exclusion criteria, as judged by the Investigator

A total of 90 patients must complete the trial. To qualify as completed, the patient must be dosed according to protocol and have blood drawn for the ADA analyses described in the protocol.

#### 7.3.3.2 Center discontinuation

The center can be closed and the trial terminated for the following reasons:

- The center is unlikely to be able to recruit sufficient patients within the agreed time frame
- The center does not respond to trial management requests
- Repeat protocol violations

#### 7.3.3.3 Trial termination

The sponsor reserves the right to modify or terminate the trial at any time. Possible reasons for termination are:

- Safety reasons – the incidence of AEs in this or any other trial using the same trial medication indicates a potential health risk for the patients.
- New scientific knowledge becomes known that makes the objectives of the trial no longer feasible/valid
- Unsatisfactory enrolment of patients

### 7.3.4 Replacement of patients

Patients prematurely withdrawn from the trial will not be replaced.

## 7.4 Investigational medicinal product(s)

### 7.4.1 Identity of investigational medicinal product(s)

The identity of the investigation products is summarized in Table 7-1.

**Table 7-1: Identity of investigational products**

| | Test product | Reference product |
|---|---|---|
| Name | Dasiglucagon | GlucaGen® |
| Active substance | ZP4207 | Recombinant glucagon hydrochloride |
| Formulation | Liquid formulation, 0.6 mL | Powder and solvent for reconstitution as 1 mL solution for injection |
| Strength | 1 mg/mL | 1 mg |
| Container | Single use pre-filled syringe | Powder and solvent for reconstitution packed together in a plastic box. A "hypo-kit" |
| Manufacturer | Zealand Pharma A/S, Glostrup (Copenhagen), Denmark | Novo Nordisk A/S, Bagsværd, Denmark |
| Storage requirements | Store between 2 and 8°C | Store between 2 and 8°C |

Handling, preparation and administration of trial medication will be done by unblinded trial personnel. All trial assessments on the trial site will be done by blinded trial personnel. However, exposure assessments and ADA assessments will be performed by unblinded personnel at the specialty laboratories, to make sure that dasiglucagon or GlucaGen administration is matched with the applicable bioanalytical assay.

The test product will be packed by the sponsor. The information on the labels will be in the local language. The label on the product will be compliant with local laws and regulations.

### 7.4.2 Treatments administered

Dasiglucagon is a stable peptide analog of human glucagon in a ready-to-use liquid formulation indicated for treatment of severe hypoglycemia in insulin dependent patients with diabetes mellitus. Dasiglucagon is in clinical development and has no marketing authorization as yet. GlucaGen is approved in the EU and US and is indicated for treatment of severe hypoglycemic reactions, which may occur in the management of insulin-treated children and adults with diabetes mellitus.

Patients in this trial have not previously been treated with dasiglucagon (ZP4207) and will be randomly assigned (1:1) to receive 1 of the following trial medications:

- 0.6 mg dasiglucagon
- 1 mg GlucaGen

Prior to administration of trial medication at all dosing visits patients must reach a target plasma glucose level of 70-150 mg/dL. If the patient presents with plasma glucose > 150 mg/dL, corrective amounts of fast acting insulin will be administered at the investigator's discretion. Plasma glucose measured after 20 and after 40 minutes should ensure that the patient has reached the targeted 70-150 mg/dL. If the patient presents with plasma glucose < 70 mg/dL glucose ingestion will be provided in order to reach target plasma glucose of 70-150 mg/dL documented by plasma glucose measurements after 20 and 40 minutes, respectively.

Following the first (Visit 2) and third (Visit 4) dose administration, patients must be fasting for 90 min after dosing. Following the 90-min blood sample draw after the administration of the first (Visit 2) and third (Visit 4) dose administration, patients may be treated individually in order to alleviate any potential side effects in order to minimize prematurely treatment discontinuation and consequently reduce the amount of missing data. This treatment can also be instituted immediately after the second dose administration (Visit 3), as pharmacodynamics will not be assessed at this visit.

In order to minimize the number of patients discontinuing treatment prematurely and consequently reduce the amount of missing data, the following treatment modalities may be used, as considered appropriate by the investigator:

At visit 2 and 4:

- 90 min after dosing patients will be allowed to eat and drink moderately to make them feel comfortable
- 90 min after dosing a moderate and individualized corrective dose of insulin to convert the induced hyperglycemia to euglycemia, after agreement with the investigator
- antiemetic treatment in the form of metoclopramide (Primperan®) as per local label, administered before or after dosing

At visit 3:

- patients will be allowed to eat and drink moderately to make them feel comfortable
- a moderate and individualized corrective dose of insulin to convert the induced hyperglycemia to euglycemia, after agreement with the investigator
- antiemetic treatment in the form of metoclopramide (Primperan®) as per local label, administered before or after dosing

Patients must be monitored for at least 5 h after dosing at the clinical site for safety observations, including blood glucose monitoring.

Patients will not be discharged until they are considered stable and with a blood glucose level in the range of 70-180 mg/dL. Before discharge, the investigator will provide instructions to the patients on management of their blood glucose levels. Each trial medication will be administered s.c. 3 times in total, with 7 days between dosing (i.e. dosing occurs at Days 0, 7, and 14; Visits 2, 3, and 4). Should the patient experience vomiting following the first or second dasiglucagon or GlucaGen administration, the investigator must ensure normal electrolytes prior to the next dosing day. An electrolyte imbalance can be corrected by administration of an electrolyte supplement or by any other treatment modality considered appropriate by the investigator.

### 7.4.3 Selection of doses in the trial

The selected dose of 1 mg GlucaGen is the recommended dose for treatment of severe hypoglycemia. Based on pre-clinical and clinical studies, it has been demonstrated that 0.6 mg of dasiglucagon results in an initial pharmacodynamic response (i.e. acute glucose mobilization) comparable to 1 mg GlucaGen.

### 7.4.4 Treatment compliance

All trial medications will be prepared and administered by unblinded trial personnel.

#### 7.4.5 Method of assigning patients to treatments or treatment sequences

Patients who meet all inclusion and none of the exclusion criteria and have given written informed consent will be randomized in a 1:1 ratio to either dasiglucagon or GlucaGen via an Interactive Web Response System (IWRS) that will assign a kit number to one of the 2 aforementioned treatment arms.

Patients with previous exogenic glucagon exposure will not be excluded from the trial, but the information on previous glucagon administration will be recorded, to enable subgroup analyses.

In the event of an emergency, e.g., when it becomes necessary for the investigator to know which treatment the patient is taking, the code for that patient can be broken by the investigator, preferably after consultation with the medical monitor. Emergency code breaks can be performed using the IWRS.

#### 7.4.6 Blinding

This is a double-blind trial. Since dasiglucagon is available as a liquid formulation and GlucaGen is available as a powder for reconstitution, and they are therefore not identical in appearance, unblinded trial personnel will be responsible for handling, preparing according to the prescription from the IWRS, administering the trial medication and keep the records strictly confidential and accessible only for unblinded staff until after database lock. To maintain double-blind conditions, all trial assessments at the trial site will be done by blinded trial personnel not involved in the administration of trial medications. However, exposure assessments and ADA assessments will be performed by unblinded personnel at the specialty laboratories, to make sure that dasiglucagon or GlucaGen administration is matched with the applicable bioanalytical assay.

#### 7.4.7 Drug accountability and disposal

Handling, preparation and administration of trial medication will be done by unblinded trial personnel. Each center will keep accurate records of the trial supplies received, stored, and dispensed, using appropriate forms. The trial supplies will be stored under appropriate conditions, locked and with restricted access.

All unused supplies and all empty and partially empty containers of trial medication will be stored until the trial closure visit has been performed and then sent to the sponsor.

#### 7.4.8 Prior and concomitant therapy

Prior glucagon exposure will be recorded in the eCRF at screening. All concomitant medications will be recorded in the eCRF at each visit.

Patients using any new concomitant medication resulting in fulfillment of a dosing day exclusion criterion will be excluded from the dosing visit, but can be rescheduled to one of the following days. Each dosing visit can only be rescheduled once. See Section 7.3.3.1 for possible reasons for patient discontinuation.

#### 7.4.9 Treatment after end of trial

Not applicable in this trial.

### 7.5 Assessments and schedule of measurements (overview)

The following assessments and measurements will be carried out at the times specified in the trial flow chart (Table 2-1).

Informed consent will be obtained prior to any trial-related procedures; see Section 11.3.

### 7.5.1 Screening examination

At screening (Visit 1), the following assessments will take place:

- Informed consent
- Check of patient eligibility
- Demographics
- Body measurement
- Medical history
- History of alcohol/drug abuse
- Concomitant illnesses
- Prior medications
- Concomitant medications
- Physical examination
- Vital signs
- ECG
- AEs
- Hematology, biochemistry, coagulation
- Pregnancy test (women of childbearing potential only)
- Urinalysis
- Urine drug screen (trial site testing)
- Alcohol breath test

### 7.5.2 Instructions to patients prior to dosing

On dosing days, patients are allowed to consume a small meal for breakfast with corresponding administration of rapid acting insulin to address the meal and in accordance with their normal management of blood glucose levels. At visit 2 and visit 4 the patient should aim to have a similar sized breakfast and corresponding rapid acting insulin dose.

Use of long-acting insulin and rate of basal continuous subcutaneous insulin infusion (CSII) will be continued according to normal glycemic management of the individual patients during dosing days.

### 7.5.3 Procedures and assessments during the treatment period of the trial

At Visit 2 (Day 0) patient eligibility is rechecked (check of changes between the screening visit and Visit 2) and patients eligible to participate will be randomized to treatment with dasiglucagon or GlucaGen.

Additionally, during the treatment period of the trial, at Visits 2 and 4 (Days 0 and 14), the following assessments will take place:

- Concomitant medication
- Check of withdrawal criteria
- Check of dosing day exclusion criteria
- Vital signs (pre-dose, and at 30, 90 and 120 min post-dosing)
- ECG (pre-dose, and at 20, 35, 45 and 60 min post-dosing)
- Local tolerability (at 0.5 and 2 h post-dosing)
- AEs
- Hematology and biochemistry (pre-dose, and at 30 and 90 min post-dosing)
- Urinalysis (pre-dose)

- Urine drug screen (pre-dose) at trial site
- Urine pregnancy test (pre-dose) at trial site (women of childbearing potential only)
- Alcohol breath test (pre-dose)
- Dasiglucagon/glucagon plasma concentrations
  (Pre-dose, 5, 10, 30, 60, and 90 min post-dosing. The actual time of blood sampling for exposure should not deviate from the nominal time by more than ±1 min. Pre-dose is defined as within 5 min prior to dosing.)
- Plasma glucose concentration
  (Pre-dose, 5, 10, 30, 60, and 90 min post-dosing. The actual time for blood sampling for plasma glucose should not deviate from the nominal time by more than ±1 min. Pre-dose is defined as within 5 min prior to dosing.)
- Antibodies against dasiglucagon/glucagon (pre-dose)
- Administration of trial medication
  Prior to administration of trial medication patients must reach a target plasma glucose level of 70-150 mg/dL. If the patient presents with plasma glucose > 150 mg/dL, corrective amounts of fast acting insulin will be administered at the investigator's discretion. Plasma glucose measured after 20 and after 40 minutes should ensure that the patient has reached the targeted 70-150 mg/dL. If the patient presents with plasma glucose < 70 mg/dL glucose ingestion will be provided in order to reach target plasma glucose of 70-150 mg/dL documented by plasma glucose measurements after 20 and 40 minutes, respectively . Patients must be fasting for 90 min after administration of trial medication, and can be treated individually to alleviate any potential side effects. See section 7.4.2. Patients will be observed for at least 5 h post-dose.

At Visit 3 of the treatment period (Day 7), only the following assessments will take place

- Concomitant medication
- Check of withdrawal criteria
- Check of dosing day exclusion criteria
- Vital signs (pre-dose, and at 30, 90 and 120 min post-dosing)
- ECG (pre-dose, and at 20, 35, 45 and 60 min post-dosing)
- Local tolerability (at 0.5 and 2 h post-dosing)
- AEs
- Urine drug screen (pre-dose) at trial site
- Urine pregnancy test (pre-dose) at trial site (women of childbearing potential only)Alcohol breath test (pre-dose)
- Antibodies against dasiglucagon/glucagon (pre-dose)
- Administration of trial medication
  Prior to administration of trial medication patients must reach a target plasma glucose level of 70-150 mg/dL. If the patient presents with plasma glucose > 150 mg/dL, corrective amounts of fast acting insulin will be administered at the investigator's discretion. Plasma glucose measured after 20 and after 40 minutes should ensure that the patient has reached the targeted 70-150 mg/dL. If the patient presents with plasma glucose < 70 mg/dL glucose ingestion will be provided in order to reach target plasma glucose of 70-150 mg/dL documented by plasma glucose measurements after 20 and 40 minutes, respectively. Patients are NOT required to be fasting after administration of trial medication, and can be treated individually to alleviate any potential side effects immediately following dosing. See section 7.4.2. Patients will be observed for at least 5 h post-dose.

### 7.5.4 Follow-up period of the trial

After the final dose of trial medication (at Visit 4, Day 14, described above), patients will be followed up until Day 104 (Visit 7). During the follow-up period, visits take place at Days 35, 60, and 104 (Visits 5, 6, and 7).

At Visits 5 and 6 during the follow-up period, the following assessments will take place:

- Concomitant medication
- Check of withdrawal criteria
- AEs
- Antibodies against dasiglucagon/glucagon

Additionally, the following assessments will take place at Visit 5 only:

- Vital signs
- ECG
- Hematology and biochemistry

At Visit 7, the final visit of the follow-up period and of the trial (EoT visit), the following assessments will take place:

- Concomitant medication
- Physical examination
- Vital signs
- ECG
- AEs
- Hematology and biochemistry
- Urinalysis
- Antibodies against dasiglucagon/glucagon

### 7.5.5 Final examination at the end of the trial

The final visit of the trial is Visit 7 (Day 104 of the follow-up period; EoT visit). See Section 7.5.4 for further details.

### 7.5.6 Unscheduled visit for ADA positive patients

A patient with a treatment induced or treatment boosted ADA response at visit 3-7 must be called in for an unscheduled visit. The procedures of this visit will be identical to the procedures performed at Visit 4 including administration of trial medication and assessments of pharmacodynamic and pharmacokinetic responses.

The visit should be scheduled as soon as the investigator is informed about a positive ADA result. The investigator should instruct the patient of the same dosing day requirements as for the regular dosing visits (Section 7.5.2) and instruct the patient to aim of having a similar sized breakfast and corresponding rapid acting insulin dose as prior Visit 2 and Visit 4.

A patient with a treatment induced or treatment boosted ADA response will be monitored until the ADA levels return to baseline.

### 7.5.7 Additional (safety) examinations

If there are any unclear symptoms or observations the responsible physician in charge may perform further medical examinations, other than outlined in this protocol, including further clinical laboratory tests, in order to clarify the relevance or to diagnose symptoms.

### 7.5.8 Safety laboratory tests

Samples obtained will be prepared and transferred to the appropriate laboratory by SynteractHCR according to Standard Operating Procedures. The parameters listed in Section 7.6.4.1 will be determined using standard methods.

The total volume of blood sampled per patient for safety analyses is 68 mL.

## 7.6 Immunogenicity, pharmacokinetic, pharmacodynamic, tolerability and safety measurements

Details of sampling for immunogenicity testing, plasma glucose concentrations, plasma trial medication concentrations, and safety laboratory testing are provided in a laboratory manual (see the Investigator site file).

### 7.6.1 Immunogenicity measurements

Antibodies against dasiglucagon/glucagon will be measured at all visits after screening. During the treatment period (Visits 2, 3, and 4) samples will be collected pre-dose as well as at unscheduled dosing visits for ADA positive patients.

The ADA samples will be analyzed at a special laboratory (refer to Appendix 1). Samples collected at the planned visits will be analyzed in batches at three occasions during the trial and any positive results will immediately be communicated to the investigator in a blinded manner.

The clinical ADA assays have been validated in accordance with existing guidelines and recommendations.[6,7,8,9,10]

Confirmed positive anti- dasiglucagon antibody samples, (treatment-induced or treatment-boosted) from anti-dasiglucagon antibody-positive patients will be evaluated for binding titer neutralizing potential and titer as well as cross-reactivity towards endogenous glucagon.

No further serum sampling will be needed as the ADA samples can be used for Nab analysis.

The *in vitro* neutralizing effect of antibodies will be measured using an assay[9,12] based on glucagon receptor transfected human embryonic kidney cells. The calculated sensitivity in previous studies was about 51.8 ng/mL. The assay was also validated for recombinant glucagon with similar results.[11,12] In case of a positive result in the Nab assay, a titer estimation will be performed. The cell-based Nab analyses will be performed by a special laboratory, BioAgilytix, Durham, NC, USA.

*In vivo* neutralizing effect will be evaluated by comparing PK and PD endpoints between ADA positive and ADA negative patients, further investigation may be performed by correlating ADA titer with PK and PD endpoints.

Residual and additional antibody serum samples may be stored until approval of market authorization by the health authorities. Further characterization of the antibody response may be requested by the health authorities.

### 7.6.2 Plasma concentrations of dasiglucagon and GlucaGen

The exposure to trial medication (dasiglucagon or GlucaGen) will be assessed based on plasma concentration data ($AUC_{0\text{-}30min}$, $AUC_{0\text{-}90\ min}$, $C_{max}$, $t_{max}$) from samples collected at Visits 2 and 4 (after administration of the first and third doses of trial medication).

Samples will be collected pre-dose, and at 5, 10, 30, 60, and 90 min post-dosing. The actual time of blood sampling for exposure should not deviate from the nominal time by more than ±1 min. Pre-dose is defined as within 5 min prior to dosing.

### 7.6.3 Pharmacodynamic measurements

The plasma glucose profile will be assessed based on plasma concentration data ($AUE_{0\text{-}30min}$, $AUE_{0\text{-}90\ min}$, $CE_{max}$, $t_{max}$) from samples collected at dosing Visits 2 and 4 (first and third dosing visit).

Samples will be collected pre-dose, and at 5, 10, 30, 60, and 90 min post-dosing. The actual time of blood sampling for evaluation of plasma glucose should not deviate from the nominal time by more than ±1 min. Pre-dose is defined as within 5 min prior to dosing.

The samples will be sent to the clinical laboratory and analyzed using a sensitive and validated assay for glucose measurements.

### 7.6.4 Safety and tolerability measurements

#### 7.6.4.1 Safety laboratory tests

Routine safety laboratory tests will be performed centrally. Samples for safety laboratory parameters will be collected at Visits 1, 2, 4, 5, and 7. Samples for urinalysis will be collected at Visits 1, 2, 4, and 7. The following parameters will be determined:

- Clinical chemistry: sodium, potassium, calcium, glucose, urea, creatinine, total bilirubin, AST (SGOT), ALT (SGPT), gamma-GT, alkaline phosphatase, total protein, C-reactive protein, $HbA_{1c}$, C-peptide
- Hematology: hemoglobin, red blood cell count (erythrocytes), hematocrit, platelet count (thrombocytes), total white blood cell count (leucocytes)
- Coagulation: international normalized ratio (INR), fibrinogen (at screening visit only)
- Urinalysis: pH, blood (leukocytes and erythrocytes), protein, glucose, ketones, nitrite

A pregnancy test will be performed at screening (Visit 1) and pre-dose at the three dosing visits (Visit 2, 3 and 4) for women of childbearing potential only.

Alcohol breath tests and urine drug screen (amphetamine, cocaine, MDMA, methamphetamine, opiate/morphine, marihuana) will be performed at Screening (Visit 1) and at Visits 2, 3, and 4.

For further details, please refer to the laboratory manual.

#### 7.6.4.2 Safety examinations

Physical examination is performed at Screening (Visit 1) and EoT (Visit 7).

AEs are assessed at all visits. Local tolerability is assessed at all dosing visits (Visits 2, 3, and 4). ECG is assessed at Screening (Visit 1) Visits 2, 3, and 4 and vital signs are assessed at Screening (Visit 1) Visits 2, 3, 4, 5, and 7.

- Physical examination includes examination of the following body systems: head, ears, eyes, nose, throat (HEENT), including the thyroid gland; heart, lung, chest; abdomen; skin; musculoskeletal system; nervous system; lymph node
- Vital signs include: pulse rate and blood pressure in a sitting position after 5 min, body temperature
- Local tolerability: skin reactions will be assessed at the injection site at 0.5 and 2 h post-dosing. Skin reactions will be reported as AEs (see Section 8).
- 12-lead ECG. Details from ECG assessments will be recorded, including PR, QRS and QT intervals.

# 8. Adverse events

## 8.1 Definitions

**Adverse Event (AE)**

An AE is any untoward medical occurrence in a trial patient administered an investigational medicinal product (IMP) and which does not necessarily have a causal relationship with the treatment. An AE can therefore be any unfavorable and unintended sign (for example, an abnormal laboratory finding), symptom, or disease temporally associated with the use of an IMP, whether or not considered related to the IMP.

Note: This includes events from the first trial related activity after the patient has signed the informed consent.

AEs include:

- A clinically significant worsening of a concomitant illness.
- A clinical laboratory abnormality which is clinically significant, i.e. any abnormality that suggests a disease and/or organ toxicity and is of a severity that requires active management. Active management includes active treatment or further investigations, for example change of medicine dose or more frequent follow-up due to the abnormality.
- Hypoglycemic episodes (see Section 8.4)
- Injection site reactions

The following should not be recorded as AEs, if recorded at screening (on Screening Form or CRF):

- Pre-planned procedure, unless the condition for which the procedure was planned has worsened from the first trial related activity after the patient has signed the informed consent.
- Pre-existing conditions found as a result of screening procedures (pre-existing conditions should be reported as medical history or concomitant illness).

**Serious adverse event (SAE)**

A SAE is any untoward medical occurrence that at any dose:

- results in death
- is life-threatening
- requires inpatient hospitalization or prolongation of existing hospitalization
- results in persistent or significant disability or incapacity
- is a congenital anomaly or birth defect
- is medically important
  Medical judgement must be exercised in deciding whether an AE is believed to be 'medically important'. Medically important event may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above.

**Suspected unexpected serious adverse reactions (SUSAR)**

An AE, fulfilling one of the criteria of seriousness and being assessed as related to IMP application, the nature or severity of which is not consistent with the applicable reference document (e.g. ZP4207 IB[3] or package leaflet/SmPC for an approved product such as GlucaGen[13]).

**Clinical event of interest**

A clinical event of interest is an event which, in the evaluation of safety, has a special focus (e.g. required by health authorities).

In this trial hemodynamic changes, as defined below, are considered clinical events of interest:

- Post-dose clinical signs or measured vital signs indicating a clinical significant drop in blood pressure including signs of orthostatic hypotension, vasovagal responses or bradycardia.
- Post-dose change in pulse or blood pressure considered an event of hypo- or hypertension as judged by the investigator.

**Intensity of an adverse event**

The maximum intensity (severity) of all AEs must be assessed by the Investigator and documented. Severity should be graded when the AE outcome is known:

Mild: A type of adverse event that is usually transient and may require only minimal treatment or therapeutic intervention. The event does not generally interfere with usual activities of daily living.

Moderate: A type of adverse event that is usually alleviated with additional specific therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort but poses no significant or permanent risk of harm to the research participant.

Severe: A type of adverse event that interrupts usual activities of daily living, or significantly affects clinical status, or may require intensive therapeutic intervention.

A 'severe' reaction does not necessarily deem the AE as 'serious' and a SAE may not be 'severe' in nature.

**Causality relationship to trial medication**

The causality of each AE should be assessed by the Investigator according to the following classification:

Probable: Good reason and sufficient documentation to assume a causal relationship.

Possible: A causal relationship is conceivable and cannot be dismissed.

Unlikely: The event is most likely related to etiology other than the trial product.

Not related: No relationship to trial product

**Outcome of an adverse event**

The outcome of all AEs must be assessed by the Investigator and documented by his/her staff. The following definitions should be used:

Recovered/resolved: The patient has fully recovered, or by medical or surgical treatment the condition has returned to the level observed at the first trial-related activity after the patient signed the informed consent.

| | |
|---|---|
| Recovering/resolving: | The condition is improving and the patient is expected to recover from the event. This term is only applicable if the patient has completed the trial. |
| Recovered/resolved with sequelae: | The patient has recovered from the condition, but with lasting effect due to a disease, injury, treatment or procedure. If a sequela meets an SAE criterion, the AE must be reported as an SAE. |
| Not recovered/ not resolved: | The condition of the patient has not improved and the symptoms are unchanged. |
| Fatal: | This term is only applicable if the patient died from a condition related to the reported AE. Outcomes of other reported AEs in a patient before he/she died should be assessed as "recovered/resolved", "recovering/resolving", "recovered/ resolved with sequelae" or "not recovered/not resolved". An AE with fatal outcome must be reported as an SAE. |
| Unknown: | This term is only applicable if the patient is lost to follow-up. |

### 8.2 Collection, recording and reporting of adverse events

All events meeting the definition of an AE must be collected and reported from the first trial related activity after the patient has signed the informed consent until the end of the post-treatment follow-up period. At each contact with the site (visit or telephone, excluding visits, where the patient is not seeing the Investigator or his staff (e.g. visits to the laboratory)) the patient must be asked about AEs. All AEs, either observed by the Investigator or reported by the patient, must be recorded by the Investigator and evaluated.

The Investigator should record the diagnosis, if possible. If no diagnosis can be made the Investigator should record each sign and symptom as individual AEs.

All AEs must be recorded by the Investigator. One single Adverse Event Form must be used per AE from start to resolution. For SAEs, the Serious Adverse Event Form must also be completed. For clinical events of interest, the Clinical Event of Interest Form must also be completed.

AE information should include the following:

- Date and time of onset and resolution
- Date and time of Investigator's first information on the (S)AE
- Seriousness
- Severity
- Causal relationship with IMP
- Interruption or withdrawal of treatment with IMP and other measures taken
- Outcome

All AEs are coded; details are described in the trial specific Data Management Plan.

If an event classifies as a clinical event of interest, the Investigator must tick the Clinical Event of Interest box on the AE form and complete the Clinical Event of Interest Form. The Clinical Event of Interest form will capture if the event was associated with any signs or symptoms and capture the highest/lowest blood pressure and pulse measured during the event.

The Investigator must report initial information in writing (fax or email) on all SAEs to the Sponsor's responsible pharmacovigilance unit (Safety CRO) immediately (within 24 hours) after

obtaining knowledge about the event. Please refer to Appendix 1 for contact details. The Safety CRO will inform the medical monitor and the sponsor about the reported SAEs.

It is the responsibility of the Safety CRO to report all SUSARs that occur in this trial to the Competent Authorities and IRBs/IECs in accordance with the local requirements in force and ICH guideline for GCP. The trial monitor must be informed accordingly.

### 8.3 Follow-up of adverse events

All AEs that are ongoing at the end of the patient's participation in the study will be followed-up until the event is resolved or reaches a satisfactory outcome as deemed by the Investigator.

Follow-up actions for all SAEs will be performed according to appropriate clinical care practices and may depend on the nature of the event. These will be determined after internal review and/or sponsor review.

The follow up information should only include new (updated and/or additional) information that reflects the situation at the time of the Investigator's signature.

Follow-up information on (S)AEs will be updated using the (S)AE Form. If a non-serious event becomes serious during the follow-up the AE Form and SAE Form have to be used and reporting timelines follow those of a SAE.

The Investigator must forward follow-up information on SAEs and if previously non-serious AEs become SAEs to the Sponsor immediately (within 24 hours) after obtaining knowledge about the new information.

### 8.4 Hypoglycemia

Hypoglycemia will be regarded as an AE and recorded and documented on an AE Form.

Hypoglycemia is defined as a fall of plasma glucose below 3.9 mmol/L (70 mg/dL).

During the dosing visits, mild to moderate symptoms of hypoglycemia, or a blood glucose (BG) value less than a threshold of <2.8 mmol/L (50 mg/dL), corresponding to a plasma glucose (PG) value of <3.1 mmol/L (56 mg/dL), will be treated by i.v. glucose solution at the investigator's discretion according to best available medical practice. Treatment is to be repeated until BG value stabilized above the threshold limit again. BG measurements will only be done due to safety concerns. Insulin-induced hypoglycemia should be recorded as an AE.

### 8.5 Pregnancy

Female patients must be instructed to notify the Investigator immediately if they become pregnant or if they suspect to be pregnant during the trial. The Sponsor has a responsibility to monitor the outcome of all pregnancies reported during the clinical trial.

Male patients must be instructed to notify the Investigator immediately if their partner becomes pregnant or suspects to be pregnant. The Sponsor has a responsibility to monitor the outcome of all pregnancies reported during the clinical trial. During an information meeting at the trial site, the patient's partner will be fully informed by a physician of her participation in capturing the outcome data, and will be asked to provide her written consent.

The Investigator must report all information on pregnancies on the initial pregnancy form. The completed initial pregnancy form must be forwarded to the Sponsor according to the procedure stated in Section 8.2. Any (S)AEs in the mother, as well as other pregnancy complications (ectopic pregnancy) fulfilling the criteria of an (S)AE, must be reported on the (S)AE form.

The following must be collected in the initial pregnancy form:

- Medical history of the mother
- Family history

- Course of the pregnancy, including expected delivery date

The Investigator must follow the pregnancy until the pregnancy outcome and the newborn infant(s) until the age of 1 month. The Investigator must collect information on the pregnancy and pregnancy complications as well as the pregnancy outcome including the health of the newborn infant(s) on the pregnancy outcome form. The completed pregnancy outcome form must be forwarded to the Sponsor according to the procedure stated in Section 8.2. Any (S)AEs in the newborn must be reported on the (S)AE form.

The SAEs that must be reported including abnormal outcome - such as congenital anomalies, fetal death and termination of pregnancy (spontaneous or elective abortion), including any anomalies of the fetus observed at gross examination or during autopsy.

The following must be collected in the pregnancy outcome form:

- Course of the pregnancy
- Outcome of the pregnancy
- Condition of the newborn
- Any AEs in the newborn infant must be followed till the age of 1 month

## 8.6 Precautions

Normal precautions taken for a human trial, including the provision of emergency equipment, will be taken during this trial. Qualified and well trained physicians and medical staff will instruct the patients. During a patient's participation in the trial, the Investigator should ensure that adequate medical care is provided to the patients for any AEs, including clinically significant laboratory values related to the trial. The Investigator should inform the patient when medical care is needed for intercurrent illnesses of which the Investigator becomes aware.

For further information on safety precautions for ZP4207 and GlucaGen, please refer to the current version of the Investigator's Brochure[3] and the SmPC for GlucaGen[13], respectively.

## 8.7 Safety Committee

The internal Zealand Pharma Safety Committee (SC) is constituted to perform ongoing safety surveillance of clinical trials with dasiglucagon, including this trial.

If safety signals are observed, either based on reported SAEs, periodic review of laboratory parameters, review of all AEs reported between the SC meetings, or on notification of significant findings, the SC will take appropriate measures to safeguard the patients.

As a minimum the SC convenes every quarter to review relevant safety information, including AEs and laboratory data.

## 9. Data management and quality control

### 9.1 Case report forms

All the information collected during the trial will be recorded in the eCRFs, which are identified by patient number. Suitable eCRFs will be designed by SynteractHCR. The investigator will ensure that the eCRFs are correctly completed. All key pages will be signed or initialed by the investigator, signifying agreement with and responsibility for the recorded data. Key pages are the following: AE-reporting form, trial medication form, concomitant medication form and trial-closure form.

Any information (e.g. results from the neutralizing antibody assay or additional unscheduled visits) may be reported separately pending availability of the results.

Data directly captured in the eCRF (i.e., data assigned or calculated automatically by the EDC system) is called e-source. This means that these data fields in the CRF are source documents. For the other CRF data fields, which are entered by medical personnel during the trial, the source data will be found in other documents (such as patients' files, worksheets, etc.), i.e. for these CRF data fields the "other" documents are the source documents.

### 9.2 Quality control

The investigator will permit trial-related monitoring, IEC review, and regulatory inspections, providing direct access to source data /documents. Sponsor-authorized quality assurance personnel may carry out audits for which the investigator must provide support.

The trial will be supervised by a monitor from SynteractHCR. The trial monitor will contact the investigator regularly to discuss the progress of the trial and to check the trial documents including the informed consent forms for completeness and consistency.

The trial monitor or a representative of the sponsor will cross-check the data entered in the eCRFs with the source data at the trial site and observe the trial procedures in order to verify adherence to the trial protocol.

The eCRFs will be checked for completeness and correctness by the monitor and data management department of SynteractHCR according to the SynteractHCR SOPs and any queries will be resolved by the investigator.

All of the clinical data will be captured via electronic data capture (EDC) using a web-based tool. The software Marvin from the company XClinical (www.xclinical.com/) is the preferred EDC software. Marvin is compliant with all legislation relevant to electronic data capture (FDA 21 CFR Part 11, GCP).

The investigator site staff will enter and edit the data via a secure network, with secure access features (username and password). A complete electronic audit trail will be maintained. The investigator will approve the data using an electronic signature (Ref: 21 CFR Part 11), and this approval is used to confirm the accuracy of the data recorded.

eCRFs will be used for all patients. The investigator's data will be accessible from the investigator's site throughout the trial. The electronic CRFs must be kept current to reflect patient status at each phase during the course of the trial. The electronic CRF will not capture personalized data. The investigator must make a separate confidential record of personalized details (name and initials) on the patient identification and enrollment log. All changes to data are done by the investigator through the EDC system.

It is the responsibility of the Principal Investigator of the respective site to ensure that all patient discontinuations or changes in trial or other medications entered on the patient's eCRF are also made on the patient's medical records.

The eCRFs for any patient leaving the trial should be completed at the time of the final visit or shortly thereafter.

### 9.3 Data management

Data management will be performed according to SynteractHCR SOPs.

# 10. Statistical methods and determination of sample size

## 10.1 Statistical and analysis plan

A separate Statistical Analysis Plan (SAP) will be finalized that details the planned statistical analysis and may include necessary adaptations to the planned statistical analysis before unblinding of the data.

Any deviations from the planned analyses will be described and justified in the final integrated trial report.

### 10.1.1 General considerations

All data obtained in this trial and documented in the eCRFs will be listed and summarized with statistics or frequency tables as appropriate. In case of termination of the trial, all data collected up to that time point will be included into the analysis.

Raw data listings and summary tables will be generated using the software SAS© Version 9 or higher.

All statistical analysis will be descriptive i.e. no formal testing will be performed.

Continuous variables will be summarized with means, standard deviations, medians, minimums, and maximums and valid cases.

Other summaries (e.g. quartiles, 95% confidence intervals) may be used as appropriate. Categorical efficacy and safety variables will be summarized by counts and by percentage of patients in corresponding categories.

### 10.1.2 Classification of patients to subsets

For the statistical analysis the randomized patients will be divided up into the following datasets:

The following definitions are applicable:

| | |
|---|---|
| **Safety analysis set (SAS)** | all patients who were randomized and received at least one dose of trial medication |
| **Full analysis set (FAS)** | all patients of the SAS with at least one measurement of the ADA titer at baseline |
| **Per Protocol set (PPS)** | all patients of the FAS for whom no relevant protocol deviations were documented |

The analysis of the primary endpoint will be based on the FAS. A secondary analysis of the primary endpoint will be based on the PPS. Safety analysis will be based on the SAS.

The decision whether a protocol deviation is relevant or not for the exclusion of patients from the PPS set will be made case-by-case in a data review meeting.

### 10.1.3 Immunogenicity data

All immunogenicity parameters will be analyzed descriptively per treatment group. No statistical tests are planned. Baseline ADA-positive patients will be calculated as a percentage of the total number of patients whose baseline samples were tested for ADA. Titer will be reported as median and interquartile range.

95% confidence intervals for incidence rates and for rate differences will be given.

Secondary immunogenicity parameters will be described with appropriate descriptive statistics for dichotomous, categorical or continuous variables.

#### 10.1.4 Exposure and PD endpoints

Plasma dasiglucagon and glucagon concentrations 0-90 min from dosing: $AUC_{0\text{-}30min}$, $AUC_{0\text{-}90\ min}$, $C_{max}$, and $t_{max}$ will be summarized with descriptive statistics.

Plasma glucose profiles 0-90 min from dosing: $AUE_{0\text{-}30min}$, $AUE_{0\text{-}90\ min}$, $CE_{max}$, and $t_{max}$ will be summarized with descriptive statistics.

Achieving a plasma glucose increase of ≥20 mg/dL (1.1 mmol/mL) within 30 minutes post-treatment will be summarized with descriptive statistics.

#### 10.1.5 Safety data

**Clinical laboratory data**

Clinical laboratory test results will be marked whether the result is below, within or above the respective reference range. The number of values outside of the reference range will be counted.

**Adverse events**

Adverse events will be tabulated by system organ class (SOC) and preferred term (PT) after medical coding using the Medical Dictionary for Regulatory Activities (MedDRA). AE summary tables will include counts and percentages of patients who experienced adverse events summarized by system organ class (SOC) and preferred term (PT).

**Other safety data**

Vital signs, physical examination, ECG and local tolerability data will be summarized with descriptive statistics.

#### 10.1.6 Further data

[redacted]

All other data obtained in this trial and documented in the eCRF will be listed.

#### 10.1.7 Withdrawals, drop-outs and missing data

In the case of drop-outs, no imputation of values for immunogenicity measurements will be done. Analysis is done on valid cases only i.e. no imputation technique like LOCF (last observation carried forward) will be applied.

#### 10.1.8 Baseline and center comparisons

Demographic and other baseline characteristics will be compared.

#### 10.1.9 Subgroup analysis

Subgroup analyses for the primariy endpoint (voerl ADA incidence) by gender, age, and race will be performed.

#### 10.1.10 Interim analysis

No interim analysis is currently planned.

### 10.2 Determination of sample size

The purpose of the present trial is to generate data describing the immunogenic potential of dasiglucagon and GlucaGen. The ADA assays to be used in this trial are both validated, but

specific for GlucaGen and dasiglucagon, respectively, and the performance of the assays are thus not directly comparable. As a consequence, a formal comparison or non-inferiority analysis will not be performed

Currently, no ADA incidences have been detected in the completed clinical trials, where up to 5 repeated doses of dasiglucagon have been administered to the same patients within a week. Across the two phase 1 clinical trials and a completed phase 2 PK/PD trial, a total of 141 subjects have been exposed to dasiglucagon and no incidences of ADA development have been observed. The obtained data indicate that dasiglucagon has a low risk for induction of ADAs in the investigated settings and as a consequence a meaningful sample size to compare both treatments cannot be estimated.
The sample size is therefore based on a certain precision of the confidence interval for the overall ADA incidence if no events are observed, respectively to ensure a certain probability for observing one event.

When no events are observed, to obtain an upper bound of 0.050 on the 90.0% confidence interval for the probability of such a rare event, would require a sample size of 45. Respectively, accepting a chance of observing at least one event of 90% and an actual probability of the event of 5% leads to a sample size of 45 patients completing the trial. In order to account for drop-outs, It is expected that 112 patients in total will be randomized and treated.

# 11. Ethics and regulations

## 11.1 Independent ethics committees and competent authorities

The clinical trial authorization (CTA) granted by the competent authority (CA) and a favorable opinion from the relevant independent ethics committee(s) (IEC(s)) /Institutional Review Board (IRB) will be obtained prior to the start of the trial. The local authorities will be notified about the trial as required by law.

The CA and the EC/IRB will be notified about the end of the trial and a report summarizing the trial results will be sent to the CA and the EC within one year after the end of the trial. If the trial is terminated early, the CA and the EC will be notified within 15 days.

The IECs and/or IRBs met the requirements of the International Conference on Harmonisation (ICH) Harmonised Tripartite Guideline for Good Clinical Practice (GCP) and local legislation. They also met the requirements of 21 CFR 312.3.

## 11.2 Ethical conduct of the trial

The trial will be conducted in accordance with the ethical principles set forth in the Declaration of Helsinki (including amendments).

## 11.3 Patient information and consent

Written informed consent will be obtained from all patients prior to entry into the trial. The investigator will explain to each patient orally and in writing (patient information sheet) the nature, significance, risks and implications of the trial before inclusion. In particular, the patients will be informed about the following:

- the possibility of withdrawing from the clinical trial at any time by revoking the consent and without any resulting disadvantage
- how personal and health-related data will be collected and used during the trial
- the patient must be informed that his/ her medical records may be examined by authorized monitors or Clinical Quality Assurance auditors appointed by the Sponsor, by appropriate IEC members, and by inspectors from regulatory authorities.

All patients will receive a copy of the patient information sheet and a copy of their signed and dated informed consent form.

All patients will be insured against injury caused by their participation in the trial according to legal requirements. They will be informed about the insurance and the resulting obligations on their part.

## 11.4 Legal and regulatory requirements

This trial will be carried out in accordance with:

- ICH guidelines for GCP, United States investigational new drug (IND) regulations (21 CFR 312), the regulations on electronic records and electronic signature (21 CFR 11), the most recent guidelines of the Declaration of Helsinki, and the relevant laws and regulations of the country in which the trial takes place.
- Standard operating procedures (SOPs for clinical investigation and documentation in force at SynteractHCR)

# 12. Trial administration

## 12.1 Responsibilities

Zealand A/S is the sponsor of this trial. SynteractHCR, a contract research organization (CRO), will organize the performance of this trial.

A list with the names and addresses of the responsible institutions and persons is provided in Appendix 1 of this protocol.

## 12.2 Protocol deviations

The investigator agrees to conduct the trial in compliance with the protocol. Prospective protocol deviations or waivers will not be granted for this trial.

Any deviation from the clinical trial protocol in the conduct of the clinical trial will be notified to the Monitor on an ongoing basis.

## 12.3 Protocol changes

Amendments to this trial protocol may be made following the procedures specified by local laws and regulations. Substantial amendments to this trial protocol may be implemented only if the approval of the CA(s) and a favorable opinion of the ethics committee(s) have been obtained.

Substantial amendments to the conduct of the clinical trial may arise from changes to the protocol or from new information relating to the scientific documents in support of the trial. Amendments to the trial are regarded as "substantial" where they are likely to have a significant impact on:

- the safety, physical health and mental integrity of the patients;
- the scientific value of the trial;
- the conduct or management of the trial; or
- the quality or safety of any investigational medicinal product used in the trial.

If a new event occurs related to the conduct of the trial or the development of the investigational product, which may affect the safety of the patients, the sponsor and the investigator will take appropriate safety measures to protect the patients against any immediate hazard. The sponsor will immediately inform the CA(s) and ethics committee(s) of the new events and the measures taken.

## 12.4 Publication of results

The original eCRFs and the data generated from the eCRFs or otherwise obtained during the trial under this trial protocol will become the property of the sponsor. Publication of the results of this trial by SynteractHCR or the investigator is possible only after written consent has been obtained from the sponsor. Any material intended for publication will be given to the sponsor at least 4 weeks before submission for publication. The sponsor will have the right to comment on the intended publication and to take any reasonable measures for patent protection.

## 12.5 Clinical trial report

After completion of the trial, the results will be tabulated, evaluated and issued as a complete final clinical trial report according to the ICH-E3 Note for guidance on structure and content of clinical trial reports.

The sponsor will send a summary of this clinical trial report to the EC and CA within one year after the end of the trial.

### 12.6 Retention of trial records

Records and documents pertaining to the conduct of the trial and the distribution of the investigational product (e.g. ICFs, laboratory slips, medication inventory records, and other pertinent information) must be retained by the Investigator according to local requirements.

To meet regulatory requirements, the eCRF data will be electronically stored at sites. The CDISC ODM (see http://www.cdisc.org/ for details) will be used to store and archive all electronic data at the sites. Since CDISC ODM is also the source for the EDC-web-based system, no transcription of data is necessary. CDISC ODM is a platform-independent standardized data format including the complete trial metadata and audit trail. The data can be reviewed at a later stage using off-the-shelf tools. CDISC provides a complete CDISC ODM Viewer for these purposes. If needed, PDF-files can be created from the ODM file.

## 13. References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9. Commissioning and validation of a method for the validation of anti-ZP4207 antibodies in human serum. Zealand Pharma Report, Study 14-173
10. Commissioning and validation of a method for the validation of anti-glucagon antibodies in human serum. Zealand Pharma Report, Study 14-174
11. Bioagilytix Labs. Validation of an Assay to detect neutralizing Antibodies to ZP4207 by Measuring Cyclic Adenosine Monophosphate (cAMP) in Glucagon Receptor Transected Cell Line, study no. BAL-14-206-004-REP. Zealand Pharma, Study 15-039. 09 Aug 2016.
12. Bioagilytix Labs. Validation of an Assay to Detect Neutralizing Antibodies to Glucagon by Measuring Cyclic Adenosine Monophosphate (cAMP) in a Glucagon Receptor Transfected Cell Line, BAL-14-206-005. Zealand Pharma, Study 15-040. 04 Jul 2016.
13. SPC Novo Nordisk GlucaGen® HypoKit 1 mg powder and solvent for solution for injection. http://www.medicines.org.uk/emc/medicine/4258/SPC/GlucaGen+Hypokit+1+mg

# APPENDIX 1:

## List of names and addresses

| | |
|---|---|
| Sponsor | Zealand Pharma A/S<br>Smedeland 36<br>2600 Glostrup, Copenhagen<br>Denmark |
| Contract Research Organization (CRO) | SynteractHCR Deutschland GmbH<br>Albrechtstr. 14<br>80636 Munich, Germany |
| Coordinating investigator | Thomas R. Pieber, MD<br>Medical University of Graz<br>Auenbruggerplatz 15<br>A-8036 Graz, Austria |
| Trial monitor | SynteractHCR Deutschland GmbH<br>Albrechtstr. 14<br>80636 Munich, Germany |
| Statistician | [redacted]<br>Senior Director Biometrics EMEA<br>SynteractHCR Deutschland GmbH<br>Albrechtstr. 14<br>80636 Munich, Germany |
| Sponsor representative | [redacted]<br>Clinical Project Manager<br>Zealand Pharma A/S<br>Smedeland 36, 2600 Glostrup, Denmark<br>Tel: [redacted]<br>E-mail: [redacted] |
| Sponsor's medical expert | [redacted] Medical OfficerZealand Pharma A/S<br>Smedeland 36, 2600 Glostrup, Denmark<br>Tel: [redacted]<br>E-mail: [redacted] |
| Sponsor representative for pharmacovigilance | [redacted]<br>PharmaLex A/S Agern Allé 24, 2970 Hørsholm, Denmark<br>Tel: [redacted] (8 a.m. to 4 p.m.)<br>[redacted] (outside 8 a.m. to 4 p.m.)<br>Fax: [redacted]<br>E-mail: drugsafety@lindeq.com |
| Clinical laboratory | MLM Medical Labs Gmbh<br>[redacted]<br>Dohrweg 63, 41066 Mönchengladbach, Germany |

| | |
|---|---|
| | Tel: [redacted]<br>E-mail: [redacted] |
| Analytical laboratory<br>(ZP4207 PK, ZP4207 ADA, Glucagon ADA) | York Bioanalytical Solutions (YBS)<br>[redacted] (ZP4207 Bioanalysis)<br>[redacted] (ADA analyses)<br>Cedar House<br>Northminster Business Park<br>Northfield Lane<br>York, YO26 6QR<br>United Kingdom<br>E-mail: [redacted]<br>E-mail: [redacted] |
| (ZP4207 and Glucagon Nab) | [redacted]<br>BioAgilytix Labs<br>2300 Englert Drive<br>Durham, NC 27713<br>USA<br>[redacted] |