16.APPENDICES

16.1 Study Information

16.1.1 Protocol and Protocol Amendments

The following protocols are provided in this Appendix:

Clinical Study Protocols and Protocol Amendments:	
Version	**Date**
Final Version 5.0	04 Apr 2018
Final Version 4.0 (Germany only)	26 Jan 2018
Final Version 3.0	06 Oct 2017
Final Version 2.0	31 Aug 2017
Final Version 1.0	29 Aug 2017

CLINICAL TRIAL PROTOCOL

A phase 3, randomized, double-blind, parallel trial to confirm the clinical efficacy and safety of dasiglucagon in the rescue treatment of hypoglycemia in subjects with type 1 diabetes mellitus (T1DM) compared to placebo and with reference to GlucaGen®

Sponsor:	Zealand Pharma A/S
Sponsor Protocol No.:	ZP4207-16137
EudraCT No.:	2017-002449-31
IND No:	127866
Trial Drug Name:	Dasiglucagon* injection
Date of Protocol:	04-Apr-2018

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

*Dasiglucagon is the proposed international nonproprietary name for ZP4207.

Updated protocol, Final version 5.0 including:
Protocol, Final version 3.0, dated 06-Oct-2017
Local protocol amendment 1 (Germany), Final version 1.0, dated 26-Jan-2018
Global protocol amendment 2, Final version 1.0, dated 04-Apr-2018

Declaration of sponsor or responsible medical officer

Title: A phase 3, randomized, double-blind, parallel trial to confirm the clinical efficacy and safety of dasiglucagon in the rescue treatment of hypoglycemia in subjects with type 1 diabetes mellitus (T1DM) compared to placebo and with reference to GlucaGen®

This trial protocol 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 Declaration of Helsinki and the guidelines on Good Clinical Practice (GCP) (1).

Name	[redacted]	Date
Title	Clinical Trial Manager	
Institution	Zealand Pharma A/S Smedeland 36 2600 Glostrup, Denmark	
Phone:	[redacted]	

Name	[redacted]	Date
Title	Medical Officer	
Institution	Zealand Pharma A/S Smedeland 36 2600 Glostrup, Denmark	
Phone:	[redacted]	

Declaration of the coordinating investigator

Title: A phase 3, randomized, double-blind, parallel trial to confirm the clinical efficacy and safety of dasiglucagon in the rescue treatment of hypoglycemia in subjects with type 1 diabetes mellitus (T1DM) compared to placebo and with reference to GlucaGen®

I have read this protocol. I approve this document and I agree that it contains all necessary details for carrying out the trial as described. I will conduct this trial in accordance with the design and specific provision of this protocol and will make a reasonable effort to complete the trial within the time designated. I will provide copies of this protocol and access to all information furnished by Zealand Pharma A/S to trial personnel under my supervision. I will discuss this material with them to ensure they are fully informed about the trial product and trial procedures. I will let them know that this information is confidential and proprietary to Zealand Pharma A/S and that it may not be further disclosed to third parties. I understand that the trial may be terminated or enrollment suspended at any time by Zealand Pharma A/S, with or without cause, or by me if it becomes necessary to protect the best interests of the trial subjects.

I agree that the trial will be carried out in accordance with GCP (1), with the Declaration of Helsinki (with amendments) (2) and with the laws and regulations of the countries in which the trial takes place.

_____ _____

Name Date
Title
Institution
Phone: +
Fax: +

Declaration of the investigator

Title: A phase 3, randomized, double-blind, parallel trial to confirm the clinical efficacy and safety of dasiglucagon in the rescue treatment of hypoglycemia in subjects with type 1 diabetes mellitus (T1DM) compared to placebo and with reference to GlucaGen®

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 this trial protocol, Investigator's Brochure, Case Report Forms, 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 subjects.

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

_____ _____

Signature Date

_____

Name (block letters)

_____

Title (block letters)

_____

Institution (block letters)

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List of abbreviations and definitions of terms

ADA	Anti-drug antibody
AE	Adverse event
AESI	Adverse event of special interest
ALT	Alanine aminotransferase
ANCOVA	Analysis of covariance
AST	Aspartate aminotransferase
AUC	Area under the concentration-time curve
$AUC_{0\text{-inf}}$	AUC from time zero to infinity
AUE	Area under the effect curve
CFB	Changes from baseline
CI	Confidence interval
C_{max}	Maximum plasma concentration
CPH	Cox proportional hazards
CRO	Contract research organization
ECG	Electrocardiogram
eCRF	Electronic case report form
EDC	Electronic data capture
EU	European Union
EudraCT	European Medicines Agency's Clinical Trials Database
FAS	Full analysis set
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HbA_{1c}	Glycated hemoglobin
ICH	International Conference on Harmonization
ID card	Identification card
IEC	Independent Ethics Committee
IM	Intramuscular(ly)
IMP	Investigational medicinal product
IRB	Institutional Review Board
IV	Intravenous(ly)

IWRS	Interactive Web Response System
MedDRA	Medical Dictionary for Regulatory Activities
NPH	Neutral protamine Hagedorn
PD	Pharmacodynamics
PK	Pharmacokinetics
PP	Per protocol
SAE	Serious adverse event
SAP	Statistical analysis plan
SAS	Safety analysis set
SC	Subcutaneous(ly)
SUSAR	Serious unexpected suspected adverse reaction
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
TEAE	Treatment-emergent adverse event
t_{max}	Time to the maximum plasma concentration
ULN	Upper limit of the normal range
US	United States of America

1 SYNOPSIS

Name of sponsor: Zealand Pharma A/S	**Trial ID:** ZP4207-16137
Title of the trial: A phase 3, randomized, double-blind, parallel trial to confirm the clinical efficacy and safety of dasiglucagon in the rescue treatment of hypoglycemia in subjects with type 1 diabetes mellitus (T1DM) compared to placebo and with reference to GlucaGen®	
Trial design: The trial is a global, multicenter, randomized, parallel-group, double-blind, clinical trial confirming the efficacy and safety of dasiglucagon for insulin-induced hypoglycemia in subjects with type 1 diabetes mellitus. The subjects will be randomized 2:1:1 to receive a single fixed subcutaneous 0.6 mg dose of dasiglucagon (hereinafter dasiglucagon), placebo for dasiglucagon (hereinafter referred to as placebo), or a 1 mg dose of GlucaGen® (hereafter referred to as GlucaGen) and followed for at least 28 days after treatment.	
Clinical phase of development: Phase 3	
Trial centers: This trial will be conducted at 4 to 6 sites in the United States of America, Canada, and Europe.	
Planned trial start (first subject first visit): Q4/2017	**Planned trial end (last subject last visit):** Q3/2018
Trial population: Male and female adult subjects with type 1 diabetes mellitus treated with insulin for at least one year	
Key objectives: **Primary objective:** • To demonstrate superiority of dasiglucagon compared to placebo following a single subcutaneous 0.6 mg dose administered to subjects with type 1 diabetes mellitus with insulin-induced hypoglycemia. **Secondary objective:** • To compare the glycemic response observed after dasiglucagon with that of GlucaGen.	
Key endpoints: **Primary endpoint:** • Time to plasma glucose recovery. Plasma glucose recovery is defined as first increase in plasma glucose of ≥20 mg/dL (1.1 mmol/L) from baseline during the hypoglycemic clamp procedure without administration of rescue intravenous (IV) glucose. **Key secondary endpoints:** • Plasma glucose recovery within 30 minutes, within 20 minutes, within 15 minutes, and within 10 minutes after study drug injection without administration of rescue IV glucose. • Plasma glucose changes from baseline within 30 minutes, within 20 minutes, within 15 minutes, and within 10 minutes after study drug injection or at the time of rescue. **Clinical efficacy (Pharmacodynamic) endpoints:** • Time to first plasma glucose concentration of ≥70 mg/dL (3.9 mmol/L) without administration of rescue IV glucose.	

- Plasma glucose response as area under the curve above baseline from time zero to 30 minutes, $AUC_{0\text{-}30min}$.

Exposure (Pharmacokinetic) endpoints:

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

Safety endpoints:

- Adverse events, clinical laboratory assessments (biochemistry, hematology, urinalysis), vital signs, physical examination, electrocardiogram, and local tolerability.
- Administration of rescue infusion of IV glucose during the hypoglycemic clamp procedure.
- Time to first rescue infusion of IV glucose during the hypoglycemic clamp procedure.

Immunogenicity endpoint:

- Occurrence of anti-drug antibodies

Exploratory endpoint:

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

Key inclusion criteria:

- Male and female subjects with type 1 diabetes mellitus treated with insulin for at least one year, diagnostic criteria as defined by the American Diabetes Association.
- Stable insulin treatment 30 days prior to screening, defined as no more than a 10-unit daily variation in total daily insulin dose.
- Hemoglobin A_{1c} <10%.
- Aged between 18 and 75 years, both inclusive.

Key exclusion criteria:

- Previously treated with dasiglucagon.
- Known or suspected allergy to trial product(s) or related products.
- History of anaphylaxis or symptoms of severe systemic allergy (such as angioedema).
- Previous participation (randomization) in this trial.

Sample size:
Approximately 156 subjects are intended to complete the trial, with 78 subjects randomized to the dasiglucagon group and 39 subjects randomized to each of the placebo and GlucaGen groups.

Investigational medicinal product:
Test product: dasiglucagon liquid formulation in pre-filled syringes.
Comparator products: Placebo and GlucaGen® lyophilized powder.
Germany only: Insulin glulisine (Apidra®), the challenge agent, will also be defined as an investigational medicinal product.

Duration of treatment:
Subjects will be randomized 2:1:1 to receive a single fixed subcutaneous dose of 0.6 mg dasiglucagon, placebo, or 1 mg GlucaGen, and followed for at least 28 days after receiving treatment.

Assessments:
The plasma glucose profile for evaluation of the primary and secondary clinical efficacy (pharmacodynamic) endpoints will be assessed based on plasma concentration data from samples collected at the dosing visit.
The exposure to trial medication (dasiglucagon, placebo, or GlucaGen) for evaluation of pharmacokinetics will also be assessed based on plasma concentration data.

Statistical methods:
Due to requirements in the size of the safety database, the sample size is set to 78 subjects treated with dasiglucagon 0.6 mg. From phase 2, the median time to an increase of 20 mg/dL of the 0.6 mg dose was approximately 10 minutes.With a 2:1:1 randomization ratio for the three treatments, dasiglucagon 0.6 mg, placebo, and GlucaGen 1 mg, and assuming an exponential time-to-recovery distributions with median times of 10 and at least 20 minutes for dasiglucagon and placebo, respectively, a two-sided log-rank test will be able to detect a difference between dasiglucagon and placebo with 90% power with a follow-up time of 45 minutes at a 5% significance level with 78 subjects treated with dasiglucagon and 39 subjects with placebo. The median time-to-recovery for placebo is expected to be longer than 20 minutes, which means that the power will be greater than 90%.

For the confirmatory analyses, the primary and key secondary endpoints for the dasiglucagon 0.6 mg and placebo treated subjects will be compared. A hierarchical procedure will be applied for the control of multiplicity in statistical inferences. The primary and secondary endpoints will be evaluated on the Full Analysis Set sample. The statistical inference comparisons with placebo will be conducted as superiority tests. The comparisons of dasiglucagon versus GlucaGen will be summarized descriptively.

The primary endpoint will be summarized using Kaplan-Meier (KM) estimates stratified by treatment group and injection site. The treatment group difference between dasiglucagon and placebo will be evaluated inferentially using a pairwise two-sided log-rank test.

In the primary analysis, recovery cannot be achieved in those subjects where IV glucose treatment is administered. Those subjects who receive IV glucose will be censored (i.e. set to ‘not recovered’) at 45 minutes after dosing.

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

The primary endpoint will additionally be analyzed using a Cox proportional hazards (CPH) time to event statistical model, if the hazards by treatment group can be assumed proportional using a graphical comparison of log-minus-log survival curves and no more than two-thirds of the recovery times are censored in each treatment group. The CPH model will be used for inferences, with treatment group and injection site modeled as categorical effects, and baseline plasma glucose modeled as a continuous covariate. The treatment group rate ratios, active versus placebo, will be estimated together with the 95% confidence intervals, and pairwise treatment group inferences (dasiglucagon vs placebo, GlucaGen vs placebo) will be evaluated using two-sided likelihood ratio tests.

The key secondary incidence variables (plasma glucose recovery within 30 minutes, within 20 minutes, within 15 minutes, within 10 minutes, in hierarchical order for inference) will be analyzed using descriptive statistics (frequency and percentage) by treatment group. Inferential treatment group comparisons will be assessed via pairwise tests of independent binomial proportions (dasiglucagon versus placebo, GlucaGen versus placebo) using Fisher's exact test for each pairwise comparison.

The key secondary endpoints of plasma glucose changes from baseline (CFB) within 30 minutes, within 20 minutes, within 15 minutes, and within 10 minutes after study drug injection or at the time of rescue, will be analyzed with the plasma glucose CFB at rescue carried forward in those subjects who require rescue IV glucose before plasma glucose ≥20 mg/dL recovery. Each of these CFB variables will be analyzed using an Analysis of Covariance model, with treatment group modeled as a fixed effect and with the baseline plasma glucose modeled as a covariate. The dasiglucagon versus placebo treatment group difference will be evaluated inferentially as a least squares means contrast, using a two-sided t-test at the 0.05 significance level, first for the 30 minute CFB, and then sequentially for the 20 minute, 15 minute, and 10 minute variables, until the first failure to reject.

The continuous clinical efficacy variables, the exploratory variables, and the pharmacokinetics variables will each be summarized descriptively by treatment group. The clinical efficacy variables will be analyzed analogous to the plasma glucose CFB variables.

The safety analyses will include by-treatment-group descriptive summaries of vital sign measurements, laboratory measures (including immunogenicity incidence), physical examination assessments, rescue IV glucose (incidence and amount of glucose infused), and adverse events. The number and percentage of subjects reporting specific events, such as nausea and vomiting, will be presented by body system and preferred term.

Further details will be included in the Statistical Analysis Plan, to be completed before database lock and treatment unmasking.

2 INTRODUCTION

2.1 Background

Zealand Pharma A/S (Zealand Pharma) is developing dasiglucagon, a physically and chemically stable peptide analog of human glucagon, in a ready-to-use liquid formulation for the acute treatment of severe hypoglycemia in patients with insulin-treated diabetes mellitus. Like native glucagon, dasiglucagon is comprised of 29 amino acids, but with 7 substitutions which improve its physical and chemical stability in aqueous media. These amino acid substitutions make dasiglucagon suitable for a liquid formulation, while providing similar efficacy and safety as compared with recombinant glucagon in the clinical setting of acute treatment of severe hypoglycemia.

2.1.1 Hypoglycemia

Hypoglycemia in patients with diabetes is defined as episodes of an abnormally low plasma glucose concentration (3). This is a common, unpredictable, and potentially dangerous side effect of treatment of diabetes mellitus, especially with 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 (4).

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 (5).

The incidence of hypoglycemic events or even the fear of hypoglycemia influences patients' adherence to prescribed treatment regimens for diabetes mellitus (6). This leads to inadequate glycemic control, which in turn may lead to an increased risk of diabetic complications (5). Serious clinically significant hypoglycemia is now defined as plasma glucose <54 mg/dL (3.0 mmol/L), while the plasma glucose alert value is defined as <70 mg/dL (3.9 mmol/L) (7). When plasma glucose falls below these values, some kind of treatment strategy is needed.

2.1.2 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) (8,9). 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 (10). Insulin decreases blood glucose levels and cases of hypoglycemia can be reversed by glucagon. Therefore, glucagon is indicated for the treatment of severe hypoglycemia.

Besides intravenous (IV) glucose administration, an injectable form of glucagon is given as first aid in cases of severe hypoglycemia, when the patient is unconscious or for other reasons cannot take glucose orally. The approved glucagon dose for an adult is 1 mg, given by intramuscular (IM), IV, or subcutaneous (SC) injection, which quickly raises blood glucose levels. As current marketed recombinant glucagon is highly unstable when dissolved in solution, the injectable form has to be reconstituted prior to use in a 9-step procedure that requires a sterile diluent to be injected into a vial containing lyophilized powdered glucagon. When dissolved in a fluid state, glucagon can form amyloid fibrils (11), or tightly woven chains of proteins made up of the individual glucagon peptides. The reconstitution process makes the use of marketed glucagon products cumbersome (12), and a more patient-friendly formulation is needed. Currently, the Food and Drug Administration (FDA) approved instructions for commercially available glucagon allow only for immediate usage of glucagon after the powder is reconstituted in aqueous solution (13). Therefore, a glucagon analog with enhanced biophysical stability may represent a leap forward in terms of convenient therapeutic applications.

2.1.3 Dasiglucagon

Dasiglucagon (ZP4207) is a stable peptide analog of human glucagon, available in a ready-to-use liquid formulation. Dasiglucagon (hereinafter referred to as dasiglucagon) 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® (hereafter referred to as GlucaGen). Dasiglucagon exhibits improved physical and chemical stability and is available in an aqueous solution at neutral pH (14).

Three clinical trials have been completed with dasiglucagon: a first-in-human dose trial in healthy volunteers and subjects with T1DM (ZP4207-14013), a multiple-dose dose-escalation trial (ZP4207-15007) evaluating the safety, tolerability, pharmacokinetics (PK) and pharmacodynamics (PD) of dasiglucagon in healthy volunteers, and a phase 2 crossover trial to assess the PK and PD of a single dose of an optimized formulation of dasiglucagon administered SC in subjects with T1DM (ZP4207-15126) (14).

2.1.3.1 Pharmacokinetics and pharmacodynamics of dasiglucagon

The results of the phase 1 and 2 clinical trials confirmed dose-proportionality for dasiglucagon PK, 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 (t_{max}) was 35 minutes for dasiglucagon compared with 20 minutes for GlucaGen.

At all dose levels in the phase 2 trial, all subjects achieved a plasma glucose level of at least 70 mg/dL (3.9 mmol/L) as well as an increase in plasma glucose by at least 20 mg/dL (1.1 mmol/L) within 30 minutes after dosing. The PD responses to 0.6 mg of dasiglucagon and 1 mg of GlucaGen were comparable.

2.1.3.2 Safety of dasiglucagon

The safety data for dasiglucagon do not give rise to any safety concerns. No new signals were observed, 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 mg (4 with dasiglucagon and 1 with GlucaGen). This is not considered a safety concern; however, hemodynamic changes after dosing will be considered an adverse event of special interest (AESI).

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's Brochure (14).

2.2 Trial rationale

The aim of the current trial is to confirm the superiority of dasiglucagon for the treatment of insulin-induced hypoglycemia in subjects with T1DM as compared to placebo for dasiglucagon (hereinafter placebo) and to compare the clinical efficacy and safety of dasiglucagon with reference to GlucaGen. A randomized, controlled trial design was used.

See Section 4.2 for justification of the design of this trial.

2.3 Risk-benefit assessment

Non-clinical experience

The nonclinical development program did not reveal any safety findings that would prohibit administration of dasiglucagon to humans. None of the safety pharmacology studies, repeated dose toxicity studies or genotoxicity studies revealed any significant toxicity findings relevant to the therapeutic use of dasiglucagon.

Clinical experience

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 IMP 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 extent in the 3 clinical studies conducted to date with dasiglucagon. As with every novel drug substance, new and as yet unknown side effects may also 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 low immunogenic potential.

Overall, dasiglucagon is judged to be a low-risk molecule, based upon the available clinical data. Administration of dasiglucagon may be associated with a risk of allergic reactions similar to those observed for other therapeutic peptides or proteins. Subjects 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 centers.

With the exception of medical examinations, a subject 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.

The development program including 141 subjects exposed to dasiglucagon to date has demonstrated that administration of dasiglucagon is efficacious and well tolerated, with a safety profile that does not give rise to specific safety concerns. Two phase 1 and one phase 2 clinical trials have been conducted to investigate the safety, tolerability, PK and PD of dasiglucagon after single and multiple dosing to healthy volunteers and subjects with T1DM under insulin-induced hypoglycemic conditions. Dasiglucagon has proven to have relevant clinical effects in the acute severe hypoglycemia rescue setting and may be an effective and reliable emergency treatment for severe hypoglycemia. Overall, the anticipated benefits for subjects entering the ZP4207-16137 trial are considered to justify the risks.

3 TRIAL OBJECTIVES AND ENDPOINTS

3.1 Primary objective

- To demonstrate superiority of dasiglucagon compared to placebo following a single subcutaneous 0.6 mg dose administered to subjects with type 1 diabetes mellitus with insulin-induced hypoglycemia.

3.2 Secondary objectives

- To compare the glycemic response observed after dasiglucagon with that of GlucaGen.

3.3 Primary endpoint

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

3.4 Key secondary endpoints

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

3.5 Other secondary endpoints

- Clinical efficacy (PD) endpoints:
  - Time to first plasma glucose concentration ≥70 mg/dL (3.9 mmol/L) without administration of rescue IV glucose.
  - Plasma glucose response as area under the curve (AUC) above baseline from time zero to 30 minutes, $AUC_{0\text{-}30min}$.
- Exposure (PK) endpoints:
  - Area under the drug concentration curve from time zero to 90 minutes, $AUC_{0\text{-}90min}$.
  - Maximum plasma drug concentration (C_{max}).
  - Time to maximum plasma drug concentration (t_{max}).

- Safety endpoints:
  - Adverse events, clinical laboratory assessments (biochemistry, hematology, urinalysis), vital signs, physical examination, electrocardiogram (ECG), and local tolerability.
  - Administration of rescue infusion of IV glucose during the hypoglycemic clamp procedure.
  - Time to first rescue infusion of IV glucose during the hypoglycemic clamp procedure.
- Immunogenicity endpoint:
  - Occurrence of anti-drug antibodies

3.6 Exploratory endpoint

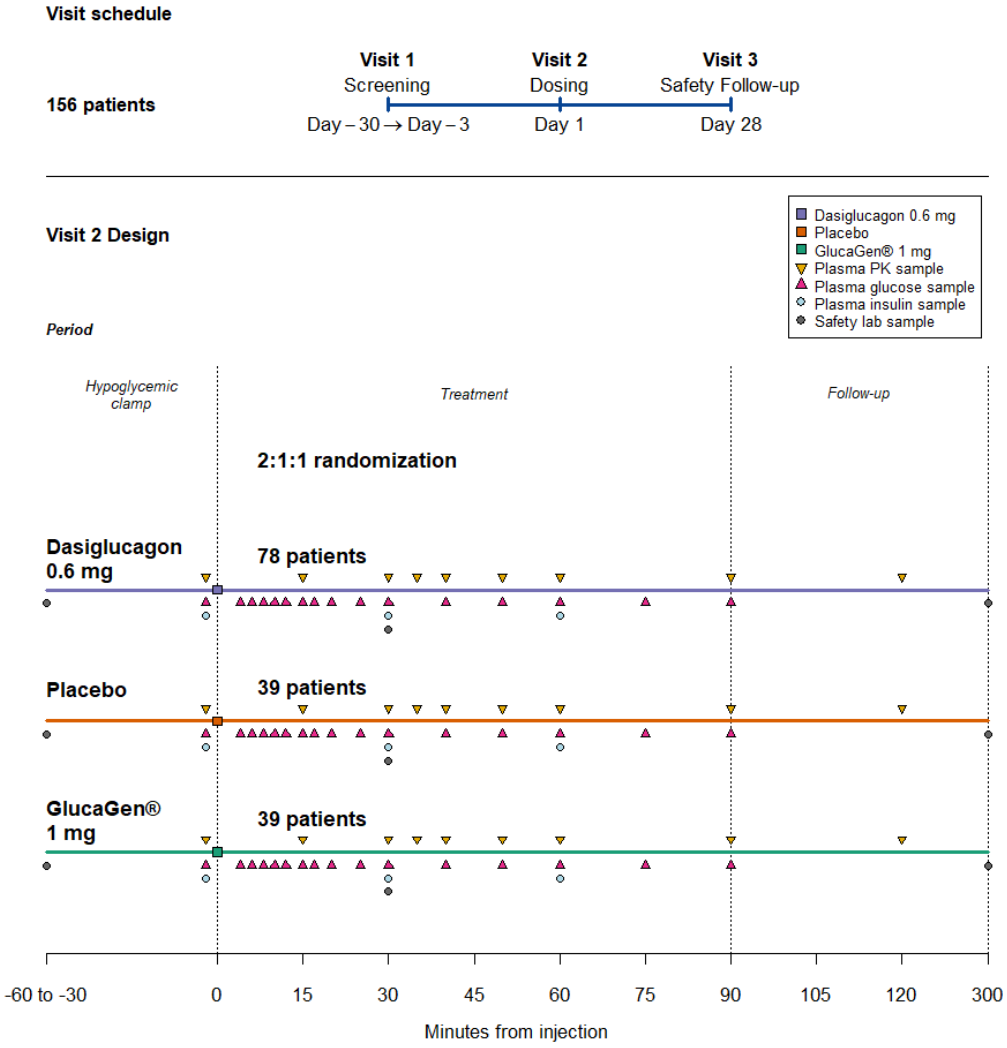
- Plasma glucose concentration ≥70 mg/dL (3.9 mmol/L) or increase of ≥20 mg/dL (1.1 mmol/L) within 30 minutes after study drug injection without administration of rescue IV glucose.
- Plasma insulin response as area under the curve above baseline from time zero to 60 minutes, $AUC_{0\text{-}60\ min}$.

4 OVERALL DESIGN AND PLAN OF THE TRIAL

4.1 Overview

This trial is a global, multicenter, randomized, parallel, double-blind, clinical trial confirming the efficacy and safety of dasiglucagon for insulin-induced hypoglycemia in subjects with T1DM. The subjects will be randomized 2:1:1 to receive a single fixed SC 0.6 mg dose of dasiglucagon, placebo, or a 1 mg dose of GlucaGen and followed for at least 28 days after receiving treatment. A total of 156 subjects with T1DM are expected to complete the treatment visit. The trial will be conducted in the European Union (EU) and North America. See Figure 1 for an overview of the trial design.

Figure 1 Trial design



4.2 Justification of the trial design

4.2.1 Justification for design and parameters

In order to avoid bias in subject selection and in the evaluation of clinical assessments, subjects will be randomly assigned 2:1:1 to either dasiglucagon, placebo, or GlucaGen and the trial will be conducted in a double-blinded manner. The randomized parallel treatment design with administration of fixed SC doses of dasiglucagon, placebo, or GlucaGen to subjects with T1DM and insulin-induced hypoglycemia allows for direct comparison of the clinical efficacy of the treatments.

The trial is double-blind to increase trial validity and to reduce bias during evaluation of the treatments. However, since the trial medications are not identical in appearance, the handling, preparation and administration of trial medication will be done by unblinded trial personnel who will not be involved in any other trial procedures or assessments. See Section 6.6 for more information about which assessments are blinded and which are not, with reasons.

A superiority trial design is used because the aim is to show that treatment with dasiglucagon is an effective treatment compared to placebo. The secondary objective was chosen to allow a comparison between treatment with dasiglucagon and an established comparator treatment for severe hypoglycemia, GlucaGen.

Administration of glucagon is intended to quickly raise blood glucose levels in subjects with T1DM with insulin-induced hypoglycemia. Therefore, in order to assess the clinical efficacy of dasiglucagon as compared to placebo and GlucaGen following a single SC dose, the primary endpoint and secondary efficacy endpoints involve the measurement of plasma glucose concentrations at different timepoints.

4.2.2 Justification for drug, route, dosage and treatment duration

Dasiglucagon and GlucaGen will be administered as 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 approved dose for treatment of severe hypoglycemia. Data from the studies conducted to date with dasiglucagon, including the phase 2 trial in subjects with T1DM, have been used to establish that 0.6 mg of dasiglucagon is an effective dose and also represents a therapeutically equivalent dose to 1 mg of GlucaGen (see also Section 6.1).

Dasiglucagon, placebo, and GlucaGen will be administered in the abdomen, buttocks, or thigh by SC injection, as this is one of the intended routes of administration for dasiglucagon, besides IM and IV.

Subjects will be followed for at least 28 days after dosing in order to perform an adequate immunogenicity evaluation of treatment.

5 TRIAL POPULATION

5.1 Rationale for trial population

Dasiglucagon is in development for the treatment of severe hypoglycemia in insulin-dependent patients with diabetes mellitus. In the present trial, subjects with T1DM are included in the evaluation of efficacy and safety of dasiglucagon under hypoglycemic conditions as this is part of the intended target population. Subjects with T1DM are selected to avoid the endogenous glucagon counter-regulatory response to insulin-induced hypoglycemia that is present in patients with T2DM. The inclusion and exclusion criteria are set to include a trial population that represents the general population of subjects with T1DM.

5.2 Planned sample size and number of trial centers

A total number of 156 subjects with T1DM are expected to complete the treatment visit (Visit 2). To qualify as completed, the subject must be dosed and have blood drawn for the PK and PD assessments described in the protocol. It is expected that up to 176 subjects will be randomized to have 156 subjects completing Visit 2. Completion of 156 subjects (78 subjects in the dasiglucagon group and 39 subjects in each of the placebo and GlucaGen groups) would be expected to provide adequate power for the primary efficacy evaluation, as described in Section 9.1.

The planned date for first subject first visit is expected to take place in Q4, 2017 and the planned date for last subject last visit is expected to take place in Q3, 2018.

This trial is planned to be conducted at 4 to 6 trial sites in the United States of America [US], Canada, and Europe.

5.3 Inclusion criteria

Subjects will be entered into this trial only if they meet 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 subject).
2. Female or male subjects with T1DM for at least 1 year, diagnostic criteria as defined by the American Diabetes Association (3).
3. Treated with insulin for T1DM for at least 1 year and with stable insulin treatment (defined as no more than a 10-unit daily variation in total daily insulin dose) 30 days prior to screening

4. Hemoglobin A_{1c} <10%.
5. Aged between 18 and 75 years, both inclusive.
6. A female subject 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 and until last follow-up visit. An acceptable method of contraception includes one of the following:
        i. Abstinence from heterosexual intercourse;
        ii. Systemic contraceptives (birth control pills, injectable/implant/ insertable hormonal birth control products, transdermal patch);
        iii. Intrauterine device (with and without hormones); or
        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).
7. A male subject must meet the following criteria: Surgically sterilized or willing to refrain from sexual intercourse from screening and until last follow-up visit or, if sexually active, uses condom and partner practices contraception during the trial from screening and until last follow-up visit.

5.4 Exclusion criteria

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

1. Previously treated with dasiglucagon (previously referred to as ZP4207).
2. Known or suspected allergy to trial product(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, are actively attempting to get pregnant, or are lactating.
6. History of hypoglycemic events associated with seizures in the last year prior to screening.
7. History of severe hypoglycemia (defined as plasma glucose <54 mg/dL [3.0 mmol/L]) in the last month prior to screening.
8. Receipt of any investigational drug within 3 months prior to screening.
9. Active malignancy within the last 5 years.
10. Congestive heart failure, New York Heart Association class II-IV.
11. Inadequately treated blood pressure, defined as systolic ≥160 mmHg or diastolic ≥90 mmHg) at screening (15).
12. Current bleeding disorder, including anti-coagulant treatment.

13. Known presence or history of pheochromocytoma (i.e. adrenal gland tumor) or insulinoma (i.e. insulin secreting pancreas tumor).
14. Use of a daily systemic beta-blocker drug, indomethacin, warfarin or anticholinergic drugs in the previous 28 days before Day 1 of this trial.
15. Aspartate aminotransferase (AST) or alanine aminotransferase (ALT) >2.5 × the upper limit of the normal range (ULN), bilirubin >1.5 × ULN, estimated glomerular filtration rate <30 mL/min/1.73 m^2 according to the Modification of Diet in Renal Disease study definition (16), or altered electrolyte values of clinical relevance for cardiac conduction, as judged by the investigator.
16. Clinically significant abnormal ECG at screening as judged by the investigator.
17. Clinically significant illness within 4 weeks before screening, as judged by the investigator.
18. Donation of blood or plasma in the past month, or in excess of 500 mL within 12 weeks prior to screening.
19. Surgery or trauma with significant blood loss within the last 2 months prior to screening.
20. 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.
21. Subjects with mental incapacity or language barriers which 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.
22. Any condition interfering with trial participation or evaluation or that could be hazardous to the subject.
23. The use of prescription or non-prescription medications known to cause QT prolongation.

5.5 Dosing day exclusion criteria

Subjects who meet one or more of the following dosing day exclusion criteria at the dosing visit will be excluded from the dosing visit, but the visit can be rescheduled 1-7 days later. The dosing visit can only be rescheduled once.

1. Atypically strenuous exercise within 4 days prior to dosing, as judged by the investigator. Exercise during the trial should follow subject's typical routine, and should not exceed a near maximum intensity for more than 20 minutes per day, or moderate intensity for more than 90 minutes per day.
2. Clinically significant illness within 4 weeks before dosing, as judged by the investigator.
3. Consumption of alcohol within 24 hours prior to dosing visit, or positive results from an alcohol breath test.

4. Not fasting from 22:00 hours the evening prior to dosing, apart from water. Small amounts of carbohydrates (up to 20 g) to prevent hypoglycemia are allowed.
5. The use of any non-prescribed systemic or topical medication, except routine vitamins and occasional use (as judged by the investigator) of acetylsalicylic acid and paracetamol within 2 weeks prior to dosing. Treatment with insulin, including analogs, is allowed.
6. Use of insulin Degludec or insulin Glargine U300 within 48 hours prior to dosing; or use of other long-acting insulins (e.g., insulin Glargine U100 or insulin Detemir) within 24 hours prior to dosing; or use of insulin Neutral protamine Hagedorn (NPH) within 16 hours prior to dosing.
7. Use of any short acting (bolus) insulin within 6 hours prior to dosing, except insulin glulisine (Apidra®).
8. Changes in medical history or concomitant medication resulting in fulfillment of clinical exclusion criteria, as judged by the investigator.
9. Plasma glucose value <50 mg/dL (2.8 mmol/L) within the last 24 hours or plasma glucose value <60 mg/dL (3.3 mmol/L) within the last 5 hours prior to initiation of the hypoglycemic procedure.

5.6 Premature treatment discontinuation and withdrawal

Participation in the trial is strictly voluntary. A subject 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 subject at any time if the investigator deems it in the subject's best interest. The reason and circumstances for withdrawal will be documented in the electronic case report form (eCRF).

5.6.1 Possible reasons for treatment visit discontinuation

A subject will be discontinued from treatment if the following applies:

- Withdrawal of consent by subject.
- If a protocol deviation occurs which, in the clinical judgement of the investigator, can invalidate the trial or endpoints or can interfere pharmacokinetically or pharmacodynamically with the trial product, the subject will be discontinued by the investigator.
- Adverse events occur which are considered unacceptable by the subject or the investigator.

If discontinuation occurs following administration of trial medication, every effort should be made to have the subject return and participate in the complete follow-up visit on Day 28 (see Table 3) to avoid missing data.

If trial participation is terminated due to an AE possibly related to any of the trial medications or trial examinations, the subject 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.

A total of 156 subjects must complete the treatment visit (Visit 2). To qualify as completed, the subject must be dosed and have blood drawn for the PK and PD assessments described in the protocol.

5.6.2 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 subjects within the agreed time frame.
- The center does not respond to trial management requests.
- Repeat protocol violations.

5.6.3 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 subjects.
- New scientific knowledge becomes known that makes the objectives of the trial no longer feasible/valid.
- Unsatisfactory enrolment of subjects.

5.7 Subject identification and randomization

Subjects who have given written informed consent and meet all inclusion and none of the exclusion criteria will be randomized in a 2:1:1 ratio to either 0.6 mg dasiglucagon (n=78), placebo (n=39), or 1 mg GlucaGen (n=39) via an Interactive Web Response System (IWRS). Randomization will continue until 156 subjects have completed Visit 2.

Subjects 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 subject is taking, the subject code can be broken by the

investigator, preferably after consultation with the medical monitor. Emergency code breaks can be performed using the IWRS.

6 TRIAL DRUG

6.1 Identity

The following trial drugs will be administered:

- Dasiglucagon, liquid formulation, 1 mg/mL, 0.6 mL delivered in a prefilled syringe.
- Placebo, liquid formulation, 0.6 mL delivered in a prefilled syringe.
- Recombinant glucagon hydrochloride, 1 mg for reconstitution (GlucaGen®, Novo Nordisk) in 1 mL sterile water.

The description of the three trial drugs is provided in Table 1. Dasiglucagon is a stable peptide analog of human glucagon in a ready-to-use liquid formulation for treatment of severe hypoglycemia in insulin-dependent patients with diabetes mellitus. 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.

Table 1 Description of trial drugs

	Test product	Placebo Product	Comparator product
Name	Dasiglucagon	Placebo	GlucaGen®
Active substance	Dasiglucagon	N/A	Recombinant glucagon hydrochloride
Formulation	Liquid formulation, 0.6 mL	Liquid formulation, 0.6 mL	Powder and solvent for reconstitution as 1 mL solution for injection
Strength	1 mg/mL	N/A	1 mg
Device	Single use pre-filled syringe	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	Zealand Pharma A/S, Glostrup (Copenhagen), Denmark	Novo Nordisk A/S, Bagsværd, Denmark

	Test product	Placebo Product	Comparator product
Storage requirements	Store between 2 and 8°C	Store between 2 and 8°C	Store between 2 and 8°C

The quantities of ingredients for dasiglucagon and placebo are provided in Table 2.

Table 2 Quantities of ingredients in dasiglucagon and placebo injection

Component	Amount per mL (dasiglucagon)	Amount per mL (placebo)	Function
Dasiglucagon*	1.0 mg	N/A	[redacted]
Sodium chloride	10.23 mg	10.23 mg	
Trometamol/Tromethamine	6.06 mg	6.06 mg	
Water for injection	To make 1 mL	To make 1 mL	
Sodium hydroxide	q.s.	q.s.	
Hydrochloric acid	q.s.	q.s.	

*The quantity of drug substance to be used is calculated according to net peptide content and purity.
q.s. = quantum sufficit (quantity required).

6.2 Treatment assignment and randomization

Subjects successfully completing screening and who fulfill entry eligibility and randomization criteria will be randomized to one of three treatment groups in a ratio of 2:1:1:

- Test treatment: Dasiglucagon, liquid formulation, 1 mg/mL, 0.6 mL delivered in a prefilled syringe.
- Placebo treatment: Placebo, liquid formulation, 0.6 mL delivered in a prefilled syringe.
- Reference treatment: Recombinant glucagon hydrochloride, 1 mg for reconstitution.

Randomization will be performed using a fixed-block randomization scheme. The randomization scheme will be generated prior to the initiation of the study by an independent statistician/programmer who will not be a member of the study team; all investigators will not be aware of the block size of the randomization scheme. Randomization will be stratified by treatment group and by injection site (abdomen, buttocks, or thigh) and controlled via the IWRS.

Subjects will be randomized to study treatment using an interactive, automated system which has been validated for the intended use under the International Society of Pharmaceutical Engineers Good Automated Manufacturing Process guidelines, 21CFR Part 11 (FDA regulation for Electronic Records and Electronic Signatures) and the International Conference on Harmonization (ICH) Guidance E6 for Industry on Good Clinical Practice (GCP).

6.3 Administration

Dasiglucagon, placebo, and GlucaGen will be administered by SC injection in the abdomen, buttocks, or thigh.

An unblinded person (appropriately trained) authorized to prepare the dose and administer the treatment in accordance with the randomization will prepare the treatment required for each subject on each dosing day. The dose will be administered by the unblinded, trained and qualified person. The content of the syringe has to be checked for clarity and absence of bubbles..

Syringes will be discarded after dose administration. Used GlucaGen vials will be stored in a lockable box (separated from unused vials) at ambient temperature.

6.4 Packaging and labelling

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

The study drug label will describe the storage conditions for study drug. The labels will supply no information about the subjects. Each treatment kit (pre-filled syringe/vial for reconstitution) will have a unique Dispensing Unit Number for drug allocation, drug accountability, and traceability purposes.

Labelling will be performed according to Annex 13 of the Good Manufacturing Practice guidelines of the European Commission, ICH GCP guidelines, and local law.

6.5 Storage of study drugs

The investigator must ensure the availability of proper storage conditions. All study drug supplies provided for this study will be stored in a secure area with restricted access at the study site.

The temperature should be monitored by recording the actual, minimum, and maximum temperatures using a calibrated thermometer or thermocouple, or by continuous recording using a qualified temperature monitoring system. The temperature should be evaluated and documented at least on working days on a temperature log. This log must be included in the Investigator Site File upon study termination.

Dasiglucagon and placebo must be stored in a refrigerator (at a temperature of 2–8°C), and should be handled in accordance with guidelines from the sponsor. GlucaGen must also be stored in a refrigerator (at a temperature of 2–8°C), and should be handled in accordance with the Summary of Product Characteristics (13).

The unblinded person responsible for study drug handling must contact the unblinded monitor in case of temperature deviations outside the acceptable range.

Please see the Pharmacy Manual for additional information on handling study drug.

6.6 Blinding and breaking the blind

This is a double-blind trial. As the trial products are not identical in appearance, dasiglucagon and placebo being available as a liquid formulation and GlucaGen as a powder for reconstitution, unblinded trial personnel will be responsible for handling, preparing (according to the prescription from the IWRS), and administering the trial medication, as well as for keeping the records strictly confidential and accessible only to unblinded staff until after the database has been locked. To maintain double-blind conditions, all trial assessments at the trial center will be done by blinded trial personnel not involved in the administration of trial medications. However, exposure assessments and anti-drug antibody (ADA) assessments will be performed by unblinded personnel at the special laboratories, to ensure that dasiglucagon, placebo, or GlucaGen administration is matched with the applicable bioanalytical assay.

Treatment assignment will be kept strictly confidential and accessible only to authorized persons until after the time of unblinding. Codes with treatment assignment will, however, be readily available in the IWRS to the blinded personnel in case of an emergency.

The code for a particular subject may be broken in a medical emergency if knowing the identity of the treatment allocation would influence the treatment of the subject. The emergency code break can be performed using the IWRS. Whenever a code is broken, the person breaking the code must record the time, date and reason as well as his/her initials in the source documents. The breaking of blinded codes in case of medical emergency for one subject should not unblind the trial personnel to the treatment information of other

subjects. The person performing the unblinding should inform as few people as possible about the result of the unblinding. All persons unblinded for a specific subject should be documented.

If the trial center needs to break the code, the medical monitor should, if at all possible, be contacted prior to breaking the code and the monitor must be notified within 24 hours after the code has been broken.

The pharmacovigilance unit (safety contract research organization [CRO]; see the list of trial personnel in Section 12.1) will be able to break the code in case of a serious unexpected suspected adverse reaction (SUSAR).

The central and specialty laboratories will be provided with a copy of the randomization list.

6.7 Drug accountability

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 for destruction. This does not apply to the used syringes as they will be discarded after dose administration. Destruction must not take place until approved by the Sponsor.

6.8 Treatment compliance

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

PK assessments will support the surveillance of compliance with IMP administration.

6.9 Prior and concomitant medications

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

Subjects using any new concomitant medication resulting in fulfillment of a dosing day exclusion criterion (see Section 5.5) will be excluded from the dosing visit, but can be

rescheduled to one of the following days (1–7 days later). The dosing visit can only be rescheduled once.

6.9.1 Prohibited medications

The use of anti-coagulant treatments and medications (prescription and non-prescription) that are known to cause QT prolongation are prohibited during the course of the trial.

Within 28 days prior to dosing, the use of daily systemic beta-blockers, indomethacin, warfarin, and anticholinergic drugs is prohibited.

Within 2 weeks prior to dosing, the use of any non-prescribed systemic or topical medication (with the exception of vitamins and the occasional use of acetylsalicylic acid and paracetamol) is prohibited.

Within 48 hours prior to dosing, the use of insulin Degludec or insulin Glargine U300 are prohibited.

Within 24 hours prior to dosing, the use of long-acting insulin (e.g., insulin Glargine U100 or insulin Detemir) is prohibited.

Within 16 hours prior to dosing, the use of insulin NPH is prohibited.

Within 6 hours prior to dosing, the use of any short acting (bolus) insulin, except insulin glulisine (Apidra®) is prohibited.

During the insulin-induced hypoglycemic procedure, continuous SC insulin infusion must be stopped.

6.10 Local German requirements for insulin glulisine (Apidra®)

Insulin glulisine (Apidra®), the insulin used to induce hypoglycemia, will according to local requirements be defined as an investigational medicinal product when used at German sites. The insulin will be provided by the local sites and drug accountability will be performed according to local procedures. In contrast to the trial drug for the randomized treatment, the insulin will be administered by the blinded trial personnel via IV infusion as described in section 8.2.3.1.

7 PARAMETERS AND METHODS OF ASSESSMENT

Overall, approximately 180 mL of blood will be drawn from each subject for PK, PD, ADA, and safety laboratory assessments.

7.1 Efficacy parameters

A description of the sample handling and sample processing at the site will be included in the laboratory manuals. Validation documentation for the assays must be available prior to sample analyses. A bioanalytical report for each analysis of trial PD and PK samples will be prepared.

7.1.1 Pharmacodynamic measurements

The plasma glucose profile for evaluation of the primary and secondary clinical efficacy endpoints will be assessed based on plasma concentration data ($AUC_{0\text{-}30min}$) from samples collected at the dosing visit (Visit 2). The samples will be sent to the clinical laboratory and analyzed using a sensitive and validated assay for glucose measurements.

Samples will be collected pre-dose, and at 4, 6, 8, 10, 12, 15, 17, 20, 25, 30, 40, 50, 60, 75, and 90 minutes after dosing (see the schedule of procedures in Table 3). The actual time of blood sampling for evaluation of plasma glucose should not deviate from the nominal time by more than ±30 seconds until the 20 minute collection time point and by more than ±1 minute for the subsequent collection time points. Pre-dose is defined as within 2 minutes prior to dosing.

7.1.2 Pharmacokinetic measurements

The exposure to trial medication (dasiglucagon or GlucaGen) for evaluation of PK will be assessed based on plasma concentration data ($AUC_{0\text{-}90\ min}$, C_{max}, t_{max}) from samples collected at the dosing visit (Visit 2).

Samples (including back-up samples) will be collected pre-dose, and at 15, 30, 35, 40, 50, 60, 90, and 120 minutes after dosing (see the schedule of procedures in Table 3). The actual time of blood sampling for exposure to trial medication should not deviate from the nominal time by more than ±1 minute. Pre-dose is defined as within 2 minutes prior to dosing.

7.2 Safety parameters

7.2.1 Adverse events

The investigator is responsible for the detection and documentation of events meeting the criteria and definition of an AE or serious adverse event (SAE), as provided in this protocol. During the trial, the investigator or center staff will be responsible for reporting AEs and SAEs, as detailed in this section of the protocol.

Based on the investigator's clinical judgment it will be determined whether an AE is related to treatment and of sufficient severity to require the subject's removal from treatment or from the trial. A subject may also voluntarily withdraw from treatment due to what he or she perceives as an intolerable AE. If either of these situations arises, the subject should be under medical supervision until symptoms cease or the condition becomes stable.

7.2.1.1 Definitions

Adverse event

An AE is any untoward medical occurrence in a trial subject given an IMP which does not necessarily have a causal relationship with the treatment. An AE can therefore be any unfavorable and/or 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.

In this trial, only treatment-emergent adverse events (TEAEs) will be collected and reported. TEAEs are events that occur from the first trial-related activity after the subject has signed the informed consent form until the end of the post-treatment follow-up period.

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 7.2.2).
- Injection site reactions (see Section 7.2.6).

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

- Pre-planned procedure, unless the condition for which the procedure was planned has worsened from the first trial related activity after the subject 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

An 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 events may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above.

Adverse drug reaction

All noxious and unintended responses to an investigational product related to any dose should be considered adverse drug reactions. The phrase responses to an investigational product means that a causal relationship between an investigational product and an adverse event is at least a reasonable possibility, i.e. the relationship cannot be ruled out.

Suspected unexpected serious adverse reactions (SUSARs)

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. dasiglucagon Investigator's Brochure or package leaflet/Summary of Product Characteristics for GlucaGen).

Adverse event of special interest

An AESI 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 AESIs:

- Post-dose clinical signs, or measured vital signs, indicating a clinically significant drop in blood pressure including signs of orthostatic hypotension, vasovagal responses or bradycardia.
- Post-dose 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 AE 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 AE 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 subject.

Severe: A type of AE 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 an SAE may not necessarily 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.

Germany only: The investigator should assess the causality to dasiglucacon/placebo/GlucaGen and to insulin glulisine (Apidra®) respectively. A special field applicable for Germany only will be present on the AE form in the eCRF to capture this data.

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 subject 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 subject signed the informed consent.
Recovering/resolving:	The condition is improving and the subject is expected to recover from the event. This term is only applicable if the subject has completed the trial.
Recovered/resolved with sequelae:	The subject 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 subject has not improved and the symptoms are unchanged.
Fatal:	This term is only applicable if the subject died from a condition related to the reported AE. Outcomes of other reported AEs in a subject 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 subject is lost to follow-up.

7.2.1.2 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 subject has signed the informed consent until the end of the post-treatment follow-up period. At each contact with the center (visit or telephone, excluding visits where the subject is not seeing the investigator or his/her staff [e.g. visits

to the laboratory]) the subject must be asked about AEs. All AEs, either observed by the investigator or reported by the subject, 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.

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 AESIs, the AESI 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 during IMP administration 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 AESI, the investigator must tick the AESI box on the AE form and complete the AESI form. The AESI 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 all AESIs to the sponsor's responsible pharmacovigilance unit (Safety CRO; see the list of trial personnel in Section 12.1) immediately (within 24 hours) after obtaining knowledge about the event.

The investigator must report initial information electronically (e.g. in PDF format) on all SAEs to the sponsor's responsible pharmacovigilance unit (Safety CRO; see the list of trial personnel in Section 12.1) immediately (within 24 hours) after obtaining knowledge about the event. 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 (see Section 7.2.1.1) that occur in this trial to the Competent Authorities and Institutional Review Boards (IRBs)/Independent Ethics Committees (IECs) in accordance with the local requirements in force and ICH guideline for GCP. The trial monitor must be informed accordingly.

7.2.1.3 Follow-up of adverse events

All AEs that are ongoing at the end of the subject's participation in the trial will be followed-up until the event is resolved or reaches a satisfactory outcome as deemed by the investigator or until the last visit of the last subject enrolled in the trial, whichever occurs first.

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 an SAE.

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

The sponsor and/or CROs acting on behalf of the sponsor can upgrade a non-serious AE to an SAE. In these situations the investigator will be informed and asked to fill out an SAE form and forward to the Safety CRO immediately (within 24 hours).

7.2.1.4 Clinical laboratory abnormalities and other abnormal assessments as adverse events or serious adverse events

Abnormal laboratory findings (e.g. biochemistry, hematology, urinalysis) or other abnormal assessments (e.g. vital signs) that are judged by the investigator as **clinically significant** will be recorded as AEs or SAEs if they meet the definition of an AE or SAE. Clinically significant abnormal laboratory findings or other abnormal assessments that are detected during the trial or are present at baseline and significantly worsen following the start of the trial will be reported as AEs or SAEs. However, clinically significant abnormal laboratory findings or other abnormal assessments that are associated with the disease being studied, unless judged by the investigator as more severe than expected for the subject's condition, or that are present or detected at the start of the trial and do not worsen, will not be reported as AEs or SAEs.

The investigator will exercise his or her medical and scientific judgment in deciding whether an abnormal laboratory finding or other abnormal assessment is clinically significant.

7.2.2 Hypoglycemia

Hypoglycemia will be regarded as an AE and recorded and documented on an AE form (and SAE form, if applicable).

Hypoglycemia is defined as a decline in plasma glucose to below 70 mg/dL (3.9 mmol/L). However, in the time period from initiation of the hypoglycemic clamp procedure (see Section 8.2.3.1) until 45 minutes after dosing, hypoglycemia is defined as a decline in plasma glucose to below 45 mg/dL (2.5 mmol/L).

During the dosing visit, prior to administration of the IMP, a plasma glucose value of <45 mg/dL (2.5 mmol/L) will be treated by an IV glucose solution. After administration of the IMP in the period from 8 minutes until 44 minutes after dosing, a plasma glucose value of <45 mg/dL (2.5 mmol/L) will be treated by an IV glucose solution, and if a plasma glucose value of ≥70 mg/dL (3.9 mmol/L) is not achieved within the 45 minutes after IMP administration, IV glucose infusion will also be initiated.

If the subject experiences symptoms of hypoglycemia, a plasma glucose measurement should be taken in order to classify the event (please refer to Section 7.4.2 for additional details).

7.2.3 Physical examination

The physical examination will be carried out at screening (Visit 1) and at the follow-up visit (Visit 3; see Table 3).

The physical examination includes examination of the following body systems: head, ears, eyes, nose, throat, including the thyroid gland; heart, lung, chest; abdomen; skin and mucosae; musculoskeletal system; nervous system; lymph node; other findings.

At the screening visit, any abnormality will be recorded and described in the eCRF, including the investigator's assessment of clinical significance ('abnormal, not clinically significant' or 'abnormal, clinically significant'). Clinically significant findings at the screening visit should be recorded as concomitant illness.

At subsequent visits, any clinically significant deterioration of a pre-existing condition as well as any new clinically significant findings will be recorded as AEs (see Section 7.2.1).

7.2.4 Vital signs

An examination of the following vital signs will be performed at screening (Visit 1), the dosing visit (Visit 2) and at the follow-up visit (Visit 3):

- Diastolic and systolic blood pressure (mmHg) will be measured after at least 5 minutes rest in a supine position. At Visit 1, blood pressure will be measured in both arms. The blood pressure from the arm with the higher systolic value is transcribed into the eCRF and this arm should be used for all subsequent measurements of the subject's blood pressure in this trial.
- Pulse (beats per min) measured after at least 5 minutes rest in a supine position.
- Body temperature (°C).

At the dosing visit, measurements will be taken prior to the start of the insulin-induced hypoglycemic procedure (within 30 minutes) and at 30, 90, and 300 minutes after dosing (see Table 3). The actual time of the assessment should not deviate from the nominal time by more than ±10 minutes. In addition to the pre-specified assessments as listed in Table 3, blood pressure and pulse may be assessed at any time during the trial at the discretion of the investigator.

7.2.5 Electrocardiogram

A standard 12-lead ECG will be performed at the screening visit (Visit 1), the dosing visit (Visit 2; prior to the start of the insulin-induced hypoglycemic procedure [within 30 minutes]) and at 20, 35, 45, 60, and 300 minutes after dosing) and at the follow-up visit (Visit 3; see Table 3). The actual time of the assessment should not deviate from the nominal time by more than ±5 minutes.

ECG parameters (heart rate, PQ, QRS, QT, QTcB) and any abnormality will be recorded and described in the eCRF including the investigator's assessment of clinical significance ('abnormal, not clinically significant' or 'abnormal, clinically significant').

At subsequent visits, any clinically significant deterioration of a pre-existing condition as well as any new clinically significant findings will be recorded as AEs (see Section 7.2.1).

7.2.6 Local tolerability

Immediately prior to treatment administration, it should be verified that the injection site is normal. To ensure all injection site assessments are performed at the injection site, the site will be marked with a pen prior to injection. Assessment of local tolerability at the injection site will be performed at the dosing visit (Visit 2; at 30, 120 and 300 minutes after dosing), and at the follow-up visit (Visit 3) (see Table 3) and more frequently, if deemed necessary by the investigator. The actual time of the assessment should not deviate from the nominal time by more than ±10 minutes. In the eCRF, the time of assessment and any injection site reaction observed will be recorded. In case of an observation, the Local Tolerability form will be completed, as well as the (S)AE form.

The local tolerability at the injection site will be evaluated by means of the following assessments: spontaneous pain, pain on palpation, itching, redness, edema, induration/infiltration, and other. Each of these assessments will be reported on a scale of 0 (none), 1 (mild), 2 (moderate), and 3 (severe). The evaluation and the actual time of the assessment will be recorded. The assessments will be performed by a trial physician or nurse.

Digital pictures will be taken of the injection site at the time of identification, and thereafter as often as judged necessary by the investigator. The pictures should include a subject identifier, visit number, time after dosing, and a ruler for scaling.

7.2.7 Clinical laboratory assessments

The safety parameters that will be assessed at the clinical laboratory are listed in Table 3. Routine clinical laboratory tests will be performed centrally. Samples for clinical laboratory parameters (biochemistry, hematology) will be collected at screening (Visit 1), the dosing visit (Visit 2, prior to the start of the insulin-induced hypoglycemic procedure [within 30 minutes]) and at 30 and 300 minutes after dosing), and at the follow-up visit (Visit 3). The actual time of the assessment should not deviate from the nominal time by more than ±10 minutes. Samples for glycated hemoglobin (HbA_{1c}), C-peptide and coagulation parameters will be collected at screening only (Visit 1). Samples for urinalysis will be collected at screening (Visit 1), at the dosing visit (Visit 2, prior to the start of the insulin-induced hypoglycemic procedure [within 120 minutes]) and at the follow-up visit (Visit 3). The following parameters will be assessed:

- Clinical biochemistry: sodium, potassium, calcium, glucose, urea, creatinine, total bilirubin, AST, ALT, gamma-glutamyltransferase, 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 (leukocytes).
- Coagulation: international normalized ratio, fibrinogen (at screening visit only).
- Urinalysis: pH, blood (leukocytes and erythrocytes), protein, glucose, ketones, nitrite.

Pregnancy tests will be performed at each visit for women of childbearing potential only. A serum pregnancy test will be performed at screening (Visit 1) and urine stick tests will be performed at the dosing visit (Visit 2, prior to the start of the insulin-induced hypoglycemic procedure) and the follow-up visit (Visit 3). Test sticks will be provided to the trial centers.

Alcohol breath tests and a urine drug screen will be performed at screening (Visit 1) and at the dosing visit (Visit 2, prior to the start of the insulin-induced hypoglycemic

procedure). Equipment for the alcohol breath test and uring drug screen will be provided to the trial centers.

Re-assessment of laboratory parameters will be allowed only if handling issues, damaged samples, or hemolyzed samples have confounded the measurement results.

For further details of the clinical laboratory assessments, please refer to the laboratory manual.

7.2.8 Pregnancy

Female subjects 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 subjects must be instructed to notify the investigator immediately if their partner becomes pregnant or suspects pregnancy. The sponsor has a responsibility to monitor the outcome of all pregnancies reported during the clinical trial. During an information meeting at the trial center, the subject'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 immediately (within 24 hours), according to the procedure stated in Section 7.2.1.2. 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 follow 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 7.2.1.2. Any (S)AEs in the newborn must be reported on the (S)AE form.

The SAEs that must be reported include 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 until the age of 1 month.

7.2.9 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 subjects. During a subject's participation in the trial, the investigator should ensure that adequate medical care is provided to the subject for any AEs, including clinically significant laboratory values related to the trial. The investigator should inform the subject when medical care is needed for intercurrent illnesses of which the investigator becomes aware.

For further information on safety precautions for dasiglucagon and GlucaGen, please refer to the current version of the Investigator's Brochure (14) and the Summary of Product Characteristics for GlucaGen (13), respectively.

7.2.10 Safety committee

The internal Zealand Pharma Safety Committee is constituted to perform ongoing blinded 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 Safety Committee meetings, or on notification of significant findings, the Safety Committee will take appropriate measures to safeguard the subjects.

7.3 Demography, concomitant illness, medical history and concomitant medication

Demographics, body measurements, concomitant illness and medical history will be assessed only at screening (Visit 1). Concomitant medication will be assessed at screening (Visit 1), the dosing visit (Visit 2, prior to the start of the insulin-induced hypoglycemic procedure) and at the follow-up visit (Visit 3).

7.3.1 Demography and body measurements

Subject demographics and body measurements will include:

- Age
- Race, ethnicity
- Sex
- Height (meters or inch), without shoes
- Body weight (kg or lb), only wearing underwear and measured using standard scales
- Body mass index (kg/m^2) calculated based on height and body weight (body weight/height^2).

7.3.2 Concomitant illness and medical history

A concomitant illness is any illness that is present at the start of the trial (i.e. at the screening visit). Concomitant illnesses present at the start of the trial will be recorded in the eCRF at screening.

Medical history is an account of medical events that the subject has experienced in the past, including prior medications. Relevant medical conditions/illnesses in the past obtained by asking the subject or by inspecting his/her medical records will be recorded in the eCRF at screening. History of alcohol or drug abuse will also be recorded.

The information collected for concomitant illness and medical history should include diagnosis, date of onset, date of resolution or continuation.

Any change to a concomitant illness should be recorded during the trial, including end date, if applicable. A clinically significant worsening of a concomitant illness must be reported according to Section 7.2.1.

All previous and concomitant diseases will be coded with Medical Dictionary for Regulatory Activities (MedDRA), the version of which will be provided in the clinical trial report.

7.3.3 Diabetes diagnosis and current treatment

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

7.3.4 Concomitant medication

A concomitant medication is any medication, other than the trial products and current diabetes treatment (including insulin glulisine [Apidra®] for diabetes therapy wash-out), which is taken during the trial, including screening and follow-up periods.

Details of any concomitant medication must be recorded at trial entry (i.e. at screening). Any changes in concomitant medication must be recorded at each visit as they occur. The information collected for each concomitant medication includes (at a minimum) trade name or generic name, indication, start date and stop date or continuation. A change in medication due to an AE must be recorded and reported according to Section 7.2.1. If the change in medication influences the subject's eligibility to continue in the trial, the sponsor and trial monitor must be informed.

7.4 Other assessments

7.4.1 Immunogenicity

Antibodies against dasiglucagon/GlucaGen will be measured at the dosing visit (Visit 2) and at follow-up (Visit 3). At the dosing visit (Visit 2), samples will be collected prior to the start of the insulin-induced hypoglycemic procedure.

The clinical ADA assays, specific for dasiglucagon and GlucaGen, respectively, have been validated in accordance with existing guidelines and recommendations (17-21).

Confirmed positive anti-dasiglucagon antibody samples (treatment-induced or treatment-boosted) from anti-dasiglucagon antibody-positive subjects 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 neutralizing antibody analysis.

The in vitro neutralizing effect of the antibodies will be measured using an assay based on glucagon receptor transfected human embryonic kidney cells (20,22). The calculated sensitivity in previous studies was about 51.8 ng/mL. The assay was also validated for recombinant glucagon with similar results (21,23). In case of a positive result in the neutralizing antibody assay, a titer estimation will be performed. The cell-based neutralizing antibody analyses will be performed by a special laboratory, BioAgilytix, Durham, NC, USA.

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.

7.4.2 Plasma glucose measurements for safety

During insulin-induced hypoglycemia, plasma glucose levels will be monitored closely at site. After the start of insulin infusion, plasma glucose should be checked approximately every 10 minutes while plasma glucose is above 110 md/dL, and approximately every 5 minutes once plasma glucose is at or below 110 mg/dL and until after dosing when the subject's plasma glucose is ≥70mg/dL (3.9 mmol/L). Hereafter, plasma glucose should be checked approximately every 30 minutes until 300 minutes (5 hours) after dosing. Plasma glucose concentrations will be measured using the US FDA-approved glucose analyzer YSI 2300, Yellow Springs Instruments, Yellow Springs, OH or the Super GL analyzer, Dr. Müller Gerätebau GmbH, Freital, Germany.

At the discretion of the investigator, additional plasma glucose measurements can be taken at any time during the trial, for example when there is a suspicion (e.g. symptoms) of a hypoglycemic episode.

Plasma glucose measurements for safety should only be recorded in the eCRF if they are related to an AE (e.g. a hypoglycemic episode).

In case of persistent post-treatment hypoglycemia, subjects will receive rescue treatment with an IV glucose infusion (see Section 8.2.3.1 for details). Blood samples for PD and PK assessments should still be taken at the specified timepoints.

7.4.3 Plasma insulin measurements

Samples for insulin assessment will be collected at the dosing visit (Visit 2, pre-dose and at 30 and 60 minutes after dosing). The actual time of blood sampling for evaluation of plasma insulin should not deviate from the nominal time by more than ±1 minute. Pre-dose is defined as within 2 minutes prior to dosing.

8 TRIAL CONDUCT

8.1 Schedule of procedures

The schedule of procedures is provided in Table 3. Informed consent will be obtained prior to any trial-related procedures; see Section 10.8.

8.2 Procedures by visit

8.2.1 Visit 1 (screening, Day -30 to Day -3)

Visit 1 will take place between 3 and 30 days before Visit 2, Day -1 to Day 1 (dosing day).

Informed consent can be obtained prior to or at Visit 1, however it must in any case be obtained prior to any trial related procedures. During the screening visit, the following assessments will take place:

- Informed consent (obtain or check)
- Check of subject eligibility (inclusion/exclusion criteria)
- Demography
- Body measurements
- Medical history, diabetes diagnosis, current diabetes treatment
- Concomitant illnesses
- Concomitant medications
- History of alcohol/drug abuse
- Physical examination
- Vital signs
- 12-lead ECG
- AEs
- Biochemistry, hematology, coagulation, HbA_{1c}
- Serum pregnancy test (women of childbearing potential only)
- Urinalysis
- Urine drug screen
- Alcohol breath test

Table 3 Schedule of procedures

Visit number	V1	V2	V3
Trial day	**-3**	**-1 and 1**	**28**
Visit type	**Screening**	**Dosing**	**Follow-up**
Window	**-30 to -3**		**+5 days**
Subject related information/assessments			
Informed consent	x		
Inclusion/exclusion criteria	x	x[1,2]	
Demography	x		
Body measurements	x		
Medical history, diabetes diagnosis, and current diabetes treatment	x		
Concomitant illnesses	x		
Concomitant medications	x	x[1]	x
History of alcohol/drug abuse	x		
Randomization		x[1]	
Withdrawal criteria		x[1]	
Dosing day exclusion criteria		x[1]	
Insulin-induced hypoglycemia		x	
Safety assessments			
Physical examination	x		x
Vital signs	x	x[3]	x
12-lead ECG	x	x[4]	x
Local tolerability		x[5]	x
Adverse events	x	x	x
Laboratory			
Biochemistry, hematology, coagulation, HbA_{1c} (coagulation and $HbA_{1c,}$ at Visit 1 only)	x	x[6]	x
Pregnancy test (women of childbearing potential only)	x[7]	x[1,8]	x[8]
Urinalysis	x	x[1]	x
Urine drug screen	x	x[1]	
Alcohol breath test	x	x[1]	
PK/Clinical efficacy			
Plasma dasiglucagon/GlucaGen		x[9]	
Plasma glucose		x[10]	
Other assessments			
Antibodies against dasiglucagon/GlucaGen		x[1]	x[11]
Plasma insulin		x[12]	
Trial material			
Administration of trial product (during hypoglycemic clamp procedure)		x	

[1]Prior to the start of the insulin-induced hypoglycemic procedure.
[2]Only check of dosing day exclusion criteria and changes between screening visit and Visit 2.
[3]Prior to the start of the insulin-induced hypoglycemic procedure (within 30 minutes), and at 30, 90 and 300 minutes after dosing. The actual time of the assessment should not deviate from the nominal time by more than ±10 minutes.

[4]Prior to the start of the insulin-induced hypoglycemic procedure (within 30 minutes), and at 20, 35, 45, 60 and 300 minutes after dosing. The actual time of the assessment should not deviate from the nominal time by more than ±5 minutes.
[5]Local tolerability assessed at 30, 120, and 300 minutes after dosing. The actual time of the assessment should not deviate from the nominal time by more than ±10 minutes.
[6]Prior to the start of the insulin-induced hypoglycemic procedure (within 30 minutes), and at 30 and 300 minutes after dosing. The actual time of the assessment should not deviate from the nominal time by more than ±10 minutes.
[7]Serum pregnancy test.
[8]Urine stick pregnancy test.
[9]Pre-dose, and at 15, 30, 35, 40, 50, 60, 90, and 120 minutes after dosing. The actual time of blood sampling should not deviate from the nominal time by more than ±1 minute. Pre-dose is defined as within 2 minutes prior to dosing.
[10]Pre-dose, and at 4, 6, 8, 10, 12, 15, 17, 20, 25, 30, 40, 50, 60, 75, 90 minutes after dosing. The actual time of blood sampling should not deviate from the nominal time by more than ±30 seconds until the 20 minute collection time point and by more than ±1 minute for the subsequent collection time points. Pre-dose is defined as within 2 minutes prior to dosing.
[11]Any subject that tests positive for ADA will be monitored until the ADA levels return to baseline levels.
[12]Pre-dose, and at 30 and 60 minutes after dosing. The actual time of blood sampling should not deviate from the nominal time by more than ±1 minute. Pre-dose is defined as within 2 minutes prior to dosing.
ADA = anti-drug antibodies; ECG = electrocardiogram; HbA_{1c} = glycated hemoglobin.

Eligible or potentially eligible subjects (laboratory results pending) will be provided with an Identification card (ID card), stating that the subject is participating in the trial and whom to contact (site address, investigator name and telephone number). The subjects should be instructed to return the ID card to the investigator at the last visit or to destroy the card after the last visit.

8.2.2 Instructions to subjects prior to the dosing visit (Visit 2)

At the screening visit, the investigator will inform the subject about the changes to his/her insulin therapy leading up to the start of the insulin-induced hypoglycemic procedure. The subject may be provided with insulin NPH in the wash-out period to cover the need of basal insulin, if deemed necessary by the investigator. The subject's current insulin therapy will be washed out as defined in Section 5.5.: 48 hours prior to dosing and during the dosing visit, treatment with insulin Degludec and insulin Glargine U300 are not allowed; 24 hours prior to dosing and during the dosing visit, treatment with other long-acting insulins (e.g., insulin Glargine U100 or insulin Detemir) is not allowed; 16 hours prior dosing and during the dosing visit treatment with insulin NPH is not allowed; 6 hours prior to dosing and during the dosing visit, treatment with any short acting (bolus) insulin, except insulin glulisine (Apidra®), is not allowed. The basal rate of insulin pumps (continuous SC insulin infusion) will be discontinued on the morning of the dosing day (if using insulin glulisine [Apidra®]) OR at least 6 hours prior to dosing (if using other insulins).

On the day prior to dosing (Day -1), the subjects will need to attend the clinical center and will be required to stay onsite overnight. On the morning of the dosing day (Day 1), patients are required to be in a fasting condition, defined as having consumed only water since 22:00 hours the night before. However, the subjects are allowed to consume small

amounts (up to 20 g) of carbohydrates to prevent hypoglycemia. The subjects must also not consume any alcohol within 24 hours prior to dosing (refer to Section 5.5 for all dosing day exclusion criteria).

8.2.3 Visit 2 (dosing visit)

Visit 2 will take place on Day -1 to Day 1.

The subjects will attend the clinical center the day prior to dosing (Day -1) and subject eligibility is rechecked (check of changes between the screening visit and Visit 2) and those subjects eligible to participate should stay onsite overnight. To target a glucose level around 90-110 mg/dL (5.0-6.1 mmol/L) the following morning, subjects may be administered insulin glulisine (Apidra®) at the investigator's discretion either by IV infusion, SC bolus or continuous SC insulin infusion. Dosing will take place the following morning (Day 1).

On Day 1 and prior to the start of the insulin-induced hypoglycemic procedure, those subjects eligible to participate will be randomized to treatment with dasiglucagon, placebo, or GlucaGen.

The following assessments will also take place:

- Document all changes in concomitant medication (prior to the start of the insulin-induced hypoglycemic procedure)
- Check of withdrawal criteria (prior to the start of the insulin-induced hypoglycemic procedure)
- Check of dosing day exclusion criteria (prior to the start of the insulin-induced hypoglycemic procedure)
- Vital signs (prior to the start [within 30 minutes] of the insulin-induced hypoglycemic procedure, and at 30, 90 and 300 minutes after dosing). 12-lead ECG (prior to the start [within 30 minutes] of the insulin-induced hypoglycemic procedure, and at 20, 35, 45, 60, and 300 minutes after dosing)
- Local tolerability (at 30, 120, and 300 minutes after dosing)
- AEs
- Biochemistry, hematology (prior to the start of the insulin-induced hypoglycemic procedure [within 30 minutes], and at 30 and 300 minutes after dosing)
- Urine stick pregnancy test (women of childbearing potential only; prior to the start of the insulin-induced hypoglycemic procedure)
- Urinalysis (prior to the start of the insulin-induced hypoglycemic procedure [within 120 minutes])
- Urine drug screen (prior to the start of the insulin-induced hypoglycemic procedure)
- Alcohol breath test (prior to the start of the insulin-induced hypoglycemic procedure)

- Dasiglucagon/GlucaGen plasma concentrations:
  - Pre-dose, 15, 30, 35, 40, 50, 60, 90, and 120 minutes after dosing. The actual time of blood sampling for exposure should not deviate from the nominal time by more than ±1 minute. Pre-dose is defined as within 2 minutes prior to dosing.
- Plasma glucose concentrations:
  - Pre-dose, 4, 6, 8 ,10, 12, 15, 17, 20, 25, 30, 40, 50, 60, 75, and 90 minutes after dosing. The actual time for blood sampling for plasma glucose should not deviate from the nominal time by more than ±30 seconds until the 20 minute collection time point and by more than ±1 minute for the subsequent collection time points. Pre-dose is defined as within 2 minutes prior to dosing.
- Antibodies against dasiglucagon/GlucaGen (prior to the start of the insulin-induced hypoglycemic procedure).
- Plasma insulin concentrations:
  - Pre-dose, 30 and 60 minutes after dosing. The actual time of blood sampling for plasma insulin should not deviate from the nominal time by more than ±1 minute. Pre-dose is defined as within 2 minutes prior to dosing.

8.2.3.1 Hypoglycemic clamp procedure and administration of trial medication

The following procedure is based on precendent procedures for hypoglycemia induction in patients with T1DM [24, 25].

The treatment day (Visit 2, Day 1) will be conducted after an overnight fast, targeting a starting plasma glucose around 90 to 110 mg/dL (5.0-6.1 mmol/L).

Subjects who meet one or more of the dosing day exclusion criteria (Section 5.5) at the dosing visit will be excluded from the dosing visit, but the visit can be rescheduled 1-7 days later. The dosing visit can only be rescheduled once.

The subject's current insulin therapy will be washed out as defined in Section 5.5. For subjects using multiple daily injections, the date, time and the dose of the last basal insulin and the last short-acting insulin (except insulin glulisine [Apidra®]) administration prior to dosing will be captured. For subjects using an insulin pump, the time of discontinuation of the basal rate will be captured. Any use of insulin glulisine (Apidra®) in the last 5 hours prior to initiation of the hypoglycemia induction procedure will also be captured.

At approximately 08:00 hours, an infusion catheter will be inserted into each arm (forearm cephalic vein) for the manual glucose clamp procedure, with the glucose infusion in one arm and the insulin infusion in the opposite arm (if IV insulin glulisine [Apidra®] has been administered during the night the same infusion catheter can be used). A third catheter for blood sampling will be placed into a metacarpel vein for blood

sampling. This hand will be warmed (55-65ºC) to arterialize venous blood. If there are issues with blood sampling from the metacarpal vein for the purpose of glucose measurements, a new and more proximal IV access may be used at the discretion of the investigator.

Hypoglycemia will be gradually induced by a fast-acting IV insulin glulisine (Apidra®) infusion (15 U [100 U/mL] in 49 mL saline and 1 mL of subject's blood or plasma), initially at 150% of the subject's usual basal rate and can be increased or decreased over a range of 0% to 200% or more as judged necessary by the investigator, to achieve a controlled decline in plasma glucose, targeting a plasma glucose level of 55 mg/dL (3.1 mmol/L).

Plasma glucose concentrations will be measured using the US FDA-approved glucose analyzer YSI 2300, Yellow Springs Instruments, Yellow Springs, OH or the Super GL analyzer, Dr. Müller Gerätebau GmbH, Freital, Germany. After the start of the insulin infusion, plasma glucose will be measured approximately every 10 minutes while plasma glucose is above 110 mg/dL, and approximately every 5 minutes once plasma glucose is at or below 110 mg/dL.

Once the glucose concentration declines to <60 mg/dL (3.3 mmol/L), the insulin infusion will be stopped, and 5 min later plasma glucose concentration will be measured at the glucose analyzer and blood samples for baseline assessment of plasma glucose, dasiglucagon/GlucaGen PK, and insulin PK will be collected. The samples are the baseline samples and should be collected within 2 minutes before IMP administration.

- If plasma glucose is ≥45 mg/dL and <60 mg/dL (2.5-3.3 mmol/L), study treatment (IMP) will be administered, defining time, t=0. The study treatment will be delivered in the abdomen, buttock, or thigh (according to stratification) via SC injection.
- If plasma glucose is <45 mg/dL (2.5 mmol/L), IV glucose solution will be administered sufficient to raise plasma glucose to within the 45-60 mg/dL target range. The run-in period will be adequately extended (at least 30 min) until the above target is achieved and new baseline samples for plasma glucose, dasiglucagon/GlucaGen PK, and insulin PK will be collected. Glucose should not be infused within 10 minutes before IMP administration. If plasma glucose is not within target range after the second attempt, the subject should be rescheduled for a new treatment visit within 7 days (+ 2 days).

Administration of IMP should not occur earlier than 9:00 hours in the morning or later than 12:00 hours.

As shown in Table 4, serial blood samples for glucose will be collected at t=0, 4, 6, 8, 10, 12, 15, 17, 20, 25, 30, 40, 50, 60, 75 and 90 minutes post-dosing. Samples for assessing

plasma dasiglucagon/GlucaGen concentration will be collected at t=0, 15, 30, 35, 40, 50, 60, 90, and 120 minutes. Samples for assessing plasma insulin concentration will be collected at t=0, 30, and 60 minutes.

Table 4 Post-treatment blood sampling times

Times	0	4	6	8	10	12	15	17	20	25	30	35	40	50	60	75	90	120
Plasma glucose	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y		Y	Y	Y	Y	Y	
PK Dasiglucagon/ GlucaGen	Y						Y				Y	Y	Y	Y	Y		Y	Y
PK Insulin	Y										Y				Y			

Refer to Section 7.2.7 for details of laboratory safety sampling and to Section 7.4.2 for details of blood glucose safety sampling.

When the t=90-minute blood sampling for plasma glucose has been collected the subjects are allowed to eat moderately. Drinking of water is allowed *ad libitum* during the entire procedure.

Hypoglycemia Rescue Provisions

During insulin-induced hypoglycemia, plasma glucose levels will be monitored closely at site for safety reasons as described in Section 7.4.2. Subjects should receive post-treatment rescue glucose infusion to ameliorate persistent hypoglycemia, as follows.

1. Glucose infusion should be initiated if a subject experiences severe alarming escalation of symptoms of hypoglycemia (e.g. symptoms suggesting a change in consciousness) at any time during the trial; glucose infusion should be initiated targeting a plasma glucose levels >70 mg/dL.
2. If plasma glucose is <45 mg/dL (2.5 mmol/L) between t=8 and t=44 minutes, rescue glucose infusion (1-2 mg/kg administered IV over about 5 seconds) should be initiated to maintain plasma glucose between 45 mg/dL and 55 mg/dL (2.5-3.1 mmol/L). Pause glucose infusion if plasma glucose is >55 mg/dL.
3. If plasma glucose is <70 mg/dL at t=45 minutes, rescue glucose infusion (2-3 mg/kg administered IV over about 5 seconds) should be initiated to maintain plasma glucose between 70 mg/dL and 80 mg/dL (3.9-4.4 mmol/L). Pause glucose infusion if plasma glucose is >75 mg/dL (4.2 mmol/L).

Subjects should remain in bed until completion of the test procedure 300 minutes after dosing (bathroom visits are allowed).

The IMP will be administered SC according to Section 6.3. The time of IMP administration will be recorded. At the timepoint when the insulin infusion is stopped, the total insulin dose which was required to induce hypoglycemia will be recorded.

AEs will be specifically recorded during the procedure at several timepoints.

The investigator must provide information to the subjects on how to resume their usual diabetes treatment.

The subject may be released from the clinical center if the investigator does not have any safety concerns based on the last safety plasma glucose value and the general condition of the subject. However, at the discretion of the investigator or on request of the subject, the subject may stay at the trial center for a longer period.

8.2.4 Visit 3 (follow-up visit)

Visit 3 will take place on Day 28 + 5 days. The subject does not need to be fasting.

At Visit 3, the following assessments will take place:

- Document all changes in concomitant medication
- Physical examination
- Vital signs
- 12-lead ECG
- Local tolerability
- AEs
- Biochemistry, hematology
- Urine stick pregnancy test (women of childbearing potential only)
- Urinalysis
- Antibodies against dasiglucagon/GlucaGen.

After the follow-up visit the End of Trial form must be completed. Even if a subject is not able to attend the follow-up visit, the End of Trial form, the eCRF Accountability/Affirmation Statement form and the Drug Accountability form must be completed.

9 STATISTICAL METHODS

Before database lock and treatment unmasking, a separate statistical analysis plan (SAP) will be finalized, providing detailed methods for the analyses outlined below. Further analysis details may be added or refined in the SAP.

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

9.1 Determination of sample size

Due to requirements in the size of the safety database, the sample size is set to 78 subjects treated with dasiglucagon 0.6 mg. From phase 2, the median time to an increase of 20 mg/dL of the 0.6 mg dose was approximately 10 minutes. With a 2:1:1 randomization ratio for the three treatments, dasiglucagon 0.6 mg, placebo, and GlucaGen 1 mg, and assuming an exponential time-to-recovery distributions with median times of 10 and at least 20 minutes for dasiglucagon and placebo, respectively, a two-sided log-rank test will be able to detect a difference between dasiglucagon and placebo with 90% power with a follow-up time of 45 minutes at a 5% significance level with 78 subjects treated with dasiglucagon and 39 subjects with placebo. The median time-to-recovery for placebo is expected to be longer than 20 minutes, which means that the power will be greater than 90%.

9.2 Trial subjects

9.2.1 Analysis samples

For presentation of data and reporting of the statistical analyses, the following analysis samples will be used, depending on the context:

- Safety analysis set (SAS): All randomized subjects who received at least one dose of trial medication.
- Full analysis set (FAS): All randomized subjects who received at least one dose of trial medication.
- Per protocol (PP) set: All subjects of the FAS for whom no relevant protocol deviations were documented. This sample will primarily be used for sensitivity analysis.

The analysis of the primary endpoint and secondary endpoints will be based on the FAS. A secondary analysis of the primary endpoint will be based on the PP set. All safety analyses will be based upon the SAS.

The decision regarding whether a protocol deviation is relevant or not for the exclusion of subjects from the PP set will be made case-by-case in a data review meeting prior to treatment unmasking and database lock (see Section 9.2.3).

9.2.2 Disposition of subjects

Subject disposition will be tabulated including the number of screened subjects, screening failures, subjects exposed to trial product, subjects completing the trial and subjects in each analysis set.

Subjects withdrawn from the trial will be listed including the primary reason for withdrawal.

9.2.3 Protocol deviations

Before data are released for statistical analysis, a treatment-masked review of all data will take place to identify protocol deviations that may potentially affect the results. This review will be performed without revealing to which trial product the subjects were assigned. The masking of the trial products will be maintained for everyone involved in allocating subjects to the analysis sets until data are released for statistical analysis. Furthermore, spurious outliers will be evaluated. In addition, protocol deviations that may potentially affect the results will be identified and it will be evaluated if subjects and/or data should be excluded from the analysis. Protocol deviations will be classified as minor or major in a consistent way. Major deviations from the protocol may lead to the exclusion of a subject from the PP set.

Major protocol deviations may include deviations related to trial inclusion or exclusion criteria, conduct of the trial, subject management or subject assessment. Unless explicitly decided otherwise during the masked data review, the following will be considered major protocol deviations:

- Violation of one or more major inclusion/exclusion criteria
- Interruption of administration of trial product
- Significant deviation from time windows
- Incorrect treatment allocation
- Missing primary endpoint.

The violation of several major inclusion/exclusion criteria or the complete absence of efficacy data might lead to exclusion of the subject from FAS. In that case, the decision should be taken at the masked data review meeting, and the exclusion from efficacy analysis justified in the signed notes of the meeting.

Obviously erroneous data points may be excluded from the analyses or re-analyzed (in case of e.g. serum concentrations). The decision to re-analyze or exclude data points from the statistical analysis is the joint responsibility of the sponsor and the trial statistician.

The subjects or observations to be excluded and the reason for their exclusion will be documented and signed by those responsible prior to database release. The documentation will be stored together with the remaining trial documentation. The subjects and observations excluded from analysis sets, and the corresponding reasons, will be described in the clinical trial report.

9.3 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 timepoint will be included in the analysis.

Raw data listings and summary tables will be generated using the software SAS© version 9.4 or higher.

Continuous variables will be summarized using means, standard deviations, medians, coefficients of variation, and minimum and maximum values.

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

9.4 Demographics and baseline characteristics

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

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

9.5 Efficacy Analyses

For the confirmatory analyses, the primary and key secondary endpoints for the dasiglucagon 0.6 mg and placebo treated subjects will be compared. A hierarchical procedure will be applied for the control of multiplicity. The primary and secondary endpoints will be evaluated on the full analysis set. In the primary analysis, those subjects

who require rescue IV glucose will be censored at the time to plasma glucose recovery. In a sensitivity analysis, the time to plasma glucose recovery will be analyzed without censoring the subjects who received rescue IV glucose.

9.5.1 Hierarchical testing procedure

For the confirmatory analyses, the following a priori defined hierarchical inferential test order will be applied for the control of the type 1 error rate across the planned multiple comparisons. The primary endpoint and key secondary endpoints will be inferentially evaluated within the FAS in the following order, where inference will proceed at the two-sided 0.05 criterion significance level until the first failure to reject the null hypothesis for a dasiglucagon versus placebo comparison:

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

The GlucaGen versus placebo comparisons will not be included in the inferential testing hierarchy, since the efficacy of GlucaGen is previously established, and these comparisons are intended to support the validity of the study for the dasiglucagon versus placebo comparisons.

Analogous supportive sensitivity analyses will be conducted in the PP set, but without inference intent.

9.5.2 Primary confirmatory endpoint

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

9.5.2.1 Primary analysis

The primary endpoint will be summarized using Kaplan-Meier (KM) estimates stratified by treatment group and injection site. The treatment group difference between dasiglucagon and placebo will be evaluated inferentially using a pairwise two-sided log-rank test.

In the primary analysis, recovery cannot be achieved in those subjects where IV glucose treatment is administered. Those subjects who receive IV glucose will be censored (i.e. set to 'not recovered') at 45 minutes after dosing.

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

The primary endpoint will additionally be analyzed using a Cox proportional hazards (CPH) time to event statistical model, if the hazards by treatment group can be assumed proportional using a graphical comparison of log-minus-log survival curves and no more than two-thirds of the recovery times are censored in each treatment group. The CPH model will be used for inferences, with treatment group and injection site modeled as categorical effects, and baseline plasma glucose modeled as a continuous covariate. The treatment group rate ratios, active versus placebo, will be estimated together with the 95% confidence intervals, and pairwise treatment group inferences (dasiglucagon vs placebo, GlucaGen vs placebo) will be evaluated using two-sided likelihood ratio tests.

9.5.3 Secondary endpoints

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

9.5.3.1 Confirmatory analysis

The key secondary incidence variables (plasma glucose recovery within 30 minutes, within 20 minutes, within 15 minutes, within 10 minutes, in hierarchical order for inference) will be analyzed using descriptive statistics (frequency and percentage) by treatment group. Inferential treatment group comparisons will be assessed via pairwise tests of independent binomial proportions (dasiglucagon versus placebo, GlucaGen versus placebo) using Fisher's exact test for each pairwise comparison.

The key secondary endpoints of plasma glucose CFB within 30 minutes, within 20 minutes, within 15 minutes, and within 10 minutes after study drug injection or at the time of rescue, will be analyzed with the plasma glucose CFB at rescue carried forward in those subjects who require rescue IV glucose before plasma glucose ≥20 mg/dL recovery. Each of these CFB variables will be analyzed using an Analysis of Covariance model, with treatment group modeled as a fixed effect and with the baseline plasma glucose modeled as a covariate. The dasiglucagon versus placebo treatment group difference will be evaluated inferentially as a least squares means contrast, using a two-sided t-test at the

0.05 signficance level, first for the 30 minute CFB, and then sequentially for the 20 minute, 15 minute, and 10 minute variables, until the first failure to reject.

9.5.4 Secondary clinical efficacy (PD) endpoints

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

Secondary clinical efficacy endpoints will be assessed based on plasma concentration data from samples collected at the dosing visit (Visit 2).

Unless otherwise stated, the population base of analysis will be the FAS.

9.5.4.1 Analysis of secondary clinical efficacy (PD) endpoints

1. Time to first plasma glucose concentration ≥70 mg/dL from baseline. This time-to-event endpoint will be evaluated using a Kaplan-Meier approach, with treatment group as a stratification factor, analogous to that used for the primary endpoint analysis. Differences between the KM curves (dasiglucagon versus placebo, GlucaGen versus placebo) will be evaluated inferentially using pairwise two-sided log rank tests. If the ≥70 mg/dL endpoint is not met within 45 minutes post-dosing, the time of the last valid plasma glucose measurement up to 45 minutes will be the censoring time.
2. The AUC will be calculated as the baseline-adjusted area under the plasma glucose profile over time:
    a. $AUC_{0\text{-}30min}$: restricting the time window to the 0 to 30 minutes interval.
3. The log-transformed AUC endpoint will be analyzed using an analysis of covariance (ANCOVA) model with treatment as fixed effect and baseline plasma glucose modeled as a covariate. The least squares means treatment group differences will be back-transformed (anti-logged) for presentation as a ratio of the treatment group geometric means, with their corresponding 95% CI.

9.5.5 Exposure (PK) endpoints

- Plasma dasiglucagon and GlucaGen concentrations from time zero to 90 minutes: $AUC_{0\text{-}90min}$, C_{max}, and t_{max}.

9.5.5.1 Analysis of exposure (PK) endpoints

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

C_{max} will be determined as the maximum of all valid plasma dasiglucagon/GlucaGen concentrations.

T_{max} will be determined as the timepoint where the maximum of all valid plasma dasiglucagon/GlucaGen concentration measurements for each measurement series is observed.

The log-transformed PK endpoints AUC and C_{max} will be analyzed in the same way as the AUC endpoints.

As t_{max} is a highly discrete endpoint, Wilcoxon's rank sum test for unpaired observations will be used to assess differences between the two treatment groups.

9.6 Exploratory analyses

Exploratory analyses will include descriptive statistics and modeling analogous to that done for key secondary endpoints. However, treatment group comparisons will be summarized without inference intent.

- Plasma glucose concentration ≥70 mg/dL (3.9 mmol/L) or increase of ≥20 mg/dL (1.1 mmol/L) within 30 minutes after study drug injection without administration of rescue IV glucose.

- Plasma insulin response as area under the curve above baseline from time zero to 60 minutes, $AUC_{0\text{-}60\ min}$.

Plasma insulin concentrations measured pre-dose and at 30 and 60 minutes after dosing (see Table 3) will be presented individually. A summary table per timepoint will be provided. The $AUC_{0\text{-}60min}$ will be determined and a summary presented.

9.7 Safety analyses

9.7.1 Adverse events

AEs will be coded using the latest available version of the MedDRA, the version of which will be provided in the clinical trial report. AE summary tables will include the number and percentage of subjects who experienced AEs summarized by system organ class and preferred term.

Germany only: The AE summary for AEs related to insulin glulisine (Apidra®) will be presented as an appendix to the clinical trial report, as insulin glulisine (Apidra®) is only defined as an IMP in Germany.

An overall summary table will be provided showing the number and percentage of subjects with any:

- TEAE
- Severe TEAE
- Serious TEAE
- Drug-related TEAE
- Drug-related severe TEAE
- Drug-related serious TEAE
- TEAE leading to withdrawal
- TEAE with outcome death
- AESI

Refer to Section 7.2.1 for the definition of TEAEs.

9.7.2 Immunogenicity data

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

9.7.3 Clinical laboratory assessments

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

9.7.4 Other safety data

Incidence of rescue infusion of IV glucose during the hypoglycemic clamp procedure will be analyzed using descriptive statistics (frequency and percentage) by treatment group. Inferential treatment group comparisons will be assessed via pairwise tests of independent binomial proportions (dasiglucagon versus placebo, GlucaGen versus placebo).

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

Vital signs, physical examination, 12-lead ECG and local tolerability data will be summarized using descriptive statistics.

9.8 Treatment compliance

Trained unblinded members of staff will perform all administrations of the IMP at the trial center. The administered doses will be recorded in the blinded Drug Accountability form in the eCRF.

PK assessments will support the surveillance of compliance with IMP administration.

9.9 Subject withdrawals and missing data

Failure is defined for the primary time-to-recovery endpoint as censored with time to recovery set to the maximum follow-up time, here 45 minutes. Individuals will be set to 'failure' in case of receiving rescue IV glucose, discontinuation due to treatment or discontinuation in connection with the induced hypoglycemia state. Only if an intermediate assessment is missing independent of rescue treatment, study treatment or an adverse event including hypoglycemia, interpolation can be applied to impute a missing value.

The same construct of imputation applies for the analysis of the plasma glucose AUC0-30min.

In the case of subject withdrawal, no imputation of values for PK or PD measurements will be done. Analyses will be done on valid cases only, i.e., no imputation techniques such as last observation carried forward will be applied.

9.10 Interim analyses

No interim analyses are currently planned.

10 ETHICAL, LEGAL, AND ADMINISTRATIVE ASPECTS

10.1 Quality assurance

The sponsor or designee will conduct a site visit to verify the qualifications of each investigator, inspect the facilities, and inform the investigator of responsibilities and the procedures for ensuring adequate and correct documentation.

The investigator is required to prepare and maintain adequate and accurate case histories designed to record all observations and other data pertinent to the trial for each trial participant. All information recorded on the eCRFs for this trial must be consistent with the subjects' source documentation (i.e. medical records).

The investigator will permit trial-related monitoring, IRB/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 monitor or a representative of the sponsor will cross-check the data entered in the eCRFs with the source data at the trial center and observe the trial procedures in order to verify adherence to the trial protocol. Any queries will be resolved by the investigator or his/her delegate.

All of the clinical data will be captured via electronic data capture (EDC) using a web-based tool.

The investigator center 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 subjects. The investigator's data will be accessible from the investigator's center throughout the trial. The eCRFs must be kept current to reflect subject status at each phase during the course of the trial. The eCRF will not capture personalized data. The investigator must make a separate confidential record of personalized details (name and initials) on the subject identification and enrollment log. All changes to data are made by the investigator or his/her delegate through the EDC system.

It is the responsibility of the principal investigator of the respective center to ensure that all subject discontinuations or changes in trial or other medications entered on the subject's eCRF are also made on the subject's medical records.

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

10.2 Electronic case report forms

Remote data capture software will be used for data collection. Following training, trial staff will be given access to the software. Access to the software is restricted to staff participating in the trial and the extent of access will depend on the participants' user role in the trial.

The subjects enrolled into the trial will be identified in the database by subject number and trial identification code. The investigator or delegate will enter subject data into the eCRF promptly. All data is to be entered in English. Data recorded in the eCRFs will be accessible to the trial staff throughout the trial.

After data entry, systematic data validation will be performed and data entry discrepancies will be presented electronically directly to the center staff. Queries for discrepant data may be generated automatically by the software upon entry and/or generated manually by the trial monitor or the trial data manager. All queries, whether generated by the system or by trial staff, will be in electronic format.

All sections of the eCRF are to be electronically approved by the investigator or a medically qualified delegate after the data has been entered and all queries have been resolved. Changes to any eCRF page subsequent to the approval require a new approval signature.

All queries and changes/corrections to the data are documented in the eCRF.

10.3 Access to source data

During the course of the trial, a trial monitor will make site visits to review protocol compliance, compare eCRFs with individual subject’s medical records, assess drug accountability, and ensure that the trial is being conducted according to pertinent regulatory requirements.

Checking of the eCRFs for completeness and clarity, and cross-checking with source documents, will be required to monitor the progress of the trial. Moreover, regulatory authorities of certain countries, IRBs/IECs, and/or the sponsor may wish to carry out such source data checks and/or on-site audit inspections. Direct access to source data will be required for these inspections and audits; they will be carried out giving due consideration to data protection and medical confidentiality.

10.4 Source documentation

All source documents from which eCRF entries are derived should be placed in the subject's medical records. If data are to be entered directly into the eCRF this must be specified in a source data agreement prior to the start of the trial.

eCRF entries will be verified with source documentation. The review of medical records will be performed in a manner to ensure that subject confidentiality is maintained.

The trial monitor will check the eCRF for accuracy and completion and perform source data verification. The trial monitor will document source data verification of all reviewed sections of the eCRF.

10.5 Data processing

The trial is run as an EDC trial, i.e. all relevant data is entered by the centers directly into the clinical database. The eCRF is designed to capture all required information in compliance with GCP standards.

10.6 Archiving trial records

According to ICH guidelines, essential documents should be retained for a minimum of 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. However, these documents should be retained for a longer period if required by the applicable legal requirements.

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

10.7 Good clinical practice

The procedures set out in this trial protocol are designed to ensure that the sponsor and investigator abide by the principles of the GCP guidelines of the ICH (1), and of the Declaration of Helsinki (2008) (2). The trial also will be carried out in keeping with local legal requirements.

10.8 Informed consent

Before each subject is admitted to the trial, written informed consent will be obtained from the subject according to the regulatory and legal requirements of the participating country. This consent form must be dated and retained by the investigator as part of the trial records. The investigator will not undertake any investigation specifically required only for the clinical trial until valid consent has been obtained. The terms of the consent and when it was obtained must also be documented in the eCRF.

The investigator will explain to each subject orally and in writing (subject information sheet) the nature, significance, risks and implications of the trial before inclusion. In particular, the subjects 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.
- That his/her medical records may be examined by authorized monitors or clinical quality assurance auditors appointed by the sponsor, by appropriate IRB/IEC members, and by inspectors from regulatory authorities.

All subjects will receive a copy of the subject information sheet and a copy of their signed and dated informed consent form, both of which will be in the subject's local language.

If a protocol amendment is required, the informed consent form may need to be revised to reflect the changes to the protocol. If the consent form is revised, it must be reviewed and approved by the appropriate IEC/IRB, and signed by all subjects subsequently enrolled in the trial as well as those currently enrolled in the trial.

10.9 Protocol approval and amendment

Before the start of the trial, the trial protocol and/or other relevant documents will be approved by the IEC/IRB/competent authorities, in accordance with local legal requirements. The sponsor must ensure that all ethical and legal requirements have been met before the first subject is enrolled in the trial.

This protocol is to be followed exactly. To alter the protocol, amendments must be written, receive approval from the appropriate personnel, and receive IRB/IEC/competent authority approval prior to implementation (if appropriate).

Administrative changes (not affecting the subject benefit/risk ratio) may be made without the need for a formal amendment. All amendments will be distributed to all protocol recipients, with appropriate instructions.

10.10 Duration of the trial

The maximum duration of the trial for each subject will be up to 63 days (including up to 30 days for screening and up to 33 days until the follow-up visit).

The trial will be closed when all subjects have completed Visit 3.

10.11 Premature termination of the trial

If the investigator, the sponsor (e.g. safety committee), or the safety medical monitor becomes aware of conditions or events that suggest a possible hazard to subjects if the trial continues, the trial may be terminated after appropriate consultation between the relevant parties. The trial may also be terminated early at the sponsor's discretion in the absence of such a finding.

Conditions that may warrant termination include, but are not limited to:

- The discovery of an unexpected, significant, or unacceptable risk to the subjects enrolled in the trial
- Failure to enroll subjects at an acceptable rate.
- A decision on the part of the sponsor to suspend or discontinue development of the drug.

The trial can be terminated prematurely by the sponsor at an individual center if:

- The center cannot comply with the requirements of the protocol.
- It is not possible for the center to comply with GCP standards.

10.12 Confidentiality

All trial findings and documents will be regarded as confidential. The investigator and members of his/her research team must not disclose such information without prior written approval from the sponsor.

The anonymity of participating subjects must be maintained. Subjects will be identified on eCRFs submitted to the sponsor by their subject number. On the SAE reports and all other source documents, the subject will be identified via subject number. Documents not

to be submitted to the sponsor that identify the subject (e.g. the signed informed consent form) must be maintained in confidence by the investigator.

10.13 Other ethical and regulatory issues

If a significant safety issue is identified, either from an individual case report or review of aggregate data, then the sponsor will issue prompt notification to all parties: regulatory authorities, investigators and IRB/IECs.

A significant safety issue is one that has a significant impact on the course of the clinical trial or program (including the potential for suspension of the trial program or amendments to protocols) or warrants immediate update of informed consent.

10.14 Liability and insurance

The sponsor will take out reasonable third-party liability insurance cover in accordance with all local legal requirements. The civil liability of the investigator, the persons instructed by him and the hospital, practice or institute in which they are employed and the liability of the sponsor with respect to financial loss due to personal injury and other damage that may arise as a result of the carrying out of this trial are governed by the applicable law.

The sponsor will arrange for liability insurance if subjects should be injured due to the participation in the trial and provided that the sponsor is legally liable for that.

Excluded from the insurance cover are injuries to health and deteriorations of illnesses already in existence which would have occurred or continued to exist even if the subject had not taken part in the clinical trial.

The insurance cover is jeopardized if the subject fails to report immediately to the investigator or responsible physician any injury to health, which might have resulted from participation in the clinical trial, or if he/she undergoes any other medical treatment without their consent before the clinical trial has been completely finished in so far as the individual subject is concerned.

Any injury to health, which might have occurred as a result of participation in the clinical trial must be reported by the subject to the investigator without delay. The investigator is obliged to make such a report in any case.

10.15 Publication policy

By signing the trial protocol, the investigator agrees with the use of results of the trial for the purposes of national and international registration, publication and information for medical and pharmaceutical professionals. If necessary, the authorities will be notified of the investigator's name, address, qualifications and extent of involvement.

A clinical trial report will be prepared and reviewed by the sponsor in co-operation with the coordinating investigator. The coordinatoing investigator will be appointed by Zealand Pharma to review and sign the clinical trial report on behalf of all participating investigators. A summary of the clinical trial report (according to CPMP/ICH137/95) should be sent to the regulatory authorities and IRB/IEC according to the relevant guidelines.

According to the Declaration of Helsinki (2) investigators and sponsors 'have ethical obligations with regard to the publication and dissemination of the results of research'.

The trial design and results may be published as one or more original research manuscripts/abstracts and presented at a scientific meeting. 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 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. Authorship on any publication(s) resulting from this clinical trial will be assigned according to the recommendations of the International Committee of Medicinal Journal Editors (26).

Participating subjects will not be identified by name in any published reports about the clinical trial.

The sponsor is responsible for trial registration at ClinicalTrials.gov (www.clinicaltrials.gov) according to FDA requirements, as well as the European Medicines Agency's Clinical Trials Database (EudraCT).

11 REFERENCE LIST

1. ICH Harmonised Tripartite Guideline. International Conference on Harmonisation. ICH Harmonised Tripartite Guideline. Integrated addendum to ICH E6(R1): Guideline for Good Clinical Practice E6 (R2), Step 4. 09-Nov-2016. Available from: URL: http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E6/E6_R2__Step_4.pdf
2. World Medical Association. Declaration of Helsinki. Ethical Principles for Medical Research Involving Human Subjects. 52nd WMA General Assembly, Edinburgh, Scotland, October 2000. Last amended with Note of Clarification on Paragraph 29 by the WMA General Assembly, Washington 2002, and Note of Clarification on Paragraph 30 by the WMA General assembly, Tokyo 2004
3. 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 May;36:1384-95
4. Cryer PE. The barrier of hypoglycemia in diabetes. Diabetes 2008;57:3169-76
5. American Diabetes Association Position Statement. Standards of medical care in diabetes - 2017. Diabetes Care 2017;40(Supplement 1):S1-S135
6. Davies MJ, Gagliardino JJ, Gray LJ, Khunti K, Mohan V, Hughes R. Real-world factors affecting adherence to insulin therapy in patients with Type 1 or Type 2 diabetes mellitus: a systematic review. Diabetic Medicine 2013;30:512-24
7. International Hypoglycaemia Study Group. Glucose concentrations of less than 3.0 mmol/L (54 mg/dL) should be reported in clinical trials: a joint position statement of the American Diabetes Association and the European Association for the Study of Diabetes. Diabetes Care 2017;40(1):155-57
8. Christensen M, Bagger JI, Vilsbøll T, Knop FK. The alpha-cell as target for type 2 diabetes therapy. Rev Diabet Stud 2011;8:369-81
9. Lund A, Bagger JI, Christensen M, Knop FK, Vilsbøll T. Glucagon and type 2 diabetes: the return of the alpha cell. Curr Diab Rep 2014;14:555
10. Habegger KM, Heppner KM, Geary N, Bartness TJ, DiMarchi R, Tschöp MH. The metabolic actions of glucagon revisited. Nat Rev Endocrinol 2010;6:689-97
11. Onoue S, Ohshima K, Debari K, Koh K, Shioda S, Iwasa S, et al. Mishandling of the therapeutic peptide glucagon generates cytotoxic amyloidogenic fibrils. Pharm Res 2004;21:1274-83

12. Fosgerau K, Hoffmann T. Peptide therapeutics: current status and future directions. Drug Discovery Today 2015;20:122-8
13. Novo Nordisk Limited. SPC for GlucaGen Hypokit 1 mg. 2015. http://www.medicines.org.uk/emc/medicine/4258/SPC/GlucaGen+Hypokit+1+mg - Accessed 13 Mar 2017 .
14. Zealand Pharma A/S. Dasiglucagon Investigator's Brochure, Edition 3, 12 Dec 2016.
15. The Task Force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). 2013 ESH/ESC guidelines for the management of arterial hypertension. European Heart Journal 2013;34:2159-219
16. Levey AS, Coresh J, Greene T, Stevens LA, Zhang Y, Hendriksen BA, et al. Using standardized serum creatinine values in the modification of diet in renal disease study equation for estimating glomerular filtration rate. Annals of Internal Medicine 2006;145:247-54
17. Food and Drug Administration (FDA). Guidance for Industry on Bioanalytical Method Validation. Food and Drug Administration (FDA) 2001
18. Viswanathan CT, Bansal S, Booth B, DeStefano AJ, Rose MJ, Sailstad J, et al. Quantitative bioanalytical methods validation and implementation: best practices for chromatographic and ligand binding assays. Pharm Res 2007 Oct;24:1962-73
19. Shankar G, Devanarayan V, Amaravadi L, Barrett YC, Bowsher 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;48:1267-81
20. Zealand Pharma report SS. Commissioning and validation of a method for the validation of anti-ZP4207 antibodies in human serum. 2014
21. Zealand Pharma report SS. Commissioning and validation of a method for the validation of anti-glucagon antibodies in human serum. 2014
22. Bioagilytix Labs. Validation of an assay to detect neutralizing antibodies to ZP4207 by measuring cyclic adenosine monophosphate (cAMP) in a glucagon receptor transfected cell line, BAL-14-206-004-REP. 2016. Report No.: Zealand Pharma, study 15-039
23. 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. 2016. Report No.: Zealand Pharma, Study 15-040

24. Amiel SA, Tamborlane WV, Simonson DC, et al. Defective glucose counterregulation after strict glycemic control of insulin-dependent diabetes mellitus. N Eng J Med 1987; 316: 1376-83.

25. Rickels MR, Ruedy KJ, Foster NC, et al. Intranasal glucagon for treatment of insulin-induced hypoglycemia in adults with type 1 diabetes: A randomized crossover noninferiority study. Diabetes Care 2016; 39: 264-70.

26. ICMJE. Recommendations for the conduct, reporting, editing, and publication of scholarly work in medical journals. Updated December 2016

12 APPENDICES

12.1 List of trial personnel

Sponsor **Clinical Trial Manager**	[redacted] Zealand Pharma Smedeland 36 2600 Glostrup, Denmark Phone: [redacted]
Medical Officer	[redacted] Zealand Pharma Smedeland 36 2600 Glostrup, Denmark Phone: [redacted]
Contract Research Organization	Chiltern International (Ltd) 171 Bath Road Slough Berkshire SL1 4AA United Kingdom Phone: +44 (0) 175351 2000
Project Manager	[redacted] Chiltern International (Ltd) 171 Bath Road Slough Berkshire SL1 4AA United Kingdom Phone: [redacted]
Senior Medical Officer and Safety medical monitor	[redacted] Chiltern International kft Canada Square Office House Ganz u

	12-14, 4 emelet 1027 Budapest Hungary Phone: [redacted]
Pharmacovigilance unit Responsible for Serious Adverse Event (SAE) Management and 24-hour SAE reporting	[redacted] PharmaLex Agern Allé 24 2970 Hørsholm, Denmark Phone: [redacted] (8 a.m. to 4 p.m.) Phone: (outside 8 a.m. to 4 p.m.) Fax: email: PV-nordic@pharmalex.com
Central laboratory	MLM Laboratory Dohrweg 63 41066 Mönchengladbach Germany
Special laboratory (GlucaGen PK, insulin PK)	MLM Laboratory Dohrweg 63 41066 Mönchengladbach Germany
Special laboratory (dasiglucagon PK, dasiglucagon ADA, GlucaGen ADA)	York Bioanalytical Solutions (YBS) Cedar House Northminster Business Park Northfield Lane York, YO26 6QR, United Kingdom
Special laboratory (neutralizing antibodies)	BioAgilytix 2300 Englert Drive Durham, NC, 27713, USA

A list of all investigators, IECs and IRBs will be provided in a separate document and in the clinical trial report.

CLINICAL TRIAL PROTOCOL

A phase 3, randomized, double-blind, parallel trial to confirm the clinical efficacy and safety of dasiglucagon in the rescue treatment of hypoglycemia in subjects with type 1 diabetes mellitus (T1DM) compared to placebo and with reference to GlucaGen®

Sponsor:	Zealand Pharma A/S
Sponsor Protocol No.:	ZP4207-16137
EudraCT No.:	2017-002449-31
IND No:	127866
Trial Drug Name:	Dasiglucagon* injection
Date of Protocol:	26-Jan 2018

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

*Dasiglucagon is the proposed international nonproprietary name for ZP4207.

This protocol version 4.0 consists of protocol version 3.0, dated 06-October-2017 and local German protocol amendment 1, dated 26-Jan-2018.

Declaration of sponsor or responsible medical officer

Title: A phase 3, randomized, double-blind, parallel trial to confirm the clinical efficacy and safety of dasiglucagon in the rescue treatment of hypoglycemia in subjects with type 1 diabetes mellitus (T1DM) compared to placebo and with reference to GlucaGen®

This trial protocol 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 Declaration of Helsinki and the guidelines on Good Clinical Practice (GCP) (1).

Name	[redacted]	Date
Title	Clinical Trial Manager	
Institution	Zealand Pharma A/S Smedeland 36 2600 Glostrup, Denmark	
Phone:	[redacted]	

Name	[redacted]	Date
Title	Medical Officer	
Institution	Zealand Pharma A/S Smedeland 36 2600 Glostrup, Denmark	
Phone:	[redacted]	

Declaration of the coordinating investigator

Title: A phase 3, randomized, double-blind, parallel trial to confirm the clinical efficacy and safety of dasiglucagon in the rescue treatment of hypoglycemia in subjects with type 1 diabetes mellitus (T1DM) compared to placebo and with reference to GlucaGen®

I have read this protocol. I approve this document and I agree that it contains all necessary details for carrying out the trial as described. I will conduct this trial in accordance with the design and specific provision of this protocol and will make a reasonable effort to complete the trial within the time designated. I will provide copies of this protocol and access to all information furnished by Zealand Pharma A/S to trial personnel under my supervision. I will discuss this material with them to ensure they are fully informed about the trial product and trial procedures. I will let them know that this information is confidential and proprietary to Zealand Pharma A/S and that it may not be further disclosed to third parties. I understand that the trial may be terminated or enrollment suspended at any time by Zealand Pharma A/S, with or without cause, or by me if it becomes necessary to protect the best interests of the trial subjects.

I agree that the trial will be carried out in accordance with GCP (1), with the Declaration of Helsinki (with amendments) (2) and with the laws and regulations of the countries in which the trial takes place.

_____ _____

Name Date
Title
Institution
Phone: +
Fax: +

Declaration of the investigator

Title: A phase 3, randomized, double-blind, parallel trial to confirm the clinical efficacy and safety of dasiglucagon in the rescue treatment of hypoglycemia in subjects with type 1 diabetes mellitus (T1DM) compared to placebo and with reference to GlucaGen®

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 this trial protocol, Investigator's Brochure, Case Report Forms, 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 subjects.

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

_____ _____

Signature Date

_____

Name (block letters)

_____

Title (block letters)

_____

Institution (block letters)

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List of abbreviations and definitions of terms

ADA	Anti-drug antibody
AE	Adverse event
AESI	Adverse event of special interest
ALT	Alanine aminotransferase
ANCOVA	Analysis of covariance
AST	Aspartate aminotransferase
AUC	Area under the concentration-time curve
$AUC_{0\text{-inf}}$	AUC from time zero to infinity
AUE	Area under the effect curve
CFB	Changes from baseline
CI	Confidence interval
C_{max}	Maximum plasma concentration
CPH	Cox proportional hazards
CRO	Contract research organization
ECG	Electrocardiogram
eCRF	Electronic case report form
EDC	Electronic data capture
EU	European Union
EudraCT	European Medicines Agency's Clinical Trials Database
FAS	Full analysis set
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HbA_{1c}	Glycated hemoglobin
ICH	International Conference on Harmonization
ID card	Identification card
IEC	Independent Ethics Committee
IM	Intramuscular(ly)
IMP	Investigational medicinal product
IRB	Institutional Review Board
IV	Intravenous(ly)

IWRS	Interactive Web Response System
MedDRA	Medical Dictionary for Regulatory Activities
NPH	Neutral protamine Hagedorn
PD	Pharmacodynamics
PK	Pharmacokinetics
PP	Per protocol
SAE	Serious adverse event
SAP	Statistical analysis plan
SAS	Safety analysis set
SC	Subcutaneous(ly)
SUSAR	Serious unexpected suspected adverse reaction
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
TEAE	Treatment-emergent adverse event
t_{max}	Time to the maximum plasma concentration
ULN	Upper limit of the normal range
US	United States of America

1 SYNOPSIS

Name of sponsor: Zealand Pharma A/S	**Trial ID:** ZP4207-16137
Title of the trial: A phase 3, randomized, double-blind, parallel trial to confirm the clinical efficacy and safety of dasiglucagon in the rescue treatment of hypoglycemia in subjects with type 1 diabetes mellitus (T1DM) compared to placebo and with reference to GlucaGen®	
Trial design: The trial is a global, multicenter, randomized, parallel-group, double-blind, clinical trial confirming the efficacy and safety of dasiglucagon for insulin-induced hypoglycemia in subjects with type 1 diabetes mellitus. The subjects will be randomized 2:1:1 to receive a single fixed subcutaneous 0.6 mg dose of dasiglucagon (hereinafter dasiglucagon), placebo for dasiglucagon (hereinafter referred to as placebo), or a 1 mg dose of GlucaGen® (hereafter referred to as GlucaGen) and followed for at least 28 days after treatment.	
Clinical phase of development: Phase 3	
Trial centers: This trial will be conducted at 4 to 6 sites in the United States of America, Canada, and Europe.	
Planned trial start (first subject first visit): Q4/2017	**Planned trial end (last subject last visit):** Q3/2018
Trial population: Male and female adult subjects with type 1 diabetes mellitus treated with insulin for at least one year	
Key objectives: **Primary objective:** • To demonstrate superiority of dasiglucagon compared to placebo following a single subcutaneous 0.6 mg dose administered to subjects with type 1 diabetes mellitus with insulin-induced hypoglycemia. **Secondary objective:** • To compare the glycemic response observed after dasiglucagon with that of GlucaGen.	
Key endpoints: **Primary endpoint:** • Time to plasma glucose recovery. Plasma glucose recovery is defined as first increase in plasma glucose of ≥20 mg/dL (1.1 mmol/L) from baseline during the hypoglycemic clamp procedure without administration of rescue intravenous (IV) glucose. **Key secondary endpoints:** • Plasma glucose recovery within 30 minutes, within 20 minutes, within 15 minutes, and within 10 minutes after study drug injection without administration of rescue IV glucose. • Plasma glucose changes from baseline within 30 minutes, within 20 minutes, within 15 minutes, and within 10 minutes after study drug injection or at the time of rescue. **Clinical efficacy (Pharmacodynamic) endpoints:** • Time to first plasma glucose concentration of ≥70 mg/dL (3.9 mmol/L) without administration of rescue IV glucose.	

- Plasma glucose response as area under the curve above baseline from time zero to 30 minutes, $AUC_{0\text{-}30min}$.

Exposure (Pharmacokinetic) endpoints:

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

Safety endpoints:

- Adverse events, clinical laboratory assessments (biochemistry, hematology, coagulation, urinalysis), vital signs, physical examination, electrocardiogram, and local tolerability.
- Administration of rescue infusion of IV glucose during the hypoglycemic clamp procedure.
- Time to first rescue infusion of IV glucose during the hypoglycemic clamp procedure.

Immunogenicity endpoint:

- Occurrence of anti-drug antibodies

Exploratory endpoint:

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

Key inclusion criteria:

- Male and female subjects with type 1 diabetes mellitus treated with insulin for at least one year, diagnostic criteria as defined by the American Diabetes Association.
- Stable insulin treatment 30 days prior to screening, defined as no more than a 10-unit daily variation in total daily insulin dose.
- Hemoglobin A_{1c} <10%.
- Aged between 18 and 75 years, both inclusive.

Key exclusion criteria:

- Previously treated with dasiglucagon.
- Known or suspected allergy to trial product(s) or related products.
- History of anaphylaxis or symptoms of severe systemic allergy (such as angioedema).
- Previous participation (randomization) in this trial.

Sample size:
Approximately 156 subjects are intended to complete the trial, with 78 subjects randomized to the dasiglucagon group and 39 subjects randomized to each of the placebo and GlucaGen groups.

Investigational medicinal product:
Test product: dasiglucagon liquid formulation in pre-filled syringes.
Comparator products: Placebo and GlucaGen® lyophilized powder.
Germany only: Insulin glulisine (Apidra®), the challenge agent, will also be defined as an investigational medicinal product

Duration of treatment:

Subjects will be randomized 2:1:1 to receive a single fixed subcutaneous dose of 0.6 mg dasiglucagon, placebo, or 1 mg GlucaGen, and followed for at least 28 days after receiving treatment.

Assessments:
The plasma glucose profile for evaluation of the primary and secondary clinical efficacy (pharmacodynamic) endpoints will be assessed based on plasma concentration data from samples collected at the dosing visit.
The exposure to trial medication (dasiglucagon, placebo, or GlucaGen) for evaluation of pharmacokinetics will also be assessed based on plasma concentration data.

Statistical methods:
Due to requirements in the size of the safety database, the sample size is set to 78 subjects treated with dasiglucagon 0.6 mg. With a 2:1:1 randomization ratio for the three treatments, dasiglucagon 0.6 mg, placebo, and GlucaGen 1 mg, 78 subjects injected with dasiglucagon and 39 subjects with placebo will yield a power of 90% at a 5% two-sided significance level to detect a treatment group difference in recovery incidence within 20 minutes, assuming as low as 80% and as high as 50% recovery incidence for the dasiglucagon and placebo groups, respectively.

For the confirmatory analyses, the primary and key secondary endpoints for the dasiglucagon 0.6 mg and placebo treated subjects will be compared. A hierarchical procedure will be applied for the control of multiplicity in statistical inferences. The primary and secondary endpoints will be evaluated on the Full Analysis Set sample. The statistical inference comparisons with placebo will be conducted as superiority tests. The comparisons of dasiglucagon versus GlucaGen will be summarized descriptively.

The primary endpoint will be summarized using Kaplan-Meier (KM) estimates stratified by treatment group and injection site. The treatment group difference between dasiglucagon and placebo will be evaluated inferentially using a pairwise two-sided log-rank test.

In the primary analysis, those subjects who require rescue IV glucose will be censored at the time of plasma glucose recovery. This approach is based on the assumption that rescue IV glucose administration will shorten the time to recovery. This assumption implies that censoring at the time of recovery after rescue therapy is valid as a time to recovery without rescue would be longer than the observed duration. If recovery has not occurred at 45 minutes after study drug injection, censoring will be applied irrespective of the use of rescue IV glucose.

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

The primary endpoint will additionally be analyzed using a Cox proportional hazards (CPH) time to event statistical model, if the hazards by treatment group can be assumed proportional using a graphical comparison of log-minus-log survival curves and no more than two-thirds of the recovery times are censored in each treatment group. The CPH model will be used for inferences, with treatment group and injection site modeled as categorical effects, and baseline plasma glucose modeled as a continuous covariate. The treatment group rate ratios, active versus placebo, will be estimated together with the 95% confidence intervals, and pairwise treatment group inferences (dasiglucagon vs placebo, GlucaGen vs placebo) will be evaluated using two-sided likelihood ratio tests.

The key secondary incidence variables (plasma glucose recovery within 30 minutes, within 20 minutes, within 15 minutes, within 10 minutes, in hierarchical order for inference) will be analyzed using descriptive

statistics (frequency and percentage) by treatment group. Inferential treatment group comparisons will be assessed via pairwise tests of independent binomial proportions (dasiglucagon versus placebo, GlucaGen versus placebo) using Fisher's exact test for each pairwise comparison.

The key secondary endpoints of plasma glucose changes from baseline (CFB) within 30 minutes, within 20 minutes, within 15 minutes, and within 10 minutes after study drug injection or at the time of rescue, will be analyzed with the plasma glucose CFB at rescue carried forward in those subjects who require rescue IV glucose before plasma glucose ≥20 mg/dL recovery. Each of these CFB variables will be analyzed using an Analysis of Covariance model, with treatment group modeled as a fixed effect and with the baseline plasma glucose modeled as a covariate. The dasiglucagon versus placebo treatment group difference will be evaluated inferentially as a least squares means contrast, using a two-sided t-test at the 0.05 significance level, first for the 30 minute CFB, and then sequentially for the 20 minute, 15 minute, and 10 minute variables, until the first failure to reject.

The continuous clinical efficacy variables, the exploratory variables, and the pharmacokinetics variables will each be summarized descriptively by treatment group. The clinical efficacy variables will be analyzed analogous to the plasma glucose CFB variables.

The safety analyses will include by-treatment-group descriptive summaries of vital sign measurements, laboratory measures (including immunogenicity incidence), physical examination assessments, rescue IV glucose (incidence and amount of glucose infused), and adverse events. The number and percentage of subjects reporting specific events, such as nausea and vomiting, will be presented by body system and preferred term.

Further details will be included in the Statistical Analysis Plan, to be completed before database lock and treatment unmasking.

2 INTRODUCTION

2.1 Background

Zealand Pharma A/S (Zealand Pharma) is developing dasiglucagon, a physically and chemically stable peptide analog of human glucagon, in a ready-to-use liquid formulation for the acute treatment of severe hypoglycemia in patients with insulin-treated diabetes mellitus. Like native glucagon, dasiglucagon is comprised of 29 amino acids, but with 7 substitutions which improve its physical and chemical stability in aqueous media. These amino acid substitutions make dasiglucagon suitable for a liquid formulation, while providing similar efficacy and safety as compared with recombinant glucagon in the clinical setting of acute treatment of severe hypoglycemia.

2.1.1 Hypoglycemia

Hypoglycemia in patients with diabetes is defined as episodes of an abnormally low plasma glucose concentration (3). This is a common, unpredictable, and potentially dangerous side effect of treatment of diabetes mellitus, especially with 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 (4).

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 (5).

The incidence of hypoglycemic events or even the fear of hypoglycemia influences patients' adherence to prescribed treatment regimens for diabetes mellitus (6). This leads to inadequate glycemic control, which in turn may lead to an increased risk of diabetic complications (5). Serious clinically significant hypoglycemia is now defined as plasma glucose <54 mg/dL (3.0 mmol/L), while the plasma glucose alert value is defined as <70 mg/dL (3.9 mmol/L) (7). When plasma glucose falls below these values, some kind of treatment strategy is needed.

2.1.2 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) (8,9). 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 (10). Insulin decreases blood glucose levels and cases of hypoglycemia can be reversed by glucagon. Therefore, glucagon is indicated for the treatment of severe hypoglycemia.

Besides intravenous (IV) glucose administration, an injectable form of glucagon is given as first aid in cases of severe hypoglycemia, when the patient is unconscious or for other reasons cannot take glucose orally. The approved glucagon dose for an adult is 1 mg, given by intramuscular (IM), IV, or subcutaneous (SC) injection, which quickly raises blood glucose levels. As current marketed recombinant glucagon is highly unstable when dissolved in solution, the injectable form has to be reconstituted prior to use in a 9-step procedure that requires a sterile diluent to be injected into a vial containing lyophilized powdered glucagon. When dissolved in a fluid state, glucagon can form amyloid fibrils (11), or tightly woven chains of proteins made up of the individual glucagon peptides. The reconstitution process makes the use of marketed glucagon products cumbersome (12), and a more patient-friendly formulation is needed. Currently, the Food and Drug Administration (FDA) approved instructions for commercially available glucagon allow only for immediate usage of glucagon after the powder is reconstituted in aqueous solution (13). Therefore, a glucagon analog with enhanced biophysical stability may represent a leap forward in terms of convenient therapeutic applications.

2.1.3 Dasiglucagon

Dasiglucagon (ZP4207) is a stable peptide analog of human glucagon, available in a ready-to-use liquid formulation. Dasiglucagon (hereinafter referred to as dasiglucagon) 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® (hereafter referred to as GlucaGen). Dasiglucagon exhibits improved physical and chemical stability and is available in an aqueous solution at neutral pH (14).

Three clinical trials have been completed with dasiglucagon: a first-in-human dose trial in healthy volunteers and subjects with T1DM (ZP4207-14013), a multiple-dose dose-escalation trial (ZP4207-15007) evaluating the safety, tolerability, pharmacokinetics (PK) and pharmacodynamics (PD) of dasiglucagon in healthy volunteers, and a phase 2 crossover trial to assess the PK and PD of a single dose of an optimized formulation of dasiglucagon administered SC in subjects with T1DM (ZP4207-15126) (14).

2.1.3.1 Pharmacokinetics and pharmacodynamics of dasiglucagon

The results of the phase 1 and 2 clinical trials confirmed dose-proportionality for dasiglucagon PK, 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 (t_{max}) was 35 minutes for dasiglucagon compared with 20 minutes for GlucaGen.

At all dose levels in the phase 2 trial, all subjects achieved a plasma glucose level of at least 70 mg/dL (3.9 mmol/L) as well as an increase in plasma glucose by at least 20 mg/dL (1.1 mmol/L) within 30 minutes after dosing. The PD responses to 0.6 mg of dasiglucagon and 1 mg of GlucaGen were comparable.

2.1.3.2 Safety of dasiglucagon

The safety data for dasiglucagon do not give rise to any safety concerns. No new signals were observed, 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 mg (4 with dasiglucagon and 1 with GlucaGen). This is not considered a safety concern; however, hemodynamic changes after dosing will be considered an adverse event of special interest (AESI).

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's Brochure (14).

2.2 Trial rationale

The aim of the current trial is to confirm the superiority of dasiglucagon for the treatment of insulin-induced hypoglycemia in subjects with T1DM as compared to placebo for dasiglucagon (hereinafter placebo) and to compare the clinical efficacy and safety of dasiglucagon with reference to GlucaGen. A randomized, controlled trial design was used.

See Section 4.2 for justification of the design of this trial.

2.3 Risk-benefit assessment

Non-clinical experience

The nonclinical development program did not reveal any safety findings that would prohibit administration of dasiglucagon to humans. None of the safety pharmacology studies, repeated dose toxicity studies or genotoxicity studies revealed any significant toxicity findings relevant to the therapeutic use of dasiglucagon.

Clinical experience

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 IMP 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 extent in the 3 clinical studies conducted to date with dasiglucagon. As with every novel drug substance, new and as yet unknown side effects may also 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 low immunogenic potential.

Overall, dasiglucagon is judged to be a low-risk molecule, based upon the available clinical data. Administration of dasiglucagon may be associated with a risk of allergic reactions similar to those observed for other therapeutic peptides or proteins. Subjects 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 centers.

With the exception of medical examinations, a subject 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.

The development program including 141 subjects exposed to dasiglucagon to date has demonstrated that administration of dasiglucagon is efficacious and well tolerated, with a safety profile that does not give rise to specific safety concerns. Two phase 1 and one phase 2 clinical trials have been conducted to investigate the safety, tolerability, PK and PD of dasiglucagon after single and multiple dosing to healthy volunteers and subjects with T1DM under insulin-induced hypoglycemic conditions. Dasiglucagon has proven to have relevant clinical effects in the acute severe hypoglycemia rescue setting and may be an effective and reliable emergency treatment for severe hypoglycemia. Overall, the anticipated benefits for subjects entering the ZP4207-16137 trial are considered to justify the risks.

3 TRIAL OBJECTIVES AND ENDPOINTS

3.1 Primary objective

- To demonstrate superiority of dasiglucagon compared to placebo following a single subcutaneous 0.6 mg dose administered to subjects with type 1 diabetes mellitus with insulin-induced hypoglycemia.

3.2 Secondary objectives

- To compare the glycemic response observed after dasiglucagon with that of GlucaGen.

3.3 Primary endpoint

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

3.4 Key secondary endpoints

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

3.5 Other secondary endpoints

- Clinical efficacy (PD) endpoints:
  - Time to first plasma glucose concentration ≥70 mg/dL (3.9 mmol/L) without administration of rescue IV glucose.
  - Plasma glucose response as area under the curve (AUC) above baseline from time zero to 30 minutes, $AUC_{0\text{-}30min}$.
- Exposure (PK) endpoints:
  - Area under the drug concentration curve from time zero to 90 minutes, $AUC_{0\text{-}90min}$.
  - Maximum plasma drug concentration (C_{max}).
  - Time to maximum plasma drug concentration (t_{max}).

- Safety endpoints:
  - Adverse events, clinical laboratory assessments (biochemistry, hematology, coagulation, urinalysis), vital signs, physical examination, electrocardiogram (ECG), and local tolerability.
  - Administration of rescue infusion of IV glucose during the hypoglycemic clamp procedure.
  - Time to first rescue infusion of IV glucose during the hypoglycemic clamp procedure.
- Immunogenicity endpoint:
  - Occurrence of anti-drug antibodies

3.6 Exploratory endpoint

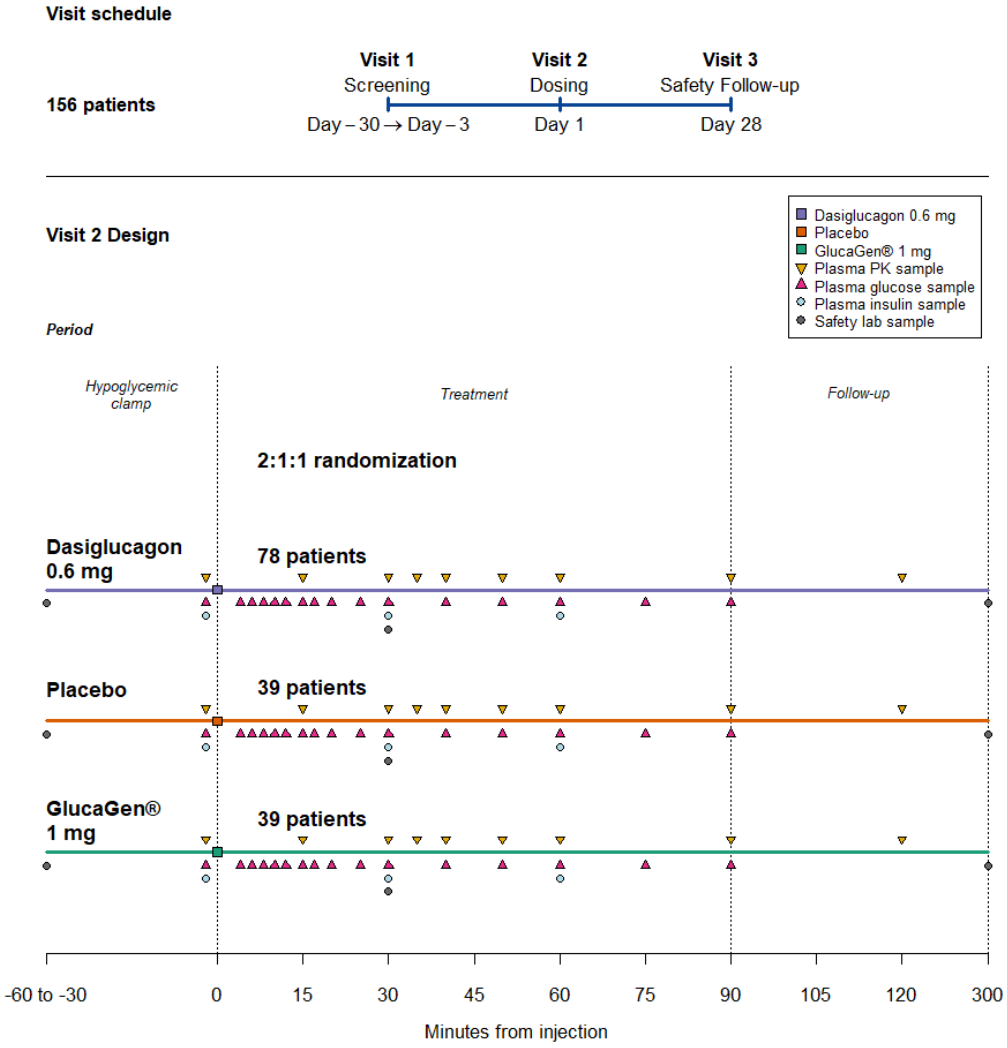
- Plasma glucose concentration ≥70 mg/dL (3.9 mmol/L) or increase of ≥20 mg/dL (1.1 mmol/L) within 30 minutes after study drug injection without administration of rescue IV glucose.
- Plasma insulin response as area under the curve above baseline from time zero to 60 minutes, $AUC_{0\text{-}60\ min}$.

4 OVERALL DESIGN AND PLAN OF THE TRIAL

4.1 Overview

This trial is a global, multicenter, randomized, parallel, double-blind, clinical trial confirming the efficacy and safety of dasiglucagon for insulin-induced hypoglycemia in subjects with T1DM. The subjects will be randomized 2:1:1 to receive a single fixed SC 0.6 mg dose of dasiglucagon, placebo, or a 1 mg dose of GlucaGen and followed for at least 28 days after receiving treatment. A total of 156 subjects with T1DM are expected to complete the treatment visit. The trial will be conducted in the European Union (EU) and North America. See Figure 1 for an overview of the trial design.

Figure 1 Trial design



4.2 Justification of the trial design

4.2.1 Justification for design and parameters

In order to avoid bias in subject selection and in the evaluation of clinical assessments, subjects will be randomly assigned 2:1:1 to either dasiglucagon, placebo, or GlucaGen and the trial will be conducted in a double-blinded manner. The randomized parallel treatment design with administration of fixed SC doses of dasiglucagon, placebo, or GlucaGen to subjects with T1DM and insulin-induced hypoglycemia allows for direct comparison of the clinical efficacy of the treatments.

The trial is double-blind to increase trial validity and to reduce bias during evaluation of the treatments. However, since the trial medications are not identical in appearance, the handling, preparation and administration of trial medication will be done by unblinded trial personnel who will not be involved in any other trial procedures or assessments. See Section 6.6 for more information about which assessments are blinded and which are not, with reasons.

A superiority trial design is used because the aim is to show that treatment with dasiglucagon is an effective treatment compared to placebo. The secondary objective was chosen to allow a comparison between treatment with dasiglucagon and an established comparator treatment for severe hypoglycemia, GlucaGen.

Administration of glucagon is intended to quickly raise blood glucose levels in subjects with T1DM with insulin-induced hypoglycemia. Therefore, in order to assess the clinical efficacy of dasiglucagon as compared to placebo and GlucaGen following a single SC dose, the primary endpoint and secondary efficacy endpoints involve the measurement of plasma glucose concentrations at different timepoints.

4.2.2 Justification for drug, route, dosage and treatment duration

Dasiglucagon and GlucaGen will be administered as 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 approved dose for treatment of severe hypoglycemia. Data from the studies conducted to date with dasiglucagon, including the phase 2 trial in subjects with T1DM, have been used to establish that 0.6 mg of dasiglucagon is an effective dose and also represents a therapeutically equivalent dose to 1 mg of GlucaGen (see also Section 6.1).

Dasiglucagon, placebo, and GlucaGen will be administered in the abdomen, buttocks, or thigh by SC injection, as this is one of the intended routes of administration for dasiglucagon, besides IM and IV.

Subjects will be followed for at least 28 days after dosing in order to perform an adequate immunogenicity evaluation of treatment.

5 TRIAL POPULATION

5.1 Rationale for trial population

Dasiglucagon is in development for the treatment of severe hypoglycemia in insulin-dependent patients with diabetes mellitus. In the present trial, subjects with T1DM are included in the evaluation of efficacy and safety of dasiglucagon under hypoglycemic conditions as this is part of the intended target population. Subjects with T1DM are selected to avoid the endogenous glucagon counter-regulatory response to insulin-induced hypoglycemia that is present in patients with T2DM. The inclusion and exclusion criteria are set to include a trial population that represents the general population of subjects with T1DM.

5.2 Planned sample size and number of trial centers

A total number of 156 subjects with T1DM are expected to complete the treatment visit (Visit 2). To qualify as completed, the subject must be dosed and have blood drawn for the PK and PD assessments described in the protocol. It is expected that up to 176 subjects will be randomized to have 156 subjects completing Visit 2. Completion of 156 subjects (78 subjects in the dasiglucagon group and 39 subjects in each of the placebo and GlucaGen groups) would be expected to provide adequate power for the primary efficacy evaluation, as described in Section 9.1.

The planned date for first subject first visit is expected to take place in Q4, 2017 and the planned date for last subject last visit is expected to take place in Q3, 2018.

This trial is planned to be conducted at 4 to 6 trial sites in the United States of America [US], Canada, and Europe.

5.3 Inclusion criteria

Subjects will be entered into this trial only if they meet 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 subject).
2. Female or male subjects with T1DM for at least 1 year, diagnostic criteria as defined by the American Diabetes Association (3).
3. Treated with insulin for T1DM for at least 1 year and with stable insulin treatment (defined as no more than a 10-unit daily variation in total daily insulin dose) 30 days prior to screening

4. Hemoglobin A_{1c} <10%.
5. Aged between 18 and 75 years, both inclusive.
6. A female subject 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 and until last follow-up visit. Additionally, if the participant is using systemic contraceptives, she must use an additional form of acceptable contraception. An acceptable method of contraception includes one of the following:
        i. Abstinence from heterosexual intercourse;
        ii. Systemic contraceptives (birth control pills, injectable/implant/ insertable hormonal birth control products, transdermal patch);
        iii. Intrauterine device (with and without hormones); or
        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).
7. A male subject must meet the following criteria: Surgically sterilized or willing to refrain from sexual intercourse from screening and until last follow-up visit or, if sexually active, uses condom and partner practices contraception during the trial from screening and until last follow-up visit.

5.4 Exclusion criteria

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

1. Previously treated with dasiglucagon (previously referred to as ZP4207).
2. Known or suspected allergy to trial product(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, are actively attempting to get pregnant, or are lactating.
6. History of hypoglycemic events associated with seizures in the last year prior to screening.
7. History of severe hypoglycemia (defined as plasma glucose <54 mg/dL [3.0 mmol/L]) in the last month prior to screening.
8. Receipt of any investigational drug within 3 months prior to screening.
9. Active malignancy within the last 5 years.
10. Congestive heart failure, New York Heart Association class II-IV.

11. Inadequately treated blood pressure, defined as systolic ≥160 mmHg or diastolic ≥90 mmHg) at screening (15).
12. Current bleeding disorder, including anti-coagulant treatment.
13. Known presence or history of pheochromocytoma (i.e. adrenal gland tumor) or insulinoma (i.e. insulin secreting pancreas tumor).
14. Use of a daily systemic beta-blocker drug, indomethacin, warfarin or anticholinergic drugs in the previous 28 days before Day 1 of this trial.
15. Aspartate aminotransferase (AST) or alanine aminotransferase (ALT) >2.5 × the upper limit of the normal range (ULN), bilirubin >1.5 × ULN, estimated glomerular filtration rate <30 mL/min/1.73 m^2 according to the Modification of Diet in Renal Disease study definition (16), or altered electrolyte values of clinical relevance for cardiac conduction, as judged by the investigator.
16. Clinically significant abnormal ECG at screening as judged by the investigator.
17. Clinically significant illness within 4 weeks before screening, as judged by the investigator.
18. Donation of blood or plasma in the past month, or in excess of 500 mL within 12 weeks prior to screening.
19. Surgery or trauma with significant blood loss within the last 2 months prior to screening.
20. 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.
21. Subjects with mental incapacity or language barriers which 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.
22. Any condition interfering with trial participation or evaluation or that could be hazardous to the subject.
23. The use of prescription or non-prescription medications known to cause QT prolongation.

5.5 Dosing day exclusion criteria

Subjects who meet one or more of the following dosing day exclusion criteria at the dosing visit will be excluded from the dosing visit, but the visit can be rescheduled 1-7 days later. The dosing visit can only be rescheduled once.

1. Atypically strenuous exercise within 4 days prior to dosing, as judged by the investigator. Exercise during the trial should follow subject's typical routine, and should not exceed a near maximum intensity for more than 20 minutes per day, or moderate intensity for more than 90 minutes per day.

2. Clinically significant illness within 4 weeks before dosing, as judged by the investigator.
3. Consumption of alcohol within 24 hours prior to dosing visit, or positive results from an alcohol breath test.
4. Not fasting from 22:00 hours the evening prior to dosing, apart from water. Small amounts of carbohydrates (up to 20 g) to prevent hypoglycemia are allowed.
5. The use of any non-prescribed systemic or topical medication, except routine vitamins and occasional use (as judged by the investigator) of acetylsalicylic acid and paracetamol within 2 weeks prior to dosing. Treatment with insulin, including analogs, is allowed.
6. Use of insulin Degludec or insulin Glargine U300 within 48 hours prior to dosing; or use of other long-acting insulins (e.g., insulin Glargine U100 or insulin Detemir) within 24 hours prior to dosing; or use of insulin Neutral protamine Hagedorn (NPH) within 16 hours prior to dosing.
7. Use of any short acting (bolus) insulin within 6 hours prior to dosing, except insulin glulisine (Apidra®).
8. Changes in medical history or concomitant medication resulting in fulfillment of clinical exclusion criteria, as judged by the investigator.
9. Plasma glucose value <50 mg/dL (2.8 mmol/L) within the last 24 hours or plasma glucose value <60 mg/dL (3.3 mmol/L) within the last 5 hours prior to initiation of the hypoglycemic procedure.

5.6 Premature treatment discontinuation and withdrawal

Participation in the trial is strictly voluntary. A subject 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 subject at any time if the investigator deems it in the subject's best interest. The reason and circumstances for withdrawal will be documented in the electronic case report form (eCRF).

5.6.1 Possible reasons for treatment visit discontinuation

A subject will be discontinued from treatment if the following applies:

- Withdrawal of consent by subject.
- If a protocol deviation occurs which, in the clinical judgement of the investigator, can invalidate the trial or endpoints or can interfere pharmacokinetically or pharmacodynamically with the trial product, the subject will be discontinued by the investigator.
- Adverse events occur which are considered unacceptable by the subject or the investigator.

If discontinuation occurs following administration of trial medication, every effort should be made to have the subject return and participate in the complete follow-up visit on Day 28 (see Table 3) to avoid missing data.

If trial participation is terminated due to an AE possibly related to any of the trial medications or trial examinations, the subject 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.

A total of 156 subjects must complete the treatment visit (Visit 2). To qualify as completed, the subject must be dosed and have blood drawn for the PK and PD assessments described in the protocol.

5.6.2 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 subjects within the agreed time frame.
- The center does not respond to trial management requests.
- Repeat protocol violations.

5.6.3 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 subjects.
- New scientific knowledge becomes known that makes the objectives of the trial no longer feasible/valid.
- Unsatisfactory enrolment of subjects.

5.7 Subject identification and randomization

Subjects who have given written informed consent and meet all inclusion and none of the exclusion criteria will be randomized in a 2:1:1 ratio to either 0.6 mg dasiglucagon (n=78), placebo (n=39), or 1 mg GlucaGen (n=39) via an Interactive Web Response System (IWRS). Randomization will continue until 156 subjects have completed Visit 2.

Subjects 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 subject is taking, the subject code can be broken by the investigator, preferably after consultation with the medical monitor. Emergency code breaks can be performed using the IWRS.

6 TRIAL DRUG

6.1 Identity

The following trial drugs will be administered:

- Dasiglucagon, liquid formulation, 1 mg/mL, 0.6 mL delivered in a prefilled syringe.
- Placebo, liquid formulation, 0.6 mL delivered in a prefilled syringe.
- Recombinant glucagon hydrochloride, 1 mg for reconstitution (GlucaGen®, Novo Nordisk) in 1 mL sterile water.

The description of the three trial drugs is provided in Table 1. Dasiglucagon is a stable peptide analog of human glucagon in a ready-to-use liquid formulation for treatment of severe hypoglycemia in insulin-dependent patients with diabetes mellitus. 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.

Table 1 Description of trial drugs

	Test product	Placebo Product	Comparator product
Name	Dasiglucagon	Placebo	GlucaGen®
Active substance	Dasiglucagon	N/A	Recombinant glucagon hydrochloride
Formulation	Liquid formulation, 0.6 mL	Liquid formulation, 0.6 mL	Powder and solvent for reconstitution as 1 mL solution for injection
Strength	1 mg/mL	N/A	1 mg
Device	Single use pre-filled syringe	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	Zealand Pharma A/S, Glostrup (Copenhagen), Denmark	Novo Nordisk A/S, Bagsværd, Denmark

	Test product	Placebo Product	Comparator product
Storage requirements	Store between 2 and 8°C	Store between 2 and 8°C	Store between 2 and 8°C

The quantities of ingredients for dasiglucagon and placebo are provided in Table 2.

Table 2 Quantities of ingredients in dasiglucagon and placebo injection

Component	Amount per mL (dasiglucagon)	Amount per mL (placebo)	Function
Dasiglucagon*	1.0 mg	N/A	[redacted]
Sodium chloride	10.23 mg	10.23 mg	
Trometamol/Tromethamine	6.06 mg	6.06 mg	
Water for injection	To make 1 mL	To make 1 mL	
Sodium hydroxide	q.s.	q.s.	
Hydrochloric acid	q.s.	q.s.	

*The quantity of drug substance to be used is calculated according to net peptide content and purity.
q.s. = quantum sufficit (quantity required).

6.2 Treatment assignment and randomization

Subjects successfully completing screening and who fulfill entry eligibility and randomization criteria will be randomized to one of three treatment groups in a ratio of 2:1:1:

- Test treatment: Dasiglucagon, liquid formulation, 1 mg/mL, 0.6 mL delivered in a prefilled syringe.
- Placebo treatment: Placebo, liquid formulation, 0.6 mL delivered in a prefilled syringe.
- Reference treatment: Recombinant glucagon hydrochloride, 1 mg for reconstitution.

Randomization will be performed using a fixed-block randomization scheme. The randomization scheme will be generated prior to the initiation of the study by an independent statistician/programmer who will not be a member of the study team; all investigators will not be aware of the block size of the randomization scheme. Randomization will be stratified by treatment group and by injection site (abdomen, buttocks, or thigh) and controlled via the IWRS.

Subjects will be randomized to study treatment using an interactive, automated system which has been validated for the intended use under the International Society of Pharmaceutical Engineers Good Automated Manufacturing Process guidelines, 21CFR Part 11 (FDA regulation for Electronic Records and Electronic Signatures) and the International Conference on Harmonization (ICH) Guidance E6 for Industry on Good Clinical Practice (GCP).

6.3 Administration

Dasiglucagon, placebo, and GlucaGen will be administered by SC injection in the abdomen, buttocks, or thigh.

An unblinded person (appropriately trained) authorized to prepare the dose and administer the treatment in accordance with the randomization will prepare the treatment required for each subject on each dosing day. The dose will be administered by the unblinded, trained and qualified person. The content of the syringe has to be checked for clarity and absence of bubbles..

Syringes will be discarded after dose administration. Used GlucaGen vials will be stored in a lockable box (separated from unused vials) at ambient temperature.

6.4 Packaging and labelling

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

The study drug label will describe the storage conditions for study drug. The labels will supply no information about the subjects. Each treatment kit (pre-filled syringe/vial for reconstitution) will have a unique Dispensing Unit Number for drug allocation, drug accountability, and traceability purposes.

Labelling will be performed according to Annex 13 of the Good Manufacturing Practice guidelines of the European Commission, ICH GCP guidelines, and local law.

6.5 Storage of study drugs

The investigator must ensure the availability of proper storage conditions. All study drug supplies provided for this study will be stored in a secure area with restricted access at the study site.

The temperature should be monitored by recording the actual, minimum, and maximum temperatures using a calibrated thermometer or thermocouple, or by continuous recording using a qualified temperature monitoring system. The temperature should be evaluated and documented at least on working days on a temperature log. This log must be included in the Investigator Site File upon study termination.

Dasiglucagon and placebo must be stored in a refrigerator (at a temperature of 2–8°C), and should be handled in accordance with guidelines from the sponsor. GlucaGen must also be stored in a refrigerator (at a temperature of 2–8°C), and should be handled in accordance with the Summary of Product Characteristics (13).

The unblinded person responsible for study drug handling must contact the unblinded monitor in case of temperature deviations outside the acceptable range.

Please see the Pharmacy Manual for additional information on handling study drug.

6.6 Blinding and breaking the blind

This is a double-blind trial. As the trial products are not identical in appearance, dasiglucagon and placebo being available as a liquid formulation and GlucaGen as a powder for reconstitution, unblinded trial personnel will be responsible for handling, preparing (according to the prescription from the IWRS), and administering the trial medication, as well as for keeping the records strictly confidential and accessible only to unblinded staff until after the database has been locked. To maintain double-blind conditions, all trial assessments at the trial center will be done by blinded trial personnel not involved in the administration of trial medications. However, exposure assessments and anti-drug antibody (ADA) assessments will be performed by unblinded personnel at the special laboratories, to ensure that dasiglucagon, placebo, or GlucaGen administration is matched with the applicable bioanalytical assay.

Treatment assignment will be kept strictly confidential and accessible only to authorized persons until after the time of unblinding. Codes with treatment assignment will, however, be readily available in the IWRS to the blinded personnel in case of an emergency.

The code for a particular subject may be broken in a medical emergency if knowing the identity of the treatment allocation would influence the treatment of the subject. The emergency code break can be performed using the IWRS. Whenever a code is broken, the person breaking the code must record the time, date and reason as well as his/her initials in the source documents. The breaking of blinded codes in case of medical emergency for one subject should not unblind the trial personnel to the treatment information of other

subjects. The person performing the unblinding should inform as few people as possible about the result of the unblinding. All persons unblinded for a specific subject should be documented.

If the trial center needs to break the code, the medical monitor should, if at all possible, be contacted prior to breaking the code and the monitor must be notified within 24 hours after the code has been broken.

The pharmacovigilance unit (safety contract research organization [CRO]; see the list of trial personnel in Section 12.1) will be able to break the code in case of a serious unexpected suspected adverse reaction (SUSAR).

The central and specialty laboratories will be provided with a copy of the randomization list.

6.7 Drug accountability

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 for destruction. This does not apply to the used syringes as they will be discarded after dose administration. Destruction must not take place until approved by the Sponsor.

6.8 Treatment compliance

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

PK assessments will support the surveillance of compliance with IMP administration.

6.9 Prior and concomitant medications

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

Subjects using any new concomitant medication resulting in fulfillment of a dosing day exclusion criterion (see Section 5.5) will be excluded from the dosing visit, but can be

rescheduled to one of the following days (1–7 days later). The dosing visit can only be rescheduled once.

6.9.1 Prohibited medications

The use of anti-coagulant treatments and medications (prescription and non-prescription) that are known to cause QT prolongation are prohibited during the course of the trial.

Within 28 days prior to dosing, the use of daily systemic beta-blockers, indomethacin, warfarin, and anticholinergic drugs is prohibited.

Within 2 weeks prior to dosing, the use of any non-prescribed systemic or topical medication (with the exception of vitamins and the occasional use of acetylsalicylic acid and paracetamol) is prohibited.

Within 48 hours prior to dosing, the use of insulin Degludec or insulin Glargine U300 are prohibited.

Within 24 hours prior to dosing, the use of long-acting insulin (e.g., insulin Glargine U100 or insulin Detemir) is prohibited.

Within 16 hours prior to dosing, the use of insulin NPH is prohibited.

Within 6 hours prior to dosing, the use of any short acting (bolus) insulin, except insulin glulisine (Apidra®) is prohibited.

During the insulin-induced hypoglycemic procedure, continuous SC insulin infusion must be stopped.

6.10 Local German requirements for insulin glulisine (Apidra®)

Insulin glulisine (Apidra), the insulin used to induce hypoglycemia, will according to local requirements be defined as an investigational medicinal product when used at German sites. The insulin will be provided by the local sites and drug accountability will be performed according to local procedures. In contrast to the trial drug for the randomized treatment, the insulin will be administered by the blinded trial personnel via IV infusion as described in section 8.2.3.1.

7 PARAMETERS AND METHODS OF ASSESSMENT

Overall, approximately 180 mL of blood will be drawn from each subject for PK, PD, ADA, and safety laboratory assessments.

7.1 Efficacy parameters

A description of the sample handling and sample processing at the site will be included in the laboratory manuals. Validation documentation for the assays must be available prior to sample analyses. A bioanalytical report for each analysis of trial PD and PK samples will be prepared.

7.1.1 Pharmacodynamic measurements

The plasma glucose profile for evaluation of the primary and secondary clinical efficacy endpoints will be assessed based on plasma concentration data ($AUC_{0\text{-}30min}$) from samples collected at the dosing visit (Visit 2). The samples will be sent to the clinical laboratory and analyzed using a sensitive and validated assay for glucose measurements.

Samples will be collected pre-dose, and at 4, 6, 8, 10, 12, 15, 17, 20, 25, 30, 40, 50, 60, 75, and 90 minutes after dosing (see the schedule of procedures in Table 3). The actual time of blood sampling for evaluation of plasma glucose should not deviate from the nominal time by more than ±30 seconds until the 20 minute collection time point and by more than ±1 minute for the subsequent collection time points. Pre-dose is defined as within 2 minutes prior to dosing.

7.1.2 Pharmacokinetic measurements

The exposure to trial medication (dasiglucagon or GlucaGen) for evaluation of PK will be assessed based on plasma concentration data ($AUC_{0\text{-}90\ min}$, C_{max}, t_{max}) from samples collected at the dosing visit (Visit 2).

Samples (including back-up samples) will be collected pre-dose, and at 15, 30, 35, 40, 50, 60, 90, and 120 minutes after dosing (see the schedule of procedures in Table 3). The actual time of blood sampling for exposure to trial medication should not deviate from the nominal time by more than ±1 minute. Pre-dose is defined as within 2 minutes prior to dosing.

7.2 Safety parameters

7.2.1 Adverse events

The investigator is responsible for the detection and documentation of events meeting the criteria and definition of an AE or serious adverse event (SAE), as provided in this protocol. During the trial, the investigator or center staff will be responsible for reporting AEs and SAEs, as detailed in this section of the protocol.

Based on the investigator's clinical judgment it will be determined whether an AE is related to treatment and of sufficient severity to require the subject's removal from treatment or from the trial. A subject may also voluntarily withdraw from treatment due to what he or she perceives as an intolerable AE. If either of these situations arises, the subject should be under medical supervision until symptoms cease or the condition becomes stable.

7.2.1.1 Definitions

Adverse event

An AE is any untoward medical occurrence in a trial subject given an IMP which does not necessarily have a causal relationship with the treatment. An AE can therefore be any unfavorable and/or 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.

In this trial, only treatment-emergent adverse events (TEAEs) will be collected and reported. TEAEs are events that occur from the first trial-related activity after the subject has signed the informed consent form until the end of the post-treatment follow-up period.

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 7.2.2).
- Injection site reactions (see Section 7.2.6).

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

- Pre-planned procedure, unless the condition for which the procedure was planned has worsened from the first trial related activity after the subject 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

An 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 events may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above.

Adverse drug reaction

All noxious and unintended responses to an investigational product related to any dose should be considered adverse drug reactions. The phrase responses to an investigational product means that a causal relationship between an investigational product and an adverse event is at least a reasonable possibility, i.e. the relationship cannot be ruled out.

Suspected unexpected serious adverse reactions (SUSARs)

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. dasiglucagon Investigator's Brochure or package leaflet/Summary of Product Characteristics for GlucaGen).

Adverse event of special interest

An AESI 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 AESIs:

- Post-dose clinical signs, or measured vital signs, indicating a clinically significant drop in blood pressure including signs of orthostatic hypotension, vasovagal responses or bradycardia.
- Post-dose 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 AE 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 AE 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 subject.
Severe:	A type of AE 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 an SAE may not necessarily 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.

Germany only: The investigator should assess the causality to dasiglucacon/placebo/GlucaGen and to insulin glulisine (Apidra®) respectively. A special field applicable for Germany only will be present on the AE form in the eCRF to capture this data.

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 subject 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 subject signed the informed consent.
Recovering/resolving:	The condition is improving and the subject is expected to recover from the event. This term is only applicable if the subject has completed the trial.
Recovered/resolved with sequelae:	The subject 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 subject has not improved and the symptoms are unchanged.
Fatal:	This term is only applicable if the subject died from a condition related to the reported AE. Outcomes of other reported AEs in a subject 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 subject is lost to follow-up.

7.2.1.2 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 subject has signed the informed consent until the end of the post-treatment follow-up period. At each contact with the center (visit or telephone, excluding visits where the subject is not seeing the investigator or his/her staff [e.g. visits

to the laboratory]) the subject must be asked about AEs. All AEs, either observed by the investigator or reported by the subject, 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.

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 AESIs, the AESI 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 during IMP administration 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 AESI, the investigator must tick the AESI box on the AE form and complete the AESI form. The AESI 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 all AESIs to the sponsor's responsible pharmacovigilance unit (Safety CRO; see the list of trial personnel in Section 12.1) immediately (within 24 hours) after obtaining knowledge about the event.

The investigator must report initial information electronically (e.g. in PDF format) on all SAEs to the sponsor's responsible pharmacovigilance unit (Safety CRO; see the list of trial personnel in Section 12.1) immediately (within 24 hours) after obtaining knowledge about the event. 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 (see Section 7.2.1.1) that occur in this trial to the Competent Authorities and Institutional Review Boards (IRBs)/Independent Ethics Committees (IECs) in accordance with the local requirements in force and ICH guideline for GCP. The trial monitor must be informed accordingly.

7.2.1.3 Follow-up of adverse events

All AEs that are ongoing at the end of the subject's participation in the trial will be followed-up until the event is resolved or reaches a satisfactory outcome as deemed by the investigator or until the last visit of the last subject enrolled in the trial, whichever occurs first.

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 an SAE.

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

The sponsor and/or CROs acting on behalf of the sponsor can upgrade a non-serious AE to an SAE. In these situations the investigator will be informed and asked to fill out an SAE form and forward to the Safety CRO immediately (within 24 hours).

7.2.1.4 Clinical laboratory abnormalities and other abnormal assessments as adverse events or serious adverse events

Abnormal laboratory findings (e.g. biochemistry, hematology, urinalysis) or other abnormal assessments (e.g. vital signs) that are judged by the investigator as **clinically significant** will be recorded as AEs or SAEs if they meet the definition of an AE or SAE. Clinically significant abnormal laboratory findings or other abnormal assessments that are detected during the trial or are present at baseline and significantly worsen following the start of the trial will be reported as AEs or SAEs. However, clinically significant abnormal laboratory findings or other abnormal assessments that are associated with the disease being studied, unless judged by the investigator as more severe than expected for the subject's condition, or that are present or detected at the start of the trial and do not worsen, will not be reported as AEs or SAEs.

The investigator will exercise his or her medical and scientific judgment in deciding whether an abnormal laboratory finding or other abnormal assessment is clinically significant.

7.2.2 Hypoglycemia

Hypoglycemia will be regarded as an AE and recorded and documented on an AE form (and SAE form, if applicable).

Hypoglycemia is defined as a decline in plasma glucose to below 70 mg/dL (3.9 mmol/L). However, in the time period from initiation of the hypoglycemic clamp procedure (see Section 8.2.3.1) until 45 minutes after dosing, hypoglycemia is defined as a decline in plasma glucose to below 45 mg/dL (2.5 mmol/L).

During the dosing visit, prior to administration of the IMP, a plasma glucose value of <45 mg/dL (2.5 mmol/L) will be treated by an IV glucose solution. After administration of the IMP in the period from 8 minutes until 44 minutes after dosing, a plasma glucose value of <45 mg/dL (2.5 mmol/L) will be treated by an IV glucose solution, and if a plasma glucose value of ≥70 mg/dL (3.9 mmol/L) is not achieved within the 45 minutes after IMP administration, IV glucose infusion will also be initiated.

If the subject experiences symptoms of hypoglycemia, a plasma glucose measurement should be taken in order to classify the event (please refer to Section 7.4.2 for additional details).

7.2.3 Physical examination

The physical examination will be carried out at screening (Visit 1) and at the follow-up visit (Visit 3; see Table 3).

The physical examination includes examination of the following body systems: head, ears, eyes, nose, throat, including the thyroid gland; heart, lung, chest; abdomen; skin and mucosae; musculoskeletal system; nervous system; lymph node; other findings.

At the screening visit, any abnormality will be recorded and described in the eCRF, including the investigator's assessment of clinical significance ('abnormal, not clinically significant' or 'abnormal, clinically significant'). Clinically significant findings at the screening visit should be recorded as concomitant illness.

At subsequent visits, any clinically significant deterioration of a pre-existing condition as well as any new clinically significant findings will be recorded as AEs (see Section 7.2.1).

7.2.4 Vital signs

An examination of the following vital signs will be performed at screening (Visit 1), the dosing visit (Visit 2) and at the follow-up visit (Visit 3):

- Diastolic and systolic blood pressure (mmHg) will be measured after at least 5 minutes rest in a supine position. At Visit 1, blood pressure will be measured in both arms. The blood pressure from the arm with the higher systolic value is transcribed into the eCRF and this arm should be used for all subsequent measurements of the subject's blood pressure in this trial.
- Pulse (beats per min) measured after at least 5 minutes rest in a supine position.
- Body temperature (°C).

At the dosing visit, measurements will be taken prior to the start of the insulin-induced hypoglycemic procedure (within 30 minutes) and at 30, 90, and 300 minutes after dosing (see Table 3). The actual time of the assessment should not deviate from the nominal time by more than ±10 minutes. In addition to the pre-specified assessments as listed in Table 3, blood pressure and pulse may be assessed at any time during the trial at the discretion of the investigator.

7.2.5 Electrocardiogram

A standard 12-lead ECG will be performed at the screening visit (Visit 1), the dosing visit (Visit 2; prior to the start of the insulin-induced hypoglycemic procedure [within 30 minutes]) and at 20, 35, 45, 60, and 300 minutes after dosing) and at the follow-up visit (Visit 3; see Table 3). The actual time of the assessment should not deviate from the nominal time by more than ±5 minutes.

ECG parameters (heart rate, PQ, QRS, QT, QTcB) and any abnormality will be recorded and described in the eCRF including the investigator's assessment of clinical significance ('abnormal, not clinically significant' or 'abnormal, clinically significant').

At subsequent visits, any clinically significant deterioration of a pre-existing condition as well as any new clinically significant findings will be recorded as AEs (see Section 7.2.1).

7.2.6 Local tolerability

Immediately prior to treatment administration, it should be verified that the injection site is normal. To ensure all injection site assessments are performed at the injection site, the site will be marked with a pen prior to injection. Assessment of local tolerability at the injection site will be performed at the dosing visit (Visit 2; at 30, 120 and 300 minutes after dosing), and at the follow-up visit (Visit 3) (see Table 3) and more frequently, if deemed necessary by the investigator. The actual time of the assessment should not deviate from the nominal time by more than ±10 minutes. In the eCRF, the time of assessment and any injection site reaction observed will be recorded. In case of an observation, the Local Tolerability form will be completed, as well as the (S)AE form.

The local tolerability at the injection site will be evaluated by means of the following assessments: spontaneous pain, pain on palpation, itching, redness, edema, induration/infiltration, and other. Each of these assessments will be reported on a scale of 0 (none), 1 (mild), 2 (moderate), and 3 (severe). The evaluation and the actual time of the assessment will be recorded. The assessments will be performed by a trial physician or nurse.

Digital pictures will be taken of the injection site at the time of identification, and thereafter as often as judged necessary by the investigator. The pictures should include a subject identifier, visit number, time after dosing, and a ruler for scaling.

7.2.7 Clinical laboratory assessments

The safety parameters that will be assessed at the clinical laboratory are listed in Table 3. Routine clinical laboratory tests will be performed centrally. Samples for clinical laboratory parameters (biochemistry, hematology, coagulation) will be collected at screening (Visit 1), the dosing visit (Visit 2, prior to the start of the insulin-induced hypoglycemic procedure [within 30 minutes]) and at 30 and 300 minutes after dosing), and at the follow-up visit (Visit 3). The actual time of the assessment should not deviate from the nominal time by more than ±10 minutes. Samples for glycated hemoglobin (HbA_{1c}) will be collected at screening only (Visit 1). Samples for urinalysis will be collected at screening (Visit 1), at the dosing visit (Visit 2, prior to the start of the insulin-induced hypoglycemic procedure [within 120 minutes]) and at the follow-up visit (Visit 3). The following parameters will be assessed:

- Clinical biochemistry: sodium, potassium, calcium, glucose, urea, creatinine, total bilirubin, AST, ALT, gamma-glutamyltransferase, 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 (leukocytes).
- Coagulation: international normalized ratio, fibrinogen (at screening visit only).
- Urinalysis: pH, blood (leukocytes and erythrocytes), protein, glucose, ketones, nitrite.

Pregnancy tests will be performed at each visit for women of childbearing potential only. A serum pregnancy test will be performed at screening (Visit 1) and urine stick tests will be performed at the dosing visit (Visit 2, prior to the start of the insulin-induced hypoglycemic procedure) and the follow-up visit (Visit 3). Test sticks will be provided to the trial centers.

Alcohol breath tests and a urine drug screen will be performed at screening (Visit 1) and at the dosing visit (Visit 2, prior to the start of the insulin-induced hypoglycemic

procedure). Equipment for the alcohol breath test and uring drug screen will be provided to the trial centers.

Re-assessment of laboratory parameters will be allowed only if handling issues, damaged samples, or hemolyzed samples have confounded the measurement results.

For further details of the clinical laboratory assessments, please refer to the laboratory manual.

7.2.8 Pregnancy

Female subjects 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 subjects must be instructed to notify the investigator immediately if their partner becomes pregnant or suspects pregnancy. The sponsor has a responsibility to monitor the outcome of all pregnancies reported during the clinical trial. During an information meeting at the trial center, the subject'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 immediately (within 24 hours), according to the procedure stated in Section 7.2.1.2. 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 follow 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 7.2.1.2. Any (S)AEs in the newborn must be reported on the (S)AE form.

The SAEs that must be reported include 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 until the age of 1 month.

7.2.9 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 subjects. During a subject's participation in the trial, the investigator should ensure that adequate medical care is provided to the subject for any AEs, including clinically significant laboratory values related to the trial. The investigator should inform the subject when medical care is needed for intercurrent illnesses of which the investigator becomes aware.

For further information on safety precautions for dasiglucagon and GlucaGen, please refer to the current version of the Investigator's Brochure (14) and the Summary of Product Characteristics for GlucaGen (13), respectively.

7.2.10 Safety committee

The internal Zealand Pharma Safety Committee is constituted to perform ongoing blinded 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 Safety Committee meetings, or on notification of significant findings, the Safety Committee will take appropriate measures to safeguard the subjects.

7.3 Demography, concomitant illness, medical history and concomitant medication

Demographics, body measurements, concomitant illness and medical history will be assessed only at screening (Visit 1). Concomitant medication will be assessed at screening (Visit 1), the dosing visit (Visit 2, prior to the start of the insulin-induced hypoglycemic procedure) and at the follow-up visit (Visit 3).

7.3.1 Demography and body measurements

Subject demographics and body measurements will include:

- Age
- Race, ethnicity
- Sex
- Height (meters or inch), without shoes
- Body weight (kg or lb), only wearing underwear and measured using standard scales
- Body mass index (kg/m^2) calculated based on height and body weight (body weight/height^2).

7.3.2 Concomitant illness and medical history

A concomitant illness is any illness that is present at the start of the trial (i.e. at the screening visit). Concomitant illnesses present at the start of the trial will be recorded in the eCRF at screening.

Medical history is an account of medical events that the subject has experienced in the past, including prior medications. Relevant medical conditions/illnesses in the past obtained by asking the subject or by inspecting his/her medical records will be recorded in the eCRF at screening. History of alcohol or drug abuse will also be recorded.

The information collected for concomitant illness and medical history should include diagnosis, date of onset, date of resolution or continuation.

Any change to a concomitant illness should be recorded during the trial, including end date, if applicable. A clinically significant worsening of a concomitant illness must be reported according to Section 7.2.1.

All previous and concomitant diseases will be coded with Medical Dictionary for Regulatory Activities (MedDRA), the version of which will be provided in the clinical trial report.

7.3.3 Diabetes diagnosis and current treatment

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

7.3.4 Concomitant medication

A concomitant medication is any medication, other than the trial products and current diabetes treatment (including insulin glulisine [Apidra®] for diabetes therapy wash-out), which is taken during the trial, including screening and follow-up periods.

Details of any concomitant medication must be recorded at trial entry (i.e. at screening). Any changes in concomitant medication must be recorded at each visit as they occur. The information collected for each concomitant medication includes (at a minimum) trade name or generic name, indication, start date and stop date or continuation. A change in medication due to an AE must be recorded and reported according to Section 7.2.1. If the change in medication influences the subject's eligibility to continue in the trial, the sponsor and trial monitor must be informed.

7.4 Other assessments

7.4.1 Immunogenicity

Antibodies against dasiglucagon/GlucaGen will be measured at the dosing visit (Visit 2) and at follow-up (Visit 3). At the dosing visit (Visit 2), samples will be collected prior to the start of the insulin-induced hypoglycemic procedure.

The clinical ADA assays, specific for dasiglucagon and GlucaGen, respectively, have been validated in accordance with existing guidelines and recommendations (17-21).

Confirmed positive anti-dasiglucagon antibody samples (treatment-induced or treatment-boosted) from anti-dasiglucagon antibody-positive subjects 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 neutralizing antibody analysis.

The in vitro neutralizing effect of the antibodies will be measured using an assay based on glucagon receptor transfected human embryonic kidney cells (20,22). The calculated sensitivity in previous studies was about 51.8 ng/mL. The assay was also validated for recombinant glucagon with similar results (21,23). In case of a positive result in the neutralizing antibody assay, a titer estimation will be performed. The cell-based neutralizing antibody analyses will be performed by a special laboratory, BioAgilytix, Durham, NC, USA.

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.

7.4.2 Plasma glucose measurements for safety

During insulin-induced hypoglycemia, plasma glucose levels will be monitored closely at site. After the start of insulin infusion, plasma glucose should be checked every 10 minutes while plasma glucose is above 110 md/dL, and every 5 minutes once plasma glucose is at or below 110 mg/dL and until after dosing when the subject's plasma glucose is ≥70mg/dL (3.9 mmol/L). Hereafter, plasma glucose should be checked every 30 minutes until 300 minutes (5 hours) after dosing. Plasma glucose concentrations will be measured using a US FDA-approved glucose analyzer (e.g., YSI 2300, Yellow Springs Instruments, Yellow Springs, OH).

At the discretion of the investigator, additional plasma glucose measurements can be taken at any time during the trial, for example when there is a suspicion (e.g. symptoms) of a hypoglycemic episode.

Plasma glucose measurements for safety should only be recorded in the eCRF if they are related to an AE (e.g. a hypoglycemic episode).

In case of persistent post-treatment hypoglycemia, subjects will receive rescue treatment with an IV glucose infusion (see Section 8.2.3.1 for details). Blood samples for PD and PK assessments should still be taken at the specified timepoints.

7.4.3 Plasma insulin measurements

Samples for insulin assessment will be collected at the dosing visit (Visit 2, pre-dose and at 30 and 60 minutes after dosing). The actual time of blood sampling for evaluation of plasma insulin should not deviate from the nominal time by more than ±1 minute. Pre-dose is defined as within 2 minutes prior to dosing.

8 TRIAL CONDUCT

8.1 Schedule of procedures

The schedule of procedures is provided in Table 3. Informed consent will be obtained prior to any trial-related procedures; see Section 10.8.

8.2 Procedures by visit

8.2.1 Visit 1 (screening, Day -30 to Day -3)

Visit 1 will take place between 3 and 30 days before Visit 2, Day -1 to Day 1 (dosing day).

Informed consent can be obtained prior to or at Visit 1, however it must in any case be obtained prior to any trial related procedures. During the screening visit, the following assessments will take place:

- Informed consent (obtain or check)
- Check of subject eligibility (inclusion/exclusion criteria)
- Demography
- Body measurements
- Medical history, diabetes diagnosis, current diabetes treatment
- Concomitant illnesses
- Concomitant medications
- History of alcohol/drug abuse
- Physical examination
- Vital signs
- 12-lead ECG
- AEs
- Biochemistry, hematology, coagulation, HbA_{1c}
- Serum pregnancy test (women of childbearing potential only)
- Urinalysis
- Urine drug screen
- Alcohol breath test

Table 3 Schedule of procedures

Visit number	V1	V2	V3
Trial day	**-3**	**-1 and 1**	**28**
Visit type	**Screening**	**Dosing**	**Follow-up**
Window	**-30 to -3**		**+5 days**
Subject related information/assessments			
Informed consent	x		
Inclusion/exclusion criteria	x	x[1,2]	
Demography	x		
Body measurements	x		
Medical history, diabetes diagnosis, and current diabetes treatment	x		
Concomitant illnesses	x		
Concomitant medications	x	x[1]	x
History of alcohol/drug abuse	x		
Randomization		x[1]	
Withdrawal criteria		x[1]	
Dosing day exclusion criteria		x[1]	
Insulin-induced hypoglycemia		x	
Safety assessments			
Physical examination	x		x
Vital signs	x	x[3]	x
12-lead ECG	x	x[4]	x
Local tolerability		x[5]	x
Adverse events	x	x	x
Laboratory			
Biochemistry, hematology, coagulation, HbA_{1c} (HbA_{1c} at Visit 1 only)	x	x[6]	x
Pregnancy test (women of childbearing potential only)	x[7]	x[1,8]	x[8]
Urinalysis	x	x[1]	x
Urine drug screen	x	x[1]	
Alcohol breath test	x	x[1]	
PK/Clinical efficacy			
Plasma dasiglucagon/GlucaGen		x[9]	
Plasma glucose		x[10]	
Other assessments			
Antibodies against dasiglucagon/GlucaGen		x[1]	x[11]
Plasma insulin		x[12]	
Trial material			
Administration of trial product (during hypoglycemic clamp procedure)		x	

[1]Prior to the start of the insulin-induced hypoglycemic procedure.
[2]Only check of dosing day exclusion criteria and changes between screening visit and Visit 2.
[3]Prior to the start of the insulin-induced hypoglycemic procedure (within 30 minutes), and at 30, 90 and 300 minutes after dosing. The actual time of the assessment should not deviate from the nominal time by more than ±10 minutes.

[4]Prior to the start of the insulin-induced hypoglycemic procedure (within 30 minutes), and at 20, 35, 45, 60 and 300 minutes after dosing. The actual time of the assessment should not deviate from the nominal time by more than ±5 minutes.
[5]Local tolerability assessed at 30, 120, and 300 minutes after dosing. The actual time of the assessment should not deviate from the nominal time by more than ±10 minutes.
[6]Prior to the start of the insulin-induced hypoglycemic procedure (within 30 minutes), and at 30 and 300 minutes after dosing. The actual time of the assessment should not deviate from the nominal time by more than ±10 minutes.
[7]Serum pregnancy test.
[8]Urine stick pregnancy test.
[9]Pre-dose, and at 15, 30, 35, 40, 50, 60, 90, and 120 minutes after dosing. The actual time of blood sampling should not deviate from the nominal time by more than ±1 minute. Pre-dose is defined as within 2 minutes prior to dosing.
[10]Pre-dose, and at 4, 6, 8, 10, 12, 15, 17, 20, 25, 30, 40, 50, 60, 75, 90 minutes after dosing. The actual time of blood sampling should not deviate from the nominal time by more than ±30 seconds until the 20 minute collection time point and by more than ±1 minute for the subsequent collection time points. Pre-dose is defined as within 2 minutes prior to dosing.
[11]Any subject that tests positive for ADA will be monitored until the ADA levels return to baseline levels.
[12]Pre-dose, and at 30 and 60 minutes after dosing. The actual time of blood sampling should not deviate from the nominal time by more than ±1 minute. Pre-dose is defined as within 2 minutes prior to dosing.
ADA = anti-drug antibodies; ECG = electrocardiogram; HbA_{1c} = glycated hemoglobin.

Eligible or potentially eligible subjects (laboratory results pending) will be provided with an Identification card (ID card), stating that the subject is participating in the trial and whom to contact (site address, investigator name and telephone number). The subjects should be instructed to return the ID card to the investigator at the last visit or to destroy the card after the last visit.

8.2.2 Instructions to subjects prior to the dosing visit (Visit 2)

At the screening visit, the investigator will inform the subject about the changes to his/her insulin therapy leading up to the start of the insulin-induced hypoglycemic procedure. The subject may be provided with insulin NPH in the wash-out period to cover the need of basal insulin, if deemed necessary by the investigator. The subject's current insulin therapy will be washed out as defined in Section 5.5.: 48 hours prior to dosing and during the dosing visit, treatment with insulin Degludec and insulin Glargine U300 are not allowed; 24 hours prior to dosing and during the dosing visit, treatment with other long-acting insulins (e.g., insulin Glargine U100 or insulin Detemir) is not allowed; 16 hours prior dosing and during the dosing visit treatment with insulin NPH is not allowed; 6 hours prior to dosing and during the dosing visit, treatment with any short acting (bolus) insulin, except insulin glulisine (Apidra®), is not allowed. The basal rate of insulin pumps (continuous SC insulin infusion) will be discontinued on the morning of the dosing day (if using insulin glulisine [Apidra®]) OR at least 6 hours prior to dosing (if using other insulins).

On the day prior to dosing (Day -1), the subjects will need to attend the clinical center and will be required to stay onsite overnight. On the morning of the dosing day (Day 1), patients are required to be in a fasting condition, defined as having consumed only water since 22:00 hours the night before. However, the subjects are allowed to consume small

amounts (up to 20 g) of carbohydrates to prevent hypoglycemia. The subjects must also not consume any alcohol within 24 hours prior to dosing (refer to Section 5.5 for all dosing day exclusion criteria).

8.2.3 Visit 2 (dosing visit)

Visit 2 will take place on Day -1 to Day 1.

The subjects will attend the clinical center the day prior to dosing (Day -1) and subject eligibility is rechecked (check of changes between the screening visit and Visit 2) and those subjects eligible to participate should stay onsite overnight. To target a glucose level around 90-110 mg/dL (5.0-6.1 mmol/L) the following morning, subjects may be administered insulin glulisine (Apidra®) at the investigator's discretion either by IV infusion, SC bolus or continuous SC insulin infusion. Dosing will take place the following morning (Day 1).

On Day 1 and prior to the start of the insulin-induced hypoglycemic procedure, those subjects eligible to participate will be randomized to treatment with dasiglucagon, placebo, or GlucaGen.

The following assessments will also take place:

- Document all changes in concomitant medication (prior to the start of the insulin-induced hypoglycemic procedure)
- Check of withdrawal criteria (prior to the start of the insulin-induced hypoglycemic procedure)
- Check of dosing day exclusion criteria (prior to the start of the insulin-induced hypoglycemic procedure)
- Vital signs (prior to the start [within 30 minutes] of the insulin-induced hypoglycemic procedure, and at 30, 90 and 300 minutes after dosing). 12-lead ECG (prior to the start [within 30 minutes] of the insulin-induced hypoglycemic procedure, and at 20, 35, 45, 60, and 300 minutes after dosing)
- Local tolerability (at 30, 120, and 300 minutes after dosing)
- AEs
- Biochemistry, hematology, coagulation (prior to the start of the insulin-induced hypoglycemic procedure [within 30 minutes], and at 30 and 300 minutes after dosing)
- Urine stick pregnancy test (women of childbearing potential only; prior to the start of the insulin-induced hypoglycemic procedure)
- Urinalysis (prior to the start of the insulin-induced hypoglycemic procedure [within 120 minutes])
- Urine drug screen (prior to the start of the insulin-induced hypoglycemic procedure)
- Alcohol breath test (prior to the start of the insulin-induced hypoglycemic procedure)

- Dasiglucagon/GlucaGen plasma concentrations:
  - Pre-dose, 15, 30, 35, 40, 50, 60, 90, and 120 minutes after dosing. The actual time of blood sampling for exposure should not deviate from the nominal time by more than ±1 minute. Pre-dose is defined as within 2 minutes prior to dosing.
- Plasma glucose concentrations:
  - Pre-dose, 4, 6, 8 ,10, 12, 15, 17, 20, 25, 30, 40, 50, 60, 75, and 90 minutes after dosing. The actual time for blood sampling for plasma glucose should not deviate from the nominal time by more than ±30 seconds until the 20 minute collection time point and by more than ±1 minute for the subsequent collection time points. Pre-dose is defined as within 2 minutes prior to dosing.
- Antibodies against dasiglucagon/GlucaGen (prior to the start of the insulin-induced hypoglycemic procedure).
- Plasma insulin concentrations:
  - Pre-dose, 30 and 60 minutes after dosing. The actual time of blood sampling for plasma insulin should not deviate from the nominal time by more than ±1 minute. Pre-dose is defined as within 2 minutes prior to dosing.

8.2.3.1 Hypoglycemic clamp procedure and administration of trial medication

The following procedure is based on precendent procedures for hypoglycemia induction in patients with T1DM [24, 25].

The treatment day (Visit 2, Day 1) will be conducted after an overnight fast of at least 8 hours, targeting a starting plasma glucose around 90 to 110 mg/dL (5.0-6.1 mmol/L).

Subjects who meet one or more of the dosing day exclusion criteria (Section 5.5) at the dosing visit will be excluded from the dosing visit, but the visit can be rescheduled 1-7 days later. The dosing visit can only be rescheduled once.

The subject's current insulin therapy will be washed out as defined in Section 5.5. For subjects using multiple daily injections, the date, time and the dose of the last basal insulin and the last short-acting insulin (except insulin glulisine [Apidra®]) administration prior to dosing will be captured. For subjects using an insulin pump, the time of discontinuation of the basal rate will be captured. Any use of insulin glulisine (Apidra®) in the last 5 hours prior to initiation of the hypoglycemia induction procedure will also be captured.

At approximately 08:00 hours, an infusion catheter will be inserted into each arm (forearm cephalic vein) for the manual glucose clamp procedure, with the glucose infusion in one arm and the insulin infusion in the opposite arm (if IV insulin glulisine [Apidra®] has been administered during the night the same infusion catheter can be used). A third catheter for blood sampling will be placed into a metacarpel vein for blood

sampling. This hand will be warmed (55-65ºC) to arterialize venous blood. If there are issues with blood sampling from the metacarpal vein for the purpose of glucose measurements, a new and more proximal IV access may be used at the discretion of the investigator.

Hypoglycemia will be gradually induced by a fast-acting IV insulin glulisine (Apidra®) infusion (15 U [100 U/mL] in 49 mL saline and 1 mL of subject's blood or plasma), initially at 150% of the subject's usual basal rate and can be increased or decreased over a range of 0% to 200% as judged necessary by the investigator, to achieve a controlled decline in plasma glucose, targeting a plasma glucose level of 55 mg/dL (3.1 mmol/L).

Plasma glucose concentrations will be measured using a US FDA-approved glucose analyzer (e.g., YSI 2300, Yellow Springs Instruments, Yellow Springs, OH). After the start of the insulin infusion, plasma glucose will be measured every 10 minutes while plasma glucose is above 110 mg/dL, and every 5 minutes once plasma glucose is at or below 110 mg/dL.

Once the glucose concentration declines to <60 mg/dL (3.3 mmol/L), the insulin infusion will be stopped, and 5 min later plasma glucose concentration will be measured at the glucose analyzer and blood samples for baseline assessment of plasma glucose, dasiglucagon/GlucaGen PK, and insulin PK will be collected. The samples are the baseline samples and should be collected within 2 minutes before IMP administration.

- If plasma glucose is ≥45 mg/dL and <60 mg/dL (2.5-3.3 mmol/L), study treatment (IMP) will be administered, defining time, t=0. The study treatment will be delivered in the abdomen, buttock, or thigh (according to stratification) via SC injection, with the subject lying in a lateral recumbent position.
- If plasma glucose is <45 mg/dL (2.5 mmol/L), IV glucose solution will be administered sufficient to raise plasma glucose to within the 45-60 mg/dL target range. The run-in period will be adequately extended (at least 30 min) until the above target is achieved and new baseline samples for plasma glucose, dasiglucagon/GlucaGen PK, and insulin PK will be collected. Glucose should not be infused within 10 minutes before IMP administration. If plasma glucose is not within target range after the second attempt, the subject should be rescheduled for a new treatment visit within 7 days (+ 2 days).

Administration of IMP should not occur earlier than 9:00 hours in the morning or later than 12:00 hours.

As shown in Table 4, serial blood samples for glucose will be collected at t=0, 4, 6, 8, 10, 12, 15, 17, 20, 25, 30, 40, 50, 60, 75 and 90 minutes post-dosing. Samples for assessing plasma dasiglucagon/GlucaGen concentration will be collected at t=0, 15, 30, 35, 40, 50,

60, 90, and 120 minutes. Samples for assessing plasma insulin concentration will be collected at t=0, 30, and 60 minutes.

Table 4 Post-treatment blood sampling times

Times	0	4	6	8	10	12	15	17	20	25	30	35	40	50	60	75	90	120
Plasma glucose	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y		Y	Y	Y	Y	Y	
PK Dasiglucagon/ GlucaGen	Y						Y				Y	Y	Y	Y	Y		Y	Y
PK Insulin	Y										Y				Y			

Refer to Section 7.2.7 for details of laboratory safety sampling and to Section 7.4.2 for details of blood glucose safety sampling.

When the t=90-minute blood sampling for plasma glucose has been collected the subjects are allowed to eat moderately. Drinking of water is allowed *ad libitum* during the entire procedure.

Hypoglycemia Rescue Provisions

During insulin-induced hypoglycemia, plasma glucose levels will be monitored closely at site for safety reasons as described in Section 7.4.2. Subjects should receive post-treatment rescue glucose infusion to ameliorate persistent hypoglycemia, as follows.

1. Glucose infusion should be initiated if a subject experiences severe alarming escalation of symptoms of hypoglycemia (e.g. symptoms suggesting a change in consciousness) at any time during the trial; glucose infusion should be initiated targeting a plasma glucose levels >70 mg/dL.
2. If plasma glucose is <45 mg/dL (2.5 mmol/L) between t=8 and t=44 minutes, rescue glucose infusion (1-2 mg/kg administered IV over about 5 seconds) should be initiated to maintain plasma glucose between 45 mg/dL and 55 mg/dL (2.5-3.1 mmol/L). Pause glucose infusion if plasma glucose is >55 mg/dL.
3. If plasma glucose is <70 mg/dL at t=45 minutes, rescue glucose infusion (2-3 mg/kg administered IV over about 5 seconds) should be initiated to maintain plasma glucose between 70 mg/dL and 80 mg/dL (3.9-4.4 mmol/L). Pause glucose infusion if plasma glucose is >75 mg/dL (4.2 mmol/L).

Subjects should remain in bed until completion of the test procedure 300 minutes after dosing (bathroom visits are allowed).

The IMP will be administered SC according to Section 6.3. The time of IMP administration will be recorded. At the timepoint when the insulin infusion is stopped, the total insulin dose which was required to induce hypoglycemia will be recorded.

AEs will be specifically recorded during the procedure at several timepoints.

The investigator must provide information to the subjects on how to resume their usual diabetes treatment.

The subject may be released from the clinical center if the investigator does not have any safety concerns based on the last safety plasma glucose value and the general condition of the subject. However, at the discretion of the investigator or on request of the subject, the subject may stay at the trial center for a longer period.

8.2.4 Visit 3 (follow-up visit)

Visit 3 will take place on Day 28 + 5 days. The subject does not need to be fasting.

At Visit 3, the following assessments will take place:

- Document all changes in concomitant medication
- Physical examination
- Vital signs
- 12-lead ECG
- Local tolerability
- AEs
- Biochemistry, hematology, coagulation
- Urine stick pregnancy test (women of childbearing potential only)
- Urinalysis
- Antibodies against dasiglucagon/GlucaGen.

After the follow-up visit the End of Trial form must be completed. Even if a subject is not able to attend the follow-up visit, the End of Trial form, the eCRF Accountability/Affirmation Statement form and the Drug Accountability form must be completed.

9 STATISTICAL METHODS

Before database lock and treatment unmasking, a separate statistical analysis plan (SAP) will be finalized, providing detailed methods for the analyses outlined below. Further analysis details may be added or refined in the SAP.

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

9.1 Determination of sample size

Due to requirements in the size of the safety database, the sample size is set to 78 subjects treated with dasiglucagon 0.6 mg. With a 2:1:1 randomization ratio for the three treatments, dasiglucagon 0.6 mg, placebo, and GlucaGen 1 mg, 78 subjects injected with dasiglucagon and 39 subjects with placebo will yield a power of 90% at a 5% two-sided significance level to detect a treatment group difference in recovery incidence within 20 minutes, assuming as low as 80% and as high as 50% recovery incidence for the dasiglucagon and placebo groups, respectively.

9.2 Trial subjects

9.2.1 Analysis samples

For presentation of data and reporting of the statistical analyses, the following analysis samples will be used, depending on the context:

- Safety analysis set (SAS): All randomized subjects who received at least one dose of trial medication.
- Full analysis set (FAS): All randomized subjects who received at least one dose of trial medication and contributed valid information for at least one post-dose endpoint.
- Per protocol (PP) set: All subjects of the FAS for whom no relevant protocol deviations were documented. This sample will primarily be used for sensitivity analysis.

The analysis of the primary endpoint and secondary endpoints will be based on the FAS. A secondary analysis of the primary endpoint will be based on the PP set. All safety analyses will be based upon the SAS.

The decision regarding whether a protocol deviation is relevant or not for the exclusion of subjects from the PP set will be made case-by-case in a data review meeting prior to treatment unmasking and database lock (see Section 9.2.3).

9.2.2 Disposition of subjects

Subject disposition will be tabulated including the number of screened subjects, screening failures, subjects exposed to trial product, subjects completing the trial and subjects in each analysis set.

Subjects withdrawn from the trial will be listed including the primary reason for withdrawal.

9.2.3 Protocol deviations

Before data are released for statistical analysis, a treatment-masked review of all data will take place to identify protocol deviations that may potentially affect the results. This review will be performed without revealing to which trial product the subjects were assigned. The masking of the trial products will be maintained for everyone involved in allocating subjects to the analysis sets until data are released for statistical analysis. Furthermore, spurious outliers will be evaluated. In addition, protocol deviations that may potentially affect the results will be identified and it will be evaluated if subjects and/or data should be excluded from the analysis. Protocol deviations will be classified as minor or major in a consistent way. Major deviations from the protocol may lead to the exclusion of a subject from the PP set.

Major protocol deviations may include deviations related to trial inclusion or exclusion criteria, conduct of the trial, subject management or subject assessment. Unless explicitly decided otherwise during the masked data review, the following will be considered major protocol deviations:

- Violation of one or more major inclusion/exclusion criteria
- Interruption of administration of trial product
- Significant deviation from time windows
- Incorrect treatment allocation
- Missing primary endpoint.

The violation of several major inclusion/exclusion criteria or the complete absence of efficacy data might lead to exclusion of the subject from FAS. In that case, the decision should be taken at the masked data review meeting, and the exclusion from efficacy analysis justified in the signed notes of the meeting.

Obviously erroneous data points may be excluded from the analyses or re-analyzed (in case of e.g. serum concentrations). The decision to re-analyze or exclude data points from the statistical analysis is the joint responsibility of the sponsor and the trial statistician.

The subjects or observations to be excluded and the reason for their exclusion will be documented and signed by those responsible prior to database release. The documentation will be stored together with the remaining trial documentation. The subjects and observations excluded from analysis sets, and the corresponding reasons, will be described in the clinical trial report.

9.3 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 timepoint will be included in the analysis.

Raw data listings and summary tables will be generated using the software SAS© version 9.4 or higher.

Continuous variables will be summarized using means, standard deviations, medians, coefficients of variation, and minimum and maximum values.

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

9.4 Demographics and baseline characteristics

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

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

9.5 Efficacy Analyses

For the confirmatory analyses, the primary and key secondary endpoints for the dasiglucagon 0.6 mg and placebo treated subjects will be compared. A hierarchical procedure will be applied for the control of multiplicity. The primary and secondary endpoints will be evaluated on the full analysis set. In the primary analysis, those subjects who require rescue IV glucose will be censored at the time to plasma glucose recovery. In a sensitivity analysis, the time to plasma glucose recovery will be analyzed without censoring the subjects who received rescue IV glucose.

9.5.1 Hierarchical testing procedure

For the confirmatory analyses, the following a priori defined hierarchical inferential test order will be applied for the control of the type 1 error rate across the planned multiple comparisons. The primary endpoint and key secondary endpoints will be inferentially evaluated within the FAS in the following order, where inference will proceed at the two-sided 0.05 criterion significance level until the first failure to reject the null hypothesis for a dasiglucagon versus placebo comparison:

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

The GlucaGen versus placebo comparisons will not be included in the inferential testing hierarchy, since the efficacy of GlucaGen is previously established, and these comparisons are intended to support the validity of the study for the dasiglucagon versus placebo comparisons.

Analogous supportive sensitivity analyses will be conducted in the PP set, but without inference intent.

9.5.2 Primary confirmatory endpoint

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

9.5.2.1 Primary analysis

The primary endpoint will be summarized using Kaplan-Meier (KM) estimates stratified by treatment group and injection site. The treatment group difference between dasiglucagon and placebo will be evaluated inferentially using a pairwise two-sided log-rank test.

In the primary analysis, those subjects who require rescue IV glucose will be censored at the time of plasma glucose recovery. This approach is based on the assumption that rescue IV glucose administration will shorten the time to recovery. This assumption implies that censoring at the time of recovey after rescue therapy is valid as a time to

recovery without rescue would be longer than the observed duration. If recovery has not occurred at 45 minutes after study drug injection, censoring will be applied irrespective of the use of rescue IV glucose.

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

The primary endpoint will additionally be analyzed using a Cox proportional hazards (CPH) time to event statistical model, if the hazards by treatment group can be assumed proportional using a graphical comparison of log-minus-log survival curves and no more than two-thirds of the recovery times are censored in each treatment group. The CPH model will be used for inferences, with treatment group and injection site modeled as categorical effects, and baseline plasma glucose modeled as a continuous covariate. The treatment group rate ratios, active versus placebo, will be estimated together with the 95% confidence intervals, and pairwise treatment group inferences (dasiglucagon vs placebo, GlucaGen vs placebo) will be evaluated using two-sided likelihood ratio tests.

9.5.3 Secondary endpoints

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

9.5.3.1 Confirmatory analysis

The key secondary incidence variables (plasma glucose recovery within 30 minutes, within 20 minutes, within 15 minutes, within 10 minutes, in hierarchical order for inference) will be analyzed using descriptive statistics (frequency and percentage) by treatment group. Inferential treatment group comparisons will be assessed via pairwise tests of independent binomial proportions (dasiglucagon versus placebo, GlucaGen versus placebo) using Fisher's exact test for each pairwise comparison.

The key secondary endpoints of plasma glucose CFB within 30 minutes, within 20 minutes, within 15 minutes, and within 10 minutes after study drug injection or at the time of rescue, will be analyzed with the plasma glucose CFB at rescue carried forward in those subjects who require rescue IV glucose before plasma glucose ≥20 mg/dL recovery. Each of these CFB variables will be analyzed using an Analysis of Covariance model, with treatment group modeled as a fixed effect and with the baseline plasma glucose modeled as a covariate. The dasiglucagon versus placebo treatment group difference will

be evaluated inferentially as a least squares means contrast, using a two-sided t-test at the 0.05 signficance level, first for the 30 minute CFB, and then sequentially for the 20 minute, 15 minute, and 10 minute variables, until the first failure to reject.

9.5.4 Secondary clinical efficacy (PD) endpoints

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

Secondary clinical efficacy endpoints will be assessed based on plasma concentration data from samples collected at the dosing visit (Visit 2).

Unless otherwise stated, the population base of analysis will be the FAS.

9.5.4.1 Analysis of secondary clinical efficacy (PD) endpoints

1. Time to first plasma glucose concentration ≥70 mg/dL from baseline. This time-to-event endpoint will be evaluated using a Kaplan-Meier approach, with treatment group as a stratification factor, analogous to that used for the primary endpoint analysis. Differences between the KM curves (dasiglucagon versus placebo, GlucaGen versus placebo) will be evaluated inferentially using pairwise two-sided log rank tests. If the ≥70 mg/dL endpoint is not met within 45 minutes post-dosing, the time of the last valid plasma glucose measurement up to 45 minutes will be the censoring time.
2. The AUC will be calculated as the baseline-adjusted area under the plasma glucose profile over time:
    a. $AUC_{0\text{-}30min}$: restricting the time window to the 0 to 30 minutes interval.
3. The log-transformed AUC endpoint will be analyzed using an analysis of covariance (ANCOVA) model with treatment as fixed effect and baseline plasma glucose modeled as a covariate. The least squares means treatment group differences will be back-transformed (anti-logged) for presentation as a ratio of the treatment group geometric means, with their corresponding 95% CI.

9.5.5 Exposure (PK) endpoints

- Plasma dasiglucagon and GlucaGen concentrations from time zero to 90 minutes: $AUC_{0\text{-}90min}$, C_{max}, and t_{max}.

9.5.5.1 Analysis of exposure (PK) endpoints

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

C_{max} will be determined as the maximum of all valid plasma dasiglucagon/GlucaGen concentrations.

T_{max} will be determined as the timepoint where the maximum of all valid plasma dasiglucagon/GlucaGen concentration measurements for each measurement series is observed.

The log-transformed PK endpoints AUC and C_{max} will be analyzed in the same way as the AUC endpoints.

As t_{max} is a highly discrete endpoint, Wilcoxon's rank sum test for unpaired observations will be used to assess differences between the two treatment groups.

9.6 Exploratory analyses

Exploratory analyses will include descriptive statistics and modeling analogous to that done for key secondary endpoints. However, treatment group comparisons will be summarized without inference intent.

- Plasma glucose concentration ≥70 mg/dL (3.9 mmol/L) or increase of ≥20 mg/dL (1.1 mmol/L) within 30 minutes after study drug injection without administration of rescue IV glucose.

- Plasma insulin response as area under the curve above baseline from time zero to 60 minutes, $AUC_{0\text{-}60\ min}$.

Plasma insulin concentrations measured pre-dose and at 30 and 60 minutes after dosing (see Table 3) will be presented individually. A summary table per timepoint will be provided. The $AUC_{0\text{-}60min}$ will be determined and a summary presented.

9.7 Safety analyses

9.7.1 Adverse events

AEs will be coded using the latest available version of the MedDRA, the version of which will be provided in the clinical trial report. AE summary tables will include the number and percentage of subjects who experienced AEs summarized by system organ class and preferred term.

Germany only: The AE summary for AEs related to insulin glulisine (Apidra®) will be presented as an appendix to the clinical trial report, as insulin glulisine (Apidra®) is only considered an IMP in Germany.

An overall summary table will be provided showing the number and percentage of subjects with any:

- TEAE
- Severe TEAE
- Serious TEAE
- Drug-related TEAE
- Drug-related severe TEAE
- Drug-related serious TEAE
- TEAE leading to withdrawal
- TEAE with outcome death
- AESI

Refer to Section 7.2.1 for the definition of TEAEs.

9.7.2 Immunogenicity data

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

9.7.3 Clinical laboratory assessments

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

9.7.4 Other safety data

Incidence of rescue infusion of IV glucose during the hypoglycemic clamp procedure will be analyzed using descriptive statistics (frequency and percentage) by treatment group. Inferential treatment group comparisons will be assessed via pairwise tests of independent binomial proportions (dasiglucagon versus placebo, GlucaGen versus placebo).

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

Vital signs, physical examination, 12-lead ECG and local tolerability data will be summarized using descriptive statistics.

9.8 Treatment compliance

Trained unblinded members of staff will perform all administrations of the IMP at the trial center. The administered doses will be recorded in the blinded Drug Accountability form in the eCRF.

PK assessments will support the surveillance of compliance with IMP administration.

9.9 Subject withdrawals and missing data

In the case of subject withdrawal, no imputation of values for PK or PD measurements will be done. Analyses will be done on valid cases only, i.e., no imputation techniques such as last observation carried forward will be applied. For the primary analysis in the FAS, missing values for the primary endpoint will be imputed by a conservative rule considering any missing value as a failure.

9.10 Interim analyses

No interim analyses are currently planned.

10 ETHICAL, LEGAL, AND ADMINISTRATIVE ASPECTS

10.1 Quality assurance

The sponsor or designee will conduct a site visit to verify the qualifications of each investigator, inspect the facilities, and inform the investigator of responsibilities and the procedures for ensuring adequate and correct documentation.

The investigator is required to prepare and maintain adequate and accurate case histories designed to record all observations and other data pertinent to the trial for each trial participant. All information recorded on the eCRFs for this trial must be consistent with the subjects' source documentation (i.e. medical records).

The investigator will permit trial-related monitoring, IRB/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 monitor or a representative of the sponsor will cross-check the data entered in the eCRFs with the source data at the trial center and observe the trial procedures in order to verify adherence to the trial protocol. Any queries will be resolved by the investigator or his/her delegate.

All of the clinical data will be captured via electronic data capture (EDC) using a web-based tool.

The investigator center 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 subjects. The investigator's data will be accessible from the investigator's center throughout the trial. The eCRFs must be kept current to reflect subject status at each phase during the course of the trial. The eCRF will not capture personalized data. The investigator must make a separate confidential record of personalized details (name and initials) on the subject identification and enrollment log. All changes to data are made by the investigator or his/her delegate through the EDC system.

It is the responsibility of the principal investigator of the respective center to ensure that all subject discontinuations or changes in trial or other medications entered on the subject's eCRF are also made on the subject's medical records.

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

10.2 Electronic case report forms

Remote data capture software will be used for data collection. Following training, trial staff will be given access to the software. Access to the software is restricted to staff participating in the trial and the extent of access will depend on the participants' user role in the trial.

The subjects enrolled into the trial will be identified in the database by subject number and trial identification code. The investigator or delegate will enter subject data into the eCRF promptly. All data is to be entered in English. Data recorded in the eCRFs will be accessible to the trial staff throughout the trial.

After data entry, systematic data validation will be performed and data entry discrepancies will be presented electronically directly to the center staff. Queries for discrepant data may be generated automatically by the software upon entry and/or generated manually by the trial monitor or the trial data manager. All queries, whether generated by the system or by trial staff, will be in electronic format.

All sections of the eCRF are to be electronically approved by the investigator or a medically qualified delegate after the data has been entered and all queries have been resolved. Changes to any eCRF page subsequent to the approval require a new approval signature.

All queries and changes/corrections to the data are documented in the eCRF.

10.3 Access to source data

During the course of the trial, a trial monitor will make site visits to review protocol compliance, compare eCRFs with individual subject’s medical records, assess drug accountability, and ensure that the trial is being conducted according to pertinent regulatory requirements.

Checking of the eCRFs for completeness and clarity, and cross-checking with source documents, will be required to monitor the progress of the trial. Moreover, regulatory authorities of certain countries, IRBs/IECs, and/or the sponsor may wish to carry out such source data checks and/or on-site audit inspections. Direct access to source data will be required for these inspections and audits; they will be carried out giving due consideration to data protection and medical confidentiality.

10.4 Source documentation

All source documents from which eCRF entries are derived should be placed in the subject's medical records. If data are to be entered directly into the eCRF this must be specified in a source data agreement prior to the start of the trial.

eCRF entries will be verified with source documentation. The review of medical records will be performed in a manner to ensure that subject confidentiality is maintained.

The trial monitor will check the eCRF for accuracy and completion and perform source data verification. The trial monitor will document source data verification of all reviewed sections of the eCRF.

10.5 Data processing

The trial is run as an EDC trial, i.e. all relevant data is entered by the centers directly into the clinical database. The eCRF is designed to capture all required information in compliance with GCP standards.

10.6 Archiving trial records

According to ICH guidelines, essential documents should be retained for a minimum of 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. However, these documents should be retained for a longer period if required by the applicable legal requirements.

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

10.7 Good clinical practice

The procedures set out in this trial protocol are designed to ensure that the sponsor and investigator abide by the principles of the GCP guidelines of the ICH (1), and of the Declaration of Helsinki (2008) (2). The trial also will be carried out in keeping with local legal requirements.

10.8 Informed consent

Before each subject is admitted to the trial, written informed consent will be obtained from the subject according to the regulatory and legal requirements of the participating country. This consent form must be dated and retained by the investigator as part of the trial records. The investigator will not undertake any investigation specifically required only for the clinical trial until valid consent has been obtained. The terms of the consent and when it was obtained must also be documented in the eCRF.

The investigator will explain to each subject orally and in writing (subject information sheet) the nature, significance, risks and implications of the trial before inclusion. In particular, the subjects 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.
- That his/her medical records may be examined by authorized monitors or clinical quality assurance auditors appointed by the sponsor, by appropriate IRB/IEC members, and by inspectors from regulatory authorities.

All subjects will receive a copy of the subject information sheet and a copy of their signed and dated informed consent form, both of which will be in the subject's local language.

If a protocol amendment is required, the informed consent form may need to be revised to reflect the changes to the protocol. If the consent form is revised, it must be reviewed and approved by the appropriate IEC/IRB, and signed by all subjects subsequently enrolled in the trial as well as those currently enrolled in the trial.

10.9 Protocol approval and amendment

Before the start of the trial, the trial protocol and/or other relevant documents will be approved by the IEC/IRB/competent authorities, in accordance with local legal requirements. The sponsor must ensure that all ethical and legal requirements have been met before the first subject is enrolled in the trial.

This protocol is to be followed exactly. To alter the protocol, amendments must be written, receive approval from the appropriate personnel, and receive IRB/IEC/competent authority approval prior to implementation (if appropriate).

Administrative changes (not affecting the subject benefit/risk ratio) may be made without the need for a formal amendment. All amendments will be distributed to all protocol recipients, with appropriate instructions.

10.10 Duration of the trial

The maximum duration of the trial for each subject will be up to 63 days (including up to 30 days for screening and up to 33 days until the follow-up visit).

The trial will be closed when all subjects have completed Visit 3.

10.11 Premature termination of the trial

If the investigator, the sponsor (e.g. safety committee), or the safety medical monitor becomes aware of conditions or events that suggest a possible hazard to subjects if the trial continues, the trial may be terminated after appropriate consultation between the relevant parties. The trial may also be terminated early at the sponsor's discretion in the absence of such a finding.

Conditions that may warrant termination include, but are not limited to:

- The discovery of an unexpected, significant, or unacceptable risk to the subjects enrolled in the trial
- Failure to enroll subjects at an acceptable rate.
- A decision on the part of the sponsor to suspend or discontinue development of the drug.

The trial can be terminated prematurely by the sponsor at an individual center if:

- The center cannot comply with the requirements of the protocol.
- It is not possible for the center to comply with GCP standards.

10.12 Confidentiality

All trial findings and documents will be regarded as confidential. The investigator and members of his/her research team must not disclose such information without prior written approval from the sponsor.

The anonymity of participating subjects must be maintained. Subjects will be identified on eCRFs submitted to the sponsor by their subject number. On the SAE reports and all other source documents, the subject will be identified via subject number. Documents not

to be submitted to the sponsor that identify the subject (e.g. the signed informed consent form) must be maintained in confidence by the investigator.

10.13 Other ethical and regulatory issues

If a significant safety issue is identified, either from an individual case report or review of aggregate data, then the sponsor will issue prompt notification to all parties: regulatory authorities, investigators and IRB/IECs.

A significant safety issue is one that has a significant impact on the course of the clinical trial or program (including the potential for suspension of the trial program or amendments to protocols) or warrants immediate update of informed consent.

10.14 Liability and insurance

The sponsor will take out reasonable third-party liability insurance cover in accordance with all local legal requirements. The civil liability of the investigator, the persons instructed by him and the hospital, practice or institute in which they are employed and the liability of the sponsor with respect to financial loss due to personal injury and other damage that may arise as a result of the carrying out of this trial are governed by the applicable law.

The sponsor will arrange for liability insurance if subjects should be injured due to the participation in the trial and provided that the sponsor is legally liable for that.

Excluded from the insurance cover are injuries to health and deteriorations of illnesses already in existence which would have occurred or continued to exist even if the subject had not taken part in the clinical trial.

The insurance cover is jeopardized if the subject fails to report immediately to the investigator or responsible physician any injury to health, which might have resulted from participation in the clinical trial, or if he/she undergoes any other medical treatment without their consent before the clinical trial has been completely finished in so far as the individual subject is concerned.

Any injury to health, which might have occurred as a result of participation in the clinical trial must be reported by the subject to the investigator without delay. The investigator is obliged to make such a report in any case.

10.15 Publication policy

By signing the trial protocol, the investigator agrees with the use of results of the trial for the purposes of national and international registration, publication and information for medical and pharmaceutical professionals. If necessary, the authorities will be notified of the investigator's name, address, qualifications and extent of involvement.

A clinical trial report will be prepared and reviewed by the sponsor in co-operation with the coordinating investigator. The coordinatoing investigator will be appointed by Zealand Pharma to review and sign the clinical trial report on behalf of all participating investigators. A summary of the clinical trial report (according to CPMP/ICH137/95) should be sent to the regulatory authorities and IRB/IEC according to the relevant guidelines.

According to the Declaration of Helsinki (2) investigators and sponsors 'have ethical obligations with regard to the publication and dissemination of the results of research'.

The trial design and results may be published as one or more original research manuscripts/abstracts and presented at a scientific meeting. 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 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. Authorship on any publication(s) resulting from this clinical trial will be assigned according to the recommendations of the International Committee of Medicinal Journal Editors (26).

Participating subjects will not be identified by name in any published reports about the clinical trial.

The sponsor is responsible for trial registration at ClinicalTrials.gov (www.clinicaltrials.gov) according to FDA requirements, as well as the European Medicines Agency's Clinical Trials Database (EudraCT).

11 REFERENCE LIST

1. ICH Harmonised Tripartite Guideline. International Conference on Harmonisation. ICH Harmonised Tripartite Guideline. Integrated addendum to ICH E6(R1): Guideline for Good Clinical Practice E6 (R2), Step 4. 09-Nov-2016. Available from: URL: http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E6/E6_R2__Step_4.pdf
2. World Medical Association. Declaration of Helsinki. Ethical Principles for Medical Research Involving Human Subjects. 52nd WMA General Assembly, Edinburgh, Scotland, October 2000. Last amended with Note of Clarification on Paragraph 29 by the WMA General Assembly, Washington 2002, and Note of Clarification on Paragraph 30 by the WMA General assembly, Tokyo 2004
3. 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 May;36:1384-95
4. Cryer PE. The barrier of hypoglycemia in diabetes. Diabetes 2008;57:3169-76
5. American Diabetes Association Position Statement. Standards of medical care in diabetes - 2017. Diabetes Care 2017;40(Supplement 1):S1-S135
6. Davies MJ, Gagliardino JJ, Gray LJ, Khunti K, Mohan V, Hughes R. Real-world factors affecting adherence to insulin therapy in patients with Type 1 or Type 2 diabetes mellitus: a systematic review. Diabetic Medicine 2013;30:512-24
7. International Hypoglycaemia Study Group. Glucose concentrations of less than 3.0 mmol/L (54 mg/dL) should be reported in clinical trials: a joint position statement of the American Diabetes Association and the European Association for the Study of Diabetes. Diabetes Care 2017;40(1):155-57
8. Christensen M, Bagger JI, Vilsbøll T, Knop FK. The alpha-cell as target for type 2 diabetes therapy. Rev Diabet Stud 2011;8:369-81
9. Lund A, Bagger JI, Christensen M, Knop FK, Vilsbøll T. Glucagon and type 2 diabetes: the return of the alpha cell. Curr Diab Rep 2014;14:555
10. Habegger KM, Heppner KM, Geary N, Bartness TJ, DiMarchi R, Tschöp MH. The metabolic actions of glucagon revisited. Nat Rev Endocrinol 2010;6:689-97
11. Onoue S, Ohshima K, Debari K, Koh K, Shioda S, Iwasa S, et al. Mishandling of the therapeutic peptide glucagon generates cytotoxic amyloidogenic fibrils. Pharm Res 2004;21:1274-83

12. Fosgerau K, Hoffmann T. Peptide therapeutics: current status and future directions. Drug Discovery Today 2015;20:122-8
13. Novo Nordisk Limited. SPC for GlucaGen Hypokit 1 mg. 2015. http://www.medicines.org.uk/emc/medicine/4258/SPC/GlucaGen+Hypokit+1+mg - Accessed 13 Mar 2017 .
14. Zealand Pharma A/S. Dasiglucagon Investigator's Brochure, Edition 3, 12 Dec 2016.
15. The Task Force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). 2013 ESH/ESC guidelines for the management of arterial hypertension. European Heart Journal 2013;34:2159-219
16. Levey AS, Coresh J, Greene T, Stevens LA, Zhang Y, Hendriksen BA, et al. Using standardized serum creatinine values in the modification of diet in renal disease study equation for estimating glomerular filtration rate. Annals of Internal Medicine 2006;145:247-54
17. Food and Drug Administration (FDA). Guidance for Industry on Bioanalytical Method Validation. Food and Drug Administration (FDA) 2001
18. Viswanathan CT, Bansal S, Booth B, DeStefano AJ, Rose MJ, Sailstad J, et al. Quantitative bioanalytical methods validation and implementation: best practices for chromatographic and ligand binding assays. Pharm Res 2007 Oct;24:1962-73
19. Shankar G, Devanarayan V, Amaravadi L, Barrett YC, Bowsher 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;48:1267-81
20. Zealand Pharma report SS. Commissioning and validation of a method for the validation of anti-ZP4207 antibodies in human serum. 2014
21. Zealand Pharma report SS. Commissioning and validation of a method for the validation of anti-glucagon antibodies in human serum. 2014
22. Bioagilytix Labs. Validation of an assay to detect neutralizing antibodies to ZP4207 by measuring cyclic adenosine monophosphate (cAMP) in a glucagon receptor transfected cell line, BAL-14-206-004-REP. 2016. Report No.: Zealand Pharma, study 15-039
23. 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. 2016. Report No.: Zealand Pharma, Study 15-040

24. Amiel SA, Tamborlane WV, Simonson DC, et al. Defective glucose counterregulation after strict glycemic control of insulin-dependent diabetes mellitus. N Eng J Med 1987; 316: 1376-83.

25. Rickels MR, Ruedy KJ, Foster NC, et al. Intranasal glucagon for treatment of insulin-induced hypoglycemia in adults with type 1 diabetes: A randomized crossover noninferiority study. Diabetes Care 2016; 39: 264-70.

26. ICMJE. Recommendations for the conduct, reporting, editing, and publication of scholarly work in medical journals. Updated December 2016

12 APPENDICES

12.1 List of trial personnel

Sponsor **Clinical Trial Manager**	[redacted] Zealand Pharma Smedeland 36 2600 Glostrup, Denmark Phone: [redacted]
Medical Officer	[redacted] Zealand Pharma Smedeland 36 2600 Glostrup, Denmark Phone: [redacted]
Contract Research Organization	Chiltern International (Ltd) 171 Bath Road Slough Berkshire SL1 4AA United Kingdom Phone: +44 (0) 175351 2000
Project Manager	[redacted] Chiltern International (Ltd) 171 Bath Road Slough Berkshire SL1 4AA United Kingdom Phone: [redacted]
Senior Medical Officer and Safety medical monitor	[redacted] Chiltern International kft Canada Square Office House Ganz u

	12-14, 4 emelet 1027 Budapest Hungary Phone: [redacted]
Pharmacovigilance unit Responsible for Serious Adverse Event (SAE) Management and 24-hour SAE reporting	[redacted] PharmaLex Agern Allé 24 2970 Hørsholm, Denmark Phone: [redacted] (8 a.m. to 4 p.m.) Phone: [redacted] (outside 8 a.m. to 4 p.m.) Fax: [redacted] email: PV-nordic@pharmalex.com
Central laboratory	MLM Laboratory Dohrweg 63 41066 Mönchengladbach Germany
Special laboratory (GlucaGen PK, insulin PK)	MLM Laboratory Dohrweg 63 41066 Mönchengladbach Germany
Special laboratory (dasiglucagon PK, dasiglucagon ADA, GlucaGen ADA)	York Bioanalytical Solutions (YBS) Cedar House Northminster Business Park Northfield Lane York, YO26 6QR, United Kingdom
Special laboratory (neutralizing antibodies)	BioAgilytix 2300 Englert Drive Durham, NC, 27713, USA

A list of all investigators, IECs and IRBs will be provided in a separate document and in the clinical trial report.

CLINICAL TRIAL PROTOCOL

A phase 3, randomized, double-blind, parallel trial to confirm the clinical efficacy and safety of dasiglucagon in the rescue treatment of hypoglycemia in subjects with type 1 diabetes mellitus (T1DM) compared to placebo and with reference to GlucaGen®

Sponsor:	Zealand Pharma A/S
Sponsor Protocol No.:	ZP4207-16137
EudraCT No.:	2017-002449-31
IND No:	127866
Trial Drug Name:	Dasiglucagon* injection
Date of Protocol:	06 Oct 2017

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

*Dasiglucagon is the proposed international nonproprietary name for ZP4207.

Declaration of sponsor or responsible medical officer

Title: A phase 3, randomized, double-blind, parallel trial to confirm the clinical efficacy and safety of dasiglucagon in the rescue treatment of hypoglycemia in subjects with type 1 diabetes mellitus (T1DM) compared to placebo and with reference to GlucaGen®

This trial protocol 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 Declaration of Helsinki and the guidelines on Good Clinical Practice (GCP) (1).

[redacted] [redacted]

Date

Title Clinical Trial Manager
Institution Zealand Pharma A/S
Smedeland 36
2600 Glostrup, Denmark
Phone: [redacted]

[redacted] [redacted]

Date

Title Medical Officer
Institution Zealand Pharma A/S
Smedeland 36
2600 Glostrup, Denmark
Phone: [redacted]

Declaration of the coordinating investigator

Title: A phase 3, randomized, double-blind, parallel trial to confirm the clinical efficacy and safety of dasiglucagon in the rescue treatment of hypoglycemia in subjects with type 1 diabetes mellitus (T1DM) compared to placebo and with reference to GlucaGen®

I have read this protocol. I approve this document and I agree that it contains all necessary details for carrying out the trial as described. I will conduct this trial in accordance with the design and specific provision of this protocol and will make a reasonable effort to complete the trial within the time designated. I will provide copies of this protocol and access to all information furnished by Zealand Pharma A/S to trial personnel under my supervision. I will discuss this material with them to ensure they are fully informed about the trial product and trial procedures. I will let them know that this information is confidential and proprietary to Zealand Pharma A/S and that it may not be further disclosed to third parties. I understand that the trial may be terminated or enrollment suspended at any time by Zealand Pharma A/S, with or without cause, or by me if it becomes necessary to protect the best interests of the trial subjects.

I agree that the trial will be carried out in accordance with GCP (1), with the Declaration of Helsinki (with amendments) (2) and with the laws and regulations of the countries in which the trial takes place.

_____ _____

Name Date
Title
Institution
Phone: +
Fax: +

Declaration of the investigator

Title: A phase 3, randomized, double-blind, parallel trial to confirm the clinical efficacy and safety of dasiglucagon in the rescue treatment of hypoglycemia in subjects with type 1 diabetes mellitus (T1DM) compared to placebo and with reference to GlucaGen®

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 this trial protocol, Investigator's Brochure, Case Report Forms, 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 subjects.

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

_____ _____

Signature Date

_____

Name (block letters)

_____

Title (block letters)

_____

Institution (block letters)

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List of abbreviations and definitions of terms

ADA	Anti-drug antibody
AE	Adverse event
AESI	Adverse event of special interest
ALT	Alanine aminotransferase
ANCOVA	Analysis of covariance
AST	Aspartate aminotransferase
AUC	Area under the concentration-time curve
$AUC_{0\text{-inf}}$	AUC from time zero to infinity
AUE	Area under the effect curve
CFB	Changes from baseline
CI	Confidence interval
C_{max}	Maximum plasma concentration
CPH	Cox proportional hazards
CRO	Contract research organization
ECG	Electrocardiogram
eCRF	Electronic case report form
EDC	Electronic data capture
EU	European Union
EudraCT	European Medicines Agency's Clinical Trials Database
FAS	Full analysis set
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HbA_{1c}	Glycated hemoglobin
ICH	International Conference on Harmonization
ID card	Identification card
IEC	Independent Ethics Committee
IM	Intramuscular(ly)
IMP	Investigational medicinal product
IRB	Institutional Review Board
IV	Intravenous(ly)

IWRS	Interactive Web Response System
MedDRA	Medical Dictionary for Regulatory Activities
NPH	Neutral protamine Hagedorn
PD	Pharmacodynamics
PK	Pharmacokinetics
PP	Per protocol
SAE	Serious adverse event
SAP	Statistical analysis plan
SAS	Safety analysis set
SC	Subcutaneous(ly)
SUSAR	Serious unexpected suspected adverse reaction
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
TEAE	Treatment-emergent adverse event
t_{max}	Time to the maximum plasma concentration
ULN	Upper limit of the normal range
US	United States of America

1 SYNOPSIS

Name of sponsor: Zealand Pharma A/S	**Trial ID:** ZP4207-16137
Title of the trial: A phase 3, randomized, double-blind, parallel trial to confirm the clinical efficacy and safety of dasiglucagon in the rescue treatment of hypoglycemia in subjects with type 1 diabetes mellitus (T1DM) compared to placebo and with reference to GlucaGen®	
Trial design: The trial is a global, multicenter, randomized, parallel-group, double-blind, clinical trial confirming the efficacy and safety of dasiglucagon for insulin-induced hypoglycemia in subjects with type 1 diabetes mellitus. The subjects will be randomized 2:1:1 to receive a single fixed subcutaneous 0.6 mg dose of dasiglucagon (hereinafter dasiglucagon), placebo for dasiglucagon (hereinafter referred to as placebo), or a 1 mg dose of GlucaGen® (hereafter referred to as GlucaGen) and followed for at least 28 days after treatment.	
Clinical phase of development: Phase 3	
Trial centers: This trial will be conducted at 4 to 6 sites in the United States of America, Canada, and Europe.	
Planned trial start (first subject first visit): Q4/2017	**Planned trial end (last subject last visit):** Q3/2018
Trial population: Male and female adult subjects with type 1 diabetes mellitus treated with insulin for at least one year	
Key objectives: **Primary objective:** • To demonstrate superiority of dasiglucagon compared to placebo following a single subcutaneous 0.6 mg dose administered to subjects with type 1 diabetes mellitus with insulin-induced hypoglycemia. **Secondary objective:** • To compare the glycemic response observed after dasiglucagon with that of GlucaGen.	
Key endpoints: **Primary endpoint:** • Time to plasma glucose recovery. Plasma glucose recovery is defined as first increase in plasma glucose of ≥20 mg/dL (1.1 mmol/L) from baseline during the hypoglycemic clamp procedure without administration of rescue intravenous (IV) glucose. **Key secondary endpoints:** • Plasma glucose recovery within 30 minutes, within 20 minutes, within 15 minutes, and within 10 minutes after study drug injection without administration of rescue IV glucose. • Plasma glucose changes from baseline within 30 minutes, within 20 minutes, within 15 minutes, and within 10 minutes after study drug injection or at the time of rescue. **Clinical efficacy (Pharmacodynamic) endpoints:** • Time to first plasma glucose concentration of ≥70 mg/dL (3.9 mmol/L) without administration of rescue IV glucose.	

- Plasma glucose response as area under the curve above baseline from time zero to 30 minutes, $AUC_{0\text{-}30min}$.

Exposure (Pharmacokinetic) endpoints:

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

Safety endpoints:

- Adverse events, clinical laboratory assessments (biochemistry, hematology, coagulation, urinalysis), vital signs, physical examination, electrocardiogram, and local tolerability.
- Administration of rescue infusion of IV glucose during the hypoglycemic clamp procedure.
- Time to first rescue infusion of IV glucose during the hypoglycemic clamp procedure.

Immunogenicity endpoint:

- Occurrence of anti-drug antibodies

Exploratory endpoint:

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

Key inclusion criteria:

- Male and female subjects with type 1 diabetes mellitus treated with insulin for at least one year, diagnostic criteria as defined by the American Diabetes Association.
- Stable insulin treatment 30 days prior to screening, defined as no more than a 10-unit daily variation in total daily insulin dose.
- Hemoglobin A_{1c} <10%.
- Aged between 18 and 75 years, both inclusive.

Key exclusion criteria:

- Previously treated with dasiglucagon.
- Known or suspected allergy to trial product(s) or related products.
- History of anaphylaxis or symptoms of severe systemic allergy (such as angioedema).
- Previous participation (randomization) in this trial.

Sample size:
Approximately 156 subjects are intended to complete the trial, with 78 subjects randomized to the dasiglucagon group and 39 subjects randomized to each of the placebo and GlucaGen groups.

Investigational medicinal product:
Test product: dasiglucagon liquid formulation in pre-filled syringes.
Comparator products: Placebo and GlucaGen® lyophilized powder.

Duration of treatment:
Subjects will be randomized 2:1:1 to receive a single fixed subcutaneous dose of 0.6 mg dasiglucagon, placebo, or 1 mg GlucaGen, and followed for at least 28 days after receiving treatment.

Assessments:
The plasma glucose profile for evaluation of the primary and secondary clinical efficacy (pharmacodynamic) endpoints will be assessed based on plasma concentration data from samples collected at the dosing visit.
The exposure to trial medication (dasiglucagon, placebo, or GlucaGen) for evaluation of pharmacokinetics will also be assessed based on plasma concentration data.

Statistical methods:
Due to requirements in the size of the safety database, the sample size is set to 78 subjects treated with dasiglucagon 0.6 mg. With a 2:1:1 randomization ratio for the three treatments, dasiglucagon 0.6 mg, placebo, and GlucaGen 1 mg, 78 subjects injected with dasiglucagon and 39 subjects with placebo will yield a power of 90% at a 5% two-sided significance level to detect a treatment group difference in recovery incidence within 20 minutes, assuming as low as 80% and as high as 50% recovery incidence for the dasiglucagon and placebo groups, respectively.

For the confirmatory analyses, the primary and key secondary endpoints for the dasiglucagon 0.6 mg and placebo treated subjects will be compared. A hierarchical procedure will be applied for the control of multiplicity in statistical inferences. The primary and secondary endpoints will be evaluated on the Full Analysis Set sample. The statistical inference comparisons with placebo will be conducted as superiority tests. The comparisons of dasiglucagon versus GlucaGen will be summarized descriptively.

The primary endpoint will be summarized using Kaplan-Meier (KM) estimates stratified by treatment group and injection site. The treatment group difference between dasiglucagon and placebo will be evaluated inferentially using a pairwise two-sided log-rank test.

In the primary analysis, those subjects who require rescue IV glucose will be censored at the time of plasma glucose recovery. This approach is based on the assumption that rescue IV glucose administration will shorten the time to recovery. This assumption implies that censoring at the time of recovery after rescue therapy is valid as a time to recovery without rescue would be longer than the observed duration. If recovery has not occurred at 45 minutes after study drug injection, censoring will be applied irrespective of the use of rescue IV glucose.

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

The primary endpoint will additionally be analyzed using a Cox proportional hazards (CPH) time to event statistical model, if the hazards by treatment group can be assumed proportional using a graphical comparison of log-minus-log survival curves and no more than two-thirds of the recovery times are censored in each treatment group. The CPH model will be used for inferences, with treatment group and injection site modeled as categorical effects, and baseline plasma glucose modeled as a continuous covariate. The treatment group rate ratios, active versus placebo, will be estimated together with the 95% confidence intervals, and pairwise treatment group inferences (dasiglucagon vs placebo, GlucaGen vs placebo) will be evaluated using two-sided likelihood ratio tests.

The key secondary incidence variables (plasma glucose recovery within 30 minutes, within 20 minutes, within 15 minutes, within 10 minutes, in hierarchical order for inference) will be analyzed using descriptive statistics (frequency and percentage) by treatment group. Inferential treatment group comparisons will be assessed via pairwise tests of independent binomial proportions (dasiglucagon versus placebo, GlucaGen

versus placebo) using Fisher's exact test for each pairwise comparison.

The key secondary endpoints of plasma glucose changes from baseline (CFB) within 30 minutes, within 20 minutes, within 15 minutes, and within 10 minutes after study drug injection or at the time of rescue, will be analyzed with the plasma glucose CFB at rescue carried forward in those subjects who require rescue IV glucose before plasma glucose ≥20 mg/dL recovery. Each of these CFB variables will be analyzed using an Analysis of Covariance model, with treatment group modeled as a fixed effect and with the baseline plasma glucose modeled as a covariate. The dasiglucagon versus placebo treatment group difference will be evaluated inferentially as a least squares means contrast, using a two-sided t-test at the 0.05 significance level, first for the 30 minute CFB, and then sequentially for the 20 minute, 15 minute, and 10 minute variables, until the first failure to reject.

The continuous clinical efficacy variables, the exploratory variables, and the pharmacokinetics variables will each be summarized descriptively by treatment group. The clinical efficacy variables will be analyzed analogous to the plasma glucose CFB variables.

The safety analyses will include by-treatment-group descriptive summaries of vital sign measurements, laboratory measures (including immunogenicity incidence), physical examination assessments, rescue IV glucose (incidence and amount of glucose infused), and adverse events. The number and percentage of subjects reporting specific events, such as nausea and vomiting, will be presented by body system and preferred term.

Further details will be included in the Statistical Analysis Plan, to be completed before database lock and treatment unmasking.

2 INTRODUCTION

2.1 Background

Zealand Pharma A/S (Zealand Pharma) is developing dasiglucagon, a physically and chemically stable peptide analog of human glucagon, in a ready-to-use liquid formulation for the acute treatment of severe hypoglycemia in patients with insulin-treated diabetes mellitus. Like native glucagon, dasiglucagon is comprised of 29 amino acids, but with 7 substitutions which improve its physical and chemical stability in aqueous media. These amino acid substitutions make dasiglucagon suitable for a liquid formulation, while providing similar efficacy and safety as compared with recombinant glucagon in the clinical setting of acute treatment of severe hypoglycemia.

2.1.1 Hypoglycemia

Hypoglycemia in patients with diabetes is defined as episodes of an abnormally low plasma glucose concentration (3). This is a common, unpredictable, and potentially dangerous side effect of treatment of diabetes mellitus, especially with 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 (4).

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 (5).

The incidence of hypoglycemic events or even the fear of hypoglycemia influences patients' adherence to prescribed treatment regimens for diabetes mellitus (6). This leads to inadequate glycemic control, which in turn may lead to an increased risk of diabetic complications (5). Serious clinically significant hypoglycemia is now defined as plasma glucose <54 mg/dL (3.0 mmol/L), while the plasma glucose alert value is defined as <70 mg/dL (3.9 mmol/L) (7). When plasma glucose falls below these values, some kind of treatment strategy is needed.

2.1.2 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) (8,9). 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 (10). Insulin decreases blood glucose levels and cases of hypoglycemia can be reversed by glucagon. Therefore, glucagon is indicated for the treatment of severe hypoglycemia.

Besides intravenous (IV) glucose administration, an injectable form of glucagon is given as first aid in cases of severe hypoglycemia, when the patient is unconscious or for other reasons cannot take glucose orally. The approved glucagon dose for an adult is 1 mg, given by intramuscular (IM), IV, or subcutaneous (SC) injection, which quickly raises blood glucose levels. As current marketed recombinant glucagon is highly unstable when dissolved in solution, the injectable form has to be reconstituted prior to use in a 9-step procedure that requires a sterile diluent to be injected into a vial containing lyophilized powdered glucagon. When dissolved in a fluid state, glucagon can form amyloid fibrils (11), or tightly woven chains of proteins made up of the individual glucagon peptides. The reconstitution process makes the use of marketed glucagon products cumbersome (12), and a more patient-friendly formulation is needed. Currently, the Food and Drug Administration (FDA) approved instructions for commercially available glucagon allow only for immediate usage of glucagon after the powder is reconstituted in aqueous solution (13). Therefore, a glucagon analog with enhanced biophysical stability may represent a leap forward in terms of convenient therapeutic applications.

2.1.3 Dasiglucagon

Dasiglucagon (ZP4207) is a stable peptide analog of human glucagon, available in a ready-to-use liquid formulation. Dasiglucagon (hereinafter referred to as dasiglucagon) 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® (hereafter referred to as GlucaGen). Dasiglucagon exhibits improved physical and chemical stability and is available in an aqueous solution at neutral pH (14).

Three clinical trials have been completed with dasiglucagon: a first-in-human dose trial in healthy volunteers and subjects with T1DM (ZP4207-14013), a multiple-dose dose-escalation trial (ZP4207-15007) evaluating the safety, tolerability, pharmacokinetics (PK) and pharmacodynamics (PD) of dasiglucagon in healthy volunteers, and a phase 2 crossover trial to assess the PK and PD of a single dose of an optimized formulation of dasiglucagon administered SC in subjects with T1DM (ZP4207-15126) (14).

2.1.3.1 Pharmacokinetics and pharmacodynamics of dasiglucagon

The results of the phase 1 and 2 clinical trials confirmed dose-proportionality for dasiglucagon PK, 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 (t_{max}) was 35 minutes for dasiglucagon compared with 20 minutes for GlucaGen.

At all dose levels in the phase 2 trial, all subjects achieved a plasma glucose level of at least 70 mg/dL (3.9 mmol/L) as well as an increase in plasma glucose by at least 20 mg/dL (1.1 mmol/L) within 30 minutes after dosing. The PD responses to 0.6 mg of dasiglucagon and 1 mg of GlucaGen were comparable.

2.1.3.2 Safety of dasiglucagon

The safety data for dasiglucagon do not give rise to any safety concerns. No new signals were observed, 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 mg (4 with dasiglucagon and 1 with GlucaGen). This is not considered a safety concern; however, hemodynamic changes after dosing will be considered an adverse event of special interest (AESI).

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's Brochure (14).

2.2 Trial rationale

The aim of the current trial is to confirm the superiority of dasiglucagon for the treatment of insulin-induced hypoglycemia in subjects with T1DM as compared to placebo for dasiglucagon (hereinafter placebo) and to compare the clinical efficacy and safety of dasiglucagon with reference to GlucaGen. A randomized, controlled trial design was used.

See Section 4.2 for justification of the design of this trial.

2.3 Risk-benefit assessment

Non-clinical experience

The nonclinical development program did not reveal any safety findings that would prohibit administration of dasiglucagon to humans. None of the safety pharmacology studies, repeated dose toxicity studies or genotoxicity studies revealed any significant toxicity findings relevant to the therapeutic use of dasiglucagon.

Clinical experience

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 IMP 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 extent in the 3 clinical studies conducted to date with dasiglucagon. As with every novel drug substance, new and as yet unknown side effects may also 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 low immunogenic potential.

Overall, dasiglucagon is judged to be a low-risk molecule, based upon the available clinical data. Administration of dasiglucagon may be associated with a risk of allergic reactions similar to those observed for other therapeutic peptides or proteins. Subjects 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 centers.

With the exception of medical examinations, a subject 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.

The development program including 141 subjects exposed to dasiglucagon to date has demonstrated that administration of dasiglucagon is efficacious and well tolerated, with a safety profile that does not give rise to specific safety concerns. Two phase 1 and one phase 2 clinical trials have been conducted to investigate the safety, tolerability, PK and PD of dasiglucagon after single and multiple dosing to healthy volunteers and subjects with T1DM under insulin-induced hypoglycemic conditions. Dasiglucagon has proven to have relevant clinical effects in the acute severe hypoglycemia rescue setting and may be an effective and reliable emergency treatment for severe hypoglycemia. Overall, the anticipated benefits for subjects entering the ZP4207-16137 trial are considered to justify the risks.

3 TRIAL OBJECTIVES AND ENDPOINTS

3.1 Primary objective

- To demonstrate superiority of dasiglucagon compared to placebo following a single subcutaneous 0.6 mg dose administered to subjects with type 1 diabetes mellitus with insulin-induced hypoglycemia.

3.2 Secondary objectives

- To compare the glycemic response observed after dasiglucagon with that of GlucaGen.

3.3 Primary endpoint

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

3.4 Key secondary endpoints

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

3.5 Other secondary endpoints

- Clinical efficacy (PD) endpoints:
  - Time to first plasma glucose concentration ≥70 mg/dL (3.9 mmol/L) without administration of rescue IV glucose.
  - Plasma glucose response as area under the curve (AUC) above baseline from time zero to 30 minutes, $AUC_{0\text{-}30min}$.
- Exposure (PK) endpoints:
  - Area under the drug concentration curve from time zero to 90 minutes, $AUC_{0\text{-}90min}$.
  - Maximum plasma drug concentration (C_{max}).
  - Time to maximum plasma drug concentration (t_{max}).

- Safety endpoints:
  - Adverse events, clinical laboratory assessments (biochemistry, hematology, coagulation, urinalysis), vital signs, physical examination, electrocardiogram (ECG), and local tolerability.
  - Administration of rescue infusion of IV glucose during the hypoglycemic clamp procedure.
  - Time to first rescue infusion of IV glucose during the hypoglycemic clamp procedure.
- Immunogenicity endpoint:
  - Occurrence of anti-drug antibodies

3.6 Exploratory endpoint

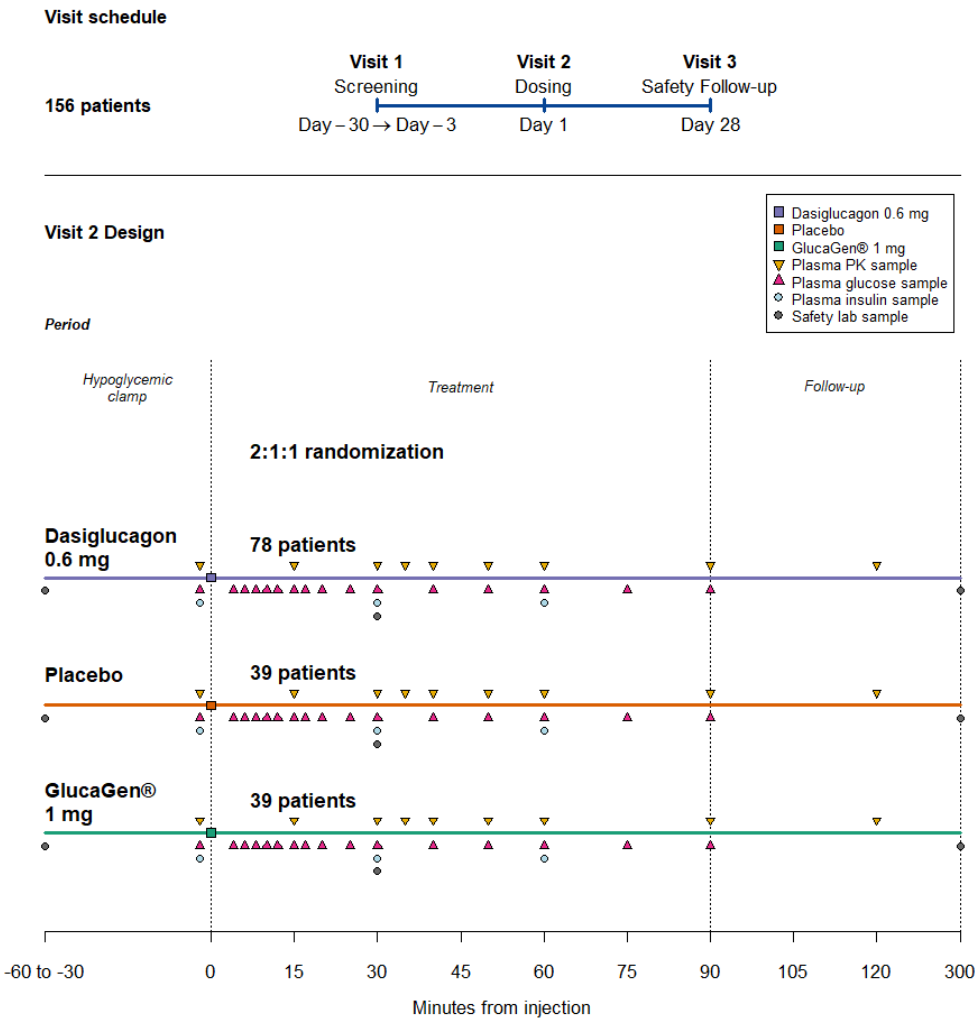
- Plasma glucose concentration ≥70 mg/dL (3.9 mmol/L) or increase of ≥20 mg/dL (1.1 mmol/L) within 30 minutes after study drug injection without administration of rescue IV glucose.
- Plasma insulin response as area under the curve above baseline from time zero to 60 minutes, $AUC_{0\text{-}60\ min}$.

4 OVERALL DESIGN AND PLAN OF THE TRIAL

4.1 Overview

This trial is a global, multicenter, randomized, parallel, double-blind, clinical trial confirming the efficacy and safety of dasiglucagon for insulin-induced hypoglycemia in subjects with T1DM. The subjects will be randomized 2:1:1 to receive a single fixed SC 0.6 mg dose of dasiglucagon, placebo, or a 1 mg dose of GlucaGen and followed for at least 28 days after receiving treatment. A total of 156 subjects with T1DM are expected to complete the treatment visit. The trial will be conducted in the European Union (EU) and North America. See Figure 1 for an overview of the trial design.

Figure 1 Trial design



4.2 Justification of the trial design

4.2.1 Justification for design and parameters

In order to avoid bias in subject selection and in the evaluation of clinical assessments, subjects will be randomly assigned 2:1:1 to either dasiglucagon, placebo, or GlucaGen and the trial will be conducted in a double-blinded manner. The randomized parallel treatment design with administration of fixed SC doses of dasiglucagon, placebo, or GlucaGen to subjects with T1DM and insulin-induced hypoglycemia allows for direct comparison of the clinical efficacy of the treatments.

The trial is double-blind to increase trial validity and to reduce bias during evaluation of the treatments. However, since the trial medications are not identical in appearance, the handling, preparation and administration of trial medication will be done by unblinded trial personnel who will not be involved in any other trial procedures or assessments. See Section 6.6 for more information about which assessments are blinded and which are not, with reasons.

A superiority trial design is used because the aim is to show that treatment with dasiglucagon is an effective treatment compared to placebo. The secondary objective was chosen to allow a comparison between treatment with dasiglucagon and an established comparator treatment for severe hypoglycemia, GlucaGen.

Administration of glucagon is intended to quickly raise blood glucose levels in subjects with T1DM with insulin-induced hypoglycemia. Therefore, in order to assess the clinical efficacy of dasiglucagon as compared to placebo and GlucaGen following a single SC dose, the primary endpoint and secondary efficacy endpoints involve the measurement of plasma glucose concentrations at different timepoints.

4.2.2 Justification for drug, route, dosage and treatment duration

Dasiglucagon and GlucaGen will be administered as 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 approved dose for treatment of severe hypoglycemia. Data from the studies conducted to date with dasiglucagon, including the phase 2 trial in subjects with T1DM, have been used to establish that 0.6 mg of dasiglucagon is an effective dose and also represents a therapeutically equivalent dose to 1 mg of GlucaGen (see also Section 6.1).

Dasiglucagon, placebo, and GlucaGen will be administered in the abdomen, buttocks, or thigh by SC injection, as this is one of the intended routes of administration for dasiglucagon, besides IM and IV.

Subjects will be followed for at least 28 days after dosing in order to perform an adequate immunogenicity evaluation of treatment.

5 TRIAL POPULATION

5.1 Rationale for trial population

Dasiglucagon is in development for the treatment of severe hypoglycemia in insulin-dependent patients with diabetes mellitus. In the present trial, subjects with T1DM are included in the evaluation of efficacy and safety of dasiglucagon under hypoglycemic conditions as this is part of the intended target population. Subjects with T1DM are selected to avoid the endogenous glucagon counter-regulatory response to insulin-induced hypoglycemia that is present in patients with T2DM. The inclusion and exclusion criteria are set to include a trial population that represents the general population of subjects with T1DM.

5.2 Planned sample size and number of trial centers

A total number of 156 subjects with T1DM are expected to complete the treatment visit (Visit 2). To qualify as completed, the subject must be dosed and have blood drawn for the PK and PD assessments described in the protocol. It is expected that up to 176 subjects will be randomized to have 156 subjects completing Visit 2. Completion of 156 subjects (78 subjects in the dasiglucagon group and 39 subjects in each of the placebo and GlucaGen groups) would be expected to provide adequate power for the primary efficacy evaluation, as described in Section 9.1.

The planned date for first subject first visit is expected to take place in Q4, 2017 and the planned date for last subject last visit is expected to take place in Q3, 2018.

This trial is planned to be conducted at 4 to 6 trial sites in the United States of America [US], Canada, and Europe.

5.3 Inclusion criteria

Subjects will be entered into this trial only if they meet 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 subject).
2. Female or male subjects with T1DM for at least 1 year, diagnostic criteria as defined by the American Diabetes Association (3).
3. Treated with insulin for T1DM for at least 1 year and with stable insulin treatment (defined as no more than a 10-unit daily variation in total daily insulin dose) 30 days prior to screening

4. Hemoglobin A_{1c} <10%.
5. Aged between 18 and 75 years, both inclusive.
6. A female subject 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 and until last follow-up visit. Additionally, if the participant is using systemic contraceptives, she must use an additional form of acceptable contraception. An acceptable method of contraception includes one of the following:
        i. Abstinence from heterosexual intercourse;
        ii. Systemic contraceptives (birth control pills, injectable/implant/ insertable hormonal birth control products, transdermal patch);
        iii. Intrauterine device (with and without hormones); or
        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).
7. A male subject must meet the following criteria: Surgically sterilized or willing to refrain from sexual intercourse from screening and until last follow-up visit or, if sexually active, uses condom and partner practices contraception during the trial from screening and until last follow-up visit.

5.4 Exclusion criteria

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

1. Previously treated with dasiglucagon (previously referred to as ZP4207).
2. Known or suspected allergy to trial product(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, are actively attempting to get pregnant, or are lactating.
6. History of hypoglycemic events associated with seizures in the last year prior to screening.
7. History of severe hypoglycemia (defined as plasma glucose <54 mg/dL [3.0 mmol/L]) in the last month prior to screening.
8. Receipt of any investigational drug within 3 months prior to screening.
9. Active malignancy within the last 5 years.
10. Congestive heart failure, New York Heart Association class II-IV.

11. Inadequately treated blood pressure, defined as systolic ≥160 mmHg or diastolic ≥90 mmHg) at screening (15).
12. Current bleeding disorder, including anti-coagulant treatment.
13. Known presence or history of pheochromocytoma (i.e. adrenal gland tumor) or insulinoma (i.e. insulin secreting pancreas tumor).
14. Use of a daily systemic beta-blocker drug, indomethacin, warfarin or anticholinergic drugs in the previous 28 days before Day 1 of this trial.
15. Aspartate aminotransferase (AST) or alanine aminotransferase (ALT) >2.5 × the upper limit of the normal range (ULN), bilirubin >1.5 × ULN, estimated glomerular filtration rate <30 mL/min/1.73 m^2 according to the Modification of Diet in Renal Disease study definition (16), or altered electrolyte values of clinical relevance for cardiac conduction, as judged by the investigator.
16. Clinically significant abnormal ECG at screening as judged by the investigator.
17. Clinically significant illness within 4 weeks before screening, as judged by the investigator.
18. Donation of blood or plasma in the past month, or in excess of 500 mL within 12 weeks prior to screening.
19. Surgery or trauma with significant blood loss within the last 2 months prior to screening.
20. 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.
21. Subjects with mental incapacity or language barriers which 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.
22. Any condition interfering with trial participation or evaluation or that could be hazardous to the subject.
23. The use of prescription or non-prescription medications known to cause QT prolongation.

5.5 Dosing day exclusion criteria

Subjects who meet one or more of the following dosing day exclusion criteria at the dosing visit will be excluded from the dosing visit, but the visit can be rescheduled 1-7 days later. The dosing visit can only be rescheduled once.

1. Atypically strenuous exercise within 4 days prior to dosing, as judged by the investigator. Exercise during the trial should follow subject's typical routine, and should not exceed a near maximum intensity for more than 20 minutes per day, or moderate intensity for more than 90 minutes per day.

2. Clinically significant illness within 4 weeks before dosing, as judged by the investigator.
3. Consumption of alcohol within 24 hours prior to dosing visit, or positive results from an alcohol breath test.
4. Not fasting from 22:00 hours the evening prior to dosing, apart from water. Small amounts of carbohydrates (up to 20 g) to prevent hypoglycemia are allowed.
5. The use of any non-prescribed systemic or topical medication, except routine vitamins and occasional use (as judged by the investigator) of acetylsalicylic acid and paracetamol within 2 weeks prior to dosing. Treatment with insulin, including analogs, is allowed.
6. Use of insulin Degludec or insulin Glargine U300 within 48 hours prior to dosing; or use of other long-acting insulins (e.g., insulin Glargine U100 or insulin Detemir) within 24 hours prior to dosing; or use of insulin Neutral protamine Hagedorn (NPH) within 16 hours prior to dosing.
7. Use of any short acting (bolus) insulin within 6 hours prior to dosing, except insulin glulisine (Apidra®).
8. Changes in medical history or concomitant medication resulting in fulfillment of clinical exclusion criteria, as judged by the investigator.
9. Plasma glucose value <50 mg/dL (2.8 mmol/L) within the last 24 hours or plasma glucose value <60 mg/dL (3.3 mmol/L) within the last 5 hours prior to initiation of the hypoglycemic procedure.

5.6 Premature treatment discontinuation and withdrawal

Participation in the trial is strictly voluntary. A subject 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 subject at any time if the investigator deems it in the subject's best interest. The reason and circumstances for withdrawal will be documented in the electronic case report form (eCRF).

5.6.1 Possible reasons for treatment visit discontinuation

A subject will be discontinued from treatment if the following applies:

- Withdrawal of consent by subject.
- If a protocol deviation occurs which, in the clinical judgement of the investigator, can invalidate the trial or endpoints or can interfere pharmacokinetically or pharmacodynamically with the trial product, the subject will be discontinued by the investigator.
- Adverse events occur which are considered unacceptable by the subject or the investigator.

If discontinuation occurs following administration of trial medication, every effort should be made to have the subject return and participate in the complete follow-up visit on Day 28 (see Table 3) to avoid missing data.

If trial participation is terminated due to an AE possibly related to any of the trial medications or trial examinations, the subject 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.

A total of 156 subjects must complete the treatment visit (Visit 2). To qualify as completed, the subject must be dosed and have blood drawn for the PK and PD assessments described in the protocol.

5.6.2 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 subjects within the agreed time frame.
- The center does not respond to trial management requests.
- Repeat protocol violations.

5.6.3 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 subjects.
- New scientific knowledge becomes known that makes the objectives of the trial no longer feasible/valid.
- Unsatisfactory enrolment of subjects.

5.7 Subject identification and randomization

Subjects who have given written informed consent and meet all inclusion and none of the exclusion criteria will be randomized in a 2:1:1 ratio to either 0.6 mg dasiglucagon (n=78), placebo (n=39), or 1 mg GlucaGen (n=39) via an Interactive Web Response System (IWRS). Randomization will continue until 156 subjects have completed Visit 2.

Subjects 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 subject is taking, the subject code can be broken by the investigator, preferably after consultation with the medical monitor. Emergency code breaks can be performed using the IWRS.

6 TRIAL DRUG

6.1 Identity

The following trial drugs will be administered:

- Dasiglucagon, liquid formulation, 1 mg/mL, 0.6 mL delivered in a prefilled syringe.
- Placebo, liquid formulation, 0.6 mL delivered in a prefilled syringe.
- Recombinant glucagon hydrochloride, 1 mg for reconstitution (GlucaGen®, Novo Nordisk) in 1 mL sterile water.

The description of the three trial drugs is provided in Table 1. Dasiglucagon is a stable peptide analog of human glucagon in a ready-to-use liquid formulation for treatment of severe hypoglycemia in insulin-dependent patients with diabetes mellitus. 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.

Table 1 Description of trial drugs

	Test product	Placebo Product	Comparator product
Name	Dasiglucagon	Placebo	GlucaGen®
Active substance	Dasiglucagon	N/A	Recombinant glucagon hydrochloride
Formulation	Liquid formulation, 0.6 mL	Liquid formulation, 0.6 mL	Powder and solvent for reconstitution as 1 mL solution for injection
Strength	1 mg/mL	N/A	1 mg
Device	Single use pre-filled syringe	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	Zealand Pharma A/S, Glostrup (Copenhagen), Denmark	Novo Nordisk A/S, Bagsværd, Denmark

	Test product	Placebo Product	Comparator product
Storage requirements	Store between 2 and 8°C	Store between 2 and 8°C	Store between 2 and 8°C

The quantities of ingredients for dasiglucagon and placebo are provided in Table 2.

Table 2 Quantities of ingredients in dasiglucagon and placebo injection

Component	Amount per mL (dasiglucagon)	Amount per mL (placebo)	Function
Dasiglucagon*	1.0 mg	N/A	[redacted]
Sodium chloride	10.23 mg	10.23 mg	
Trometamol/Tromethamine	6.06 mg	6.06 mg	
Water for injection	To make 1 mL	To make 1 mL	
Sodium hydroxide	q.s.	q.s.	
Hydrochloric acid	q.s.	q.s.	

*The quantity of drug substance to be used is calculated according to net peptide content and purity.
q.s. = quantum sufficit (quantity required).

6.2 Treatment assignment and randomization

Subjects successfully completing screening and who fulfill entry eligibility and randomization criteria will be randomized to one of three treatment groups in a ratio of 2:1:1:

- Test treatment: Dasiglucagon, liquid formulation, 1 mg/mL, 0.6 mL delivered in a prefilled syringe.
- Placebo treatment: Placebo, liquid formulation, 0.6 mL delivered in a prefilled syringe.
- Reference treatment: Recombinant glucagon hydrochloride, 1 mg for reconstitution.

Randomization will be performed using a fixed-block randomization scheme. The randomization scheme will be generated prior to the initiation of the study by an independent statistician/programmer who will not be a member of the study team; all investigators will not be aware of the block size of the randomization scheme. Randomization will be stratified by treatment group and by injection site (abdomen, buttocks, or thigh) and controlled via the IWRS.

Subjects will be randomized to study treatment using an interactive, automated system which has been validated for the intended use under the International Society of Pharmaceutical Engineers Good Automated Manufacturing Process guidelines, 21CFR Part 11 (FDA regulation for Electronic Records and Electronic Signatures) and the International Conference on Harmonization (ICH) Guidance E6 for Industry on Good Clinical Practice (GCP).

6.3 Administration

Dasiglucagon, placebo, and GlucaGen will be administered by SC injection in the abdomen, buttocks, or thigh.

An unblinded person (appropriately trained) authorized to prepare the dose and administer the treatment in accordance with the randomization will prepare the treatment required for each subject on each dosing day. The dose will be administered by the unblinded, trained and qualified person. The content of the syringe has to be checked for clarity and absence of bubbles..

Syringes will be discarded after dose administration. Used GlucaGen vials will be stored in a lockable box (separated from unused vials) at ambient temperature.

6.4 Packaging and labelling

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

The study drug label will describe the storage conditions for study drug. The labels will supply no information about the subjects. Each treatment kit (pre-filled syringe/vial for reconstitution) will have a unique Dispensing Unit Number for drug allocation, drug accountability, and traceability purposes.

Labelling will be performed according to Annex 13 of the Good Manufacturing Practice guidelines of the European Commission, ICH GCP guidelines, and local law.

6.5 Storage of study drugs

The investigator must ensure the availability of proper storage conditions. All study drug supplies provided for this study will be stored in a secure area with restricted access at the study site.

The temperature should be monitored by recording the actual, minimum, and maximum temperatures using a calibrated thermometer or thermocouple, or by continuous recording using a qualified temperature monitoring system. The temperature should be evaluated and documented at least on working days on a temperature log. This log must be included in the Investigator Site File upon study termination.

Dasiglucagon and placebo must be stored in a refrigerator (at a temperature of 2–8°C), and should be handled in accordance with guidelines from the sponsor. GlucaGen must also be stored in a refrigerator (at a temperature of 2–8°C), and should be handled in accordance with the Summary of Product Characteristics (13).

The unblinded person responsible for study drug handling must contact the unblinded monitor in case of temperature deviations outside the acceptable range.

Please see the Pharmacy Manual for additional information on handling study drug.

6.6 Blinding and breaking the blind

This is a double-blind trial. As the trial products are not identical in appearance, dasiglucagon and placebo being available as a liquid formulation and GlucaGen as a powder for reconstitution, unblinded trial personnel will be responsible for handling, preparing (according to the prescription from the IWRS), and administering the trial medication, as well as for keeping the records strictly confidential and accessible only to unblinded staff until after the database has been locked. To maintain double-blind conditions, all trial assessments at the trial center will be done by blinded trial personnel not involved in the administration of trial medications. However, exposure assessments and anti-drug antibody (ADA) assessments will be performed by unblinded personnel at the special laboratories, to ensure that dasiglucagon, placebo, or GlucaGen administration is matched with the applicable bioanalytical assay.

Treatment assignment will be kept strictly confidential and accessible only to authorized persons until after the time of unblinding. Codes with treatment assignment will, however, be readily available in the IWRS to the blinded personnel in case of an emergency.

The code for a particular subject may be broken in a medical emergency if knowing the identity of the treatment allocation would influence the treatment of the subject. The emergency code break can be performed using the IWRS. Whenever a code is broken, the person breaking the code must record the time, date and reason as well as his/her initials in the source documents. The breaking of blinded codes in case of medical emergency for one subject should not unblind the trial personnel to the treatment information of other

subjects. The person performing the unblinding should inform as few people as possible about the result of the unblinding. All persons unblinded for a specific subject should be documented.

If the trial center needs to break the code, the medical monitor should, if at all possible, be contacted prior to breaking the code and the monitor must be notified within 24 hours after the code has been broken.

The pharmacovigilance unit (safety contract research organization [CRO]; see the list of trial personnel in Section 12.1) will be able to break the code in case of a serious unexpected suspected adverse reaction (SUSAR).

The central and specialty laboratories will be provided with a copy of the randomization list.

6.7 Drug accountability

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 for destruction. This does not apply to the used syringes as they will be discarded after dose administration. Destruction must not take place until approved by the Sponsor.

6.8 Treatment compliance

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

PK assessments will support the surveillance of compliance with IMP administration.

6.9 Prior and concomitant medications

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

Subjects using any new concomitant medication resulting in fulfillment of a dosing day exclusion criterion (see Section 5.5) will be excluded from the dosing visit, but can be

rescheduled to one of the following days (1–7 days later). The dosing visit can only be rescheduled once.

6.9.1 Prohibited medications

The use of anti-coagulant treatments and medications (prescription and non-prescription) that are known to cause QT prolongation are prohibited during the course of the trial.

Within 28 days prior to dosing, the use of daily systemic beta-blockers, indomethacin, warfarin, and anticholinergic drugs is prohibited.

Within 2 weeks prior to dosing, the use of any non-prescribed systemic or topical medication (with the exception of vitamins and the occasional use of acetylsalicylic acid and paracetamol) is prohibited.

Within 48 hours prior to dosing, the use of insulin Degludec or insulin Glargine U300 are prohibited.

Within 24 hours prior to dosing, the use of long-acting insulin (e.g., insulin Glargine U100 or insulin Detemir) is prohibited.

Within 16 hours prior to dosing, the use of insulin NPH is prohibited.

Within 6 hours prior to dosing, the use of any short acting (bolus) insulin, except insulin glulisine (Apidra®) is prohibited.

During the insulin-induced hypoglycemic procedure, continuous SC insulin infusion must be stopped.

7 PARAMETERS AND METHODS OF ASSESSMENT

Overall, approximately 180 mL of blood will be drawn from each subject for PK, PD, ADA, and safety laboratory assessments.

7.1 Efficacy parameters

A description of the sample handling and sample processing at the site will be included in the laboratory manuals. Validation documentation for the assays must be available prior to sample analyses. A bioanalytical report for each analysis of trial PD and PK samples will be prepared.

7.1.1 Pharmacodynamic measurements

The plasma glucose profile for evaluation of the primary and secondary clinical efficacy endpoints will be assessed based on plasma concentration data ($AUC_{0\text{-}30min}$) from samples collected at the dosing visit (Visit 2). The samples will be sent to the clinical laboratory and analyzed using a sensitive and validated assay for glucose measurements.

Samples will be collected pre-dose, and at 4, 6, 8, 10, 12, 15, 17, 20, 25, 30, 40, 50, 60, 75, and 90 minutes after dosing (see the schedule of procedures in Table 3). The actual time of blood sampling for evaluation of plasma glucose should not deviate from the nominal time by more than ±30 seconds until the 20 minute collection time point and by more than ±1 minute for the subsequent collection time points. Pre-dose is defined as within 2 minutes prior to dosing.

7.1.2 Pharmacokinetic measurements

The exposure to trial medication (dasiglucagon or GlucaGen) for evaluation of PK will be assessed based on plasma concentration data ($AUC_{0\text{-}90\ min}$, C_{max}, t_{max}) from samples collected at the dosing visit (Visit 2).

Samples (including back-up samples) will be collected pre-dose, and at 15, 30, 35, 40, 50, 60, 90, and 120 minutes after dosing (see the schedule of procedures in Table 3). The actual time of blood sampling for exposure to trial medication should not deviate from the nominal time by more than ±1 minute. Pre-dose is defined as within 2 minutes prior to dosing.

7.2 Safety parameters

7.2.1 Adverse events

The investigator is responsible for the detection and documentation of events meeting the criteria and definition of an AE or serious adverse event (SAE), as provided in this protocol. During the trial, the investigator or center staff will be responsible for reporting AEs and SAEs, as detailed in this section of the protocol.

Based on the investigator's clinical judgment it will be determined whether an AE is related to treatment and of sufficient severity to require the subject's removal from treatment or from the trial. A subject may also voluntarily withdraw from treatment due to what he or she perceives as an intolerable AE. If either of these situations arises, the subject should be under medical supervision until symptoms cease or the condition becomes stable.

7.2.1.1 Definitions

Adverse event

An AE is any untoward medical occurrence in a trial subject given an IMP which does not necessarily have a causal relationship with the treatment. An AE can therefore be any unfavorable and/or 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.

In this trial, only treatment-emergent adverse events (TEAEs) will be collected and reported. TEAEs are events that occur from the first trial-related activity after the subject has signed the informed consent form until the end of the post-treatment follow-up period.

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 7.2.2).
- Injection site reactions (see Section 7.2.6).

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

- Pre-planned procedure, unless the condition for which the procedure was planned has worsened from the first trial related activity after the subject 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

An 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 events may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above.

Adverse drug reaction

All noxious and unintended responses to an investigational product related to any dose should be considered adverse drug reactions. The phrase responses to an investigational product means that a causal relationship between an investigational product and an adverse event is at least a reasonable possibility, i.e. the relationship cannot be ruled out.

Suspected unexpected serious adverse reactions (SUSARs)

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. dasiglucagon Investigator's Brochure or package leaflet/Summary of Product Characteristics for GlucaGen).

Adverse event of special interest

An AESI 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 AESIs:

- Post-dose clinical signs, or measured vital signs, indicating a clinically significant drop in blood pressure including signs of orthostatic hypotension, vasovagal responses or bradycardia.
- Post-dose 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 AE 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 AE 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 subject.

Severe: A type of AE 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 an SAE may not necessarily 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 subject 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 subject signed the informed consent.
Recovering/resolving:	The condition is improving and the subject is expected to recover from the event. This term is only applicable if the subject has completed the trial.
Recovered/resolved with sequelae:	The subject 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 subject has not improved and the symptoms are unchanged.
Fatal:	This term is only applicable if the subject died from a condition related to the reported AE. Outcomes of other reported AEs in a subject 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 subject is lost to follow-up.

7.2.1.2 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 subject has signed the informed consent until the end of the post-treatment follow-up period. At each contact with the center (visit or telephone, excluding visits where the subject is not seeing the investigator or his/her staff [e.g. visits to the laboratory]) the subject must be asked about AEs. All AEs, either observed by the investigator or reported by the subject, 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.

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 AESIs, the AESI 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 during IMP administration 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 AESI, the investigator must tick the AESI box on the AE form and complete the AESI form. The AESI 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 all AESIs to the sponsor's responsible pharmacovigilance unit (Safety CRO; see the list of trial personnel in Section 12.1) immediately (within 24 hours) after obtaining knowledge about the event.

The investigator must report initial information electronically (e.g. in PDF format) on all SAEs to the sponsor's responsible pharmacovigilance unit (Safety CRO; see the list of trial personnel in Section 12.1) immediately (within 24 hours) after obtaining knowledge about the event. 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 (see Section 7.2.1.1) that occur in this trial to the Competent Authorities and Institutional Review Boards (IRBs)/Independent Ethics Committees (IECs) in accordance with the local requirements in force and ICH guideline for GCP. The trial monitor must be informed accordingly.

7.2.1.3 Follow-up of adverse events

All AEs that are ongoing at the end of the subject's participation in the trial will be followed-up until the event is resolved or reaches a satisfactory outcome as deemed by the investigator or until the last visit of the last subject enrolled in the trial, whichever occurs first.

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 an SAE.

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

The sponsor and/or CROs acting on behalf of the sponsor can upgrade a non-serious AE to an SAE. In these situations the investigator will be informed and asked to fill out an SAE form and forward to the Safety CRO immediately (within 24 hours).

7.2.1.4 Clinical laboratory abnormalities and other abnormal assessments as adverse events or serious adverse events

Abnormal laboratory findings (e.g. biochemistry, hematology, urinalysis) or other abnormal assessments (e.g. vital signs) that are judged by the investigator as **clinically significant** will be recorded as AEs or SAEs if they meet the definition of an AE or SAE. Clinically significant abnormal laboratory findings or other abnormal assessments that are detected during the trial or are present at baseline and significantly worsen following the start of the trial will be reported as AEs or SAEs. However, clinically significant abnormal laboratory findings or other abnormal assessments that are associated with the disease being studied, unless judged by the investigator as more severe than expected for the subject's condition, or that are present or detected at the start of the trial and do not worsen, will not be reported as AEs or SAEs.

The investigator will exercise his or her medical and scientific judgment in deciding whether an abnormal laboratory finding or other abnormal assessment is clinically significant.

7.2.2 Hypoglycemia

Hypoglycemia will be regarded as an AE and recorded and documented on an AE form (and SAE form, if applicable).

Hypoglycemia is defined as a decline in plasma glucose to below 70 mg/dL (3.9 mmol/L). However, in the time period from initiation of the hypoglycemic clamp procedure (see Section 8.2.3.1) until 45 minutes after dosing, hypoglycemia is defined as a decline in plasma glucose to below 45 mg/dL (2.5 mmol/L).

During the dosing visit, prior to administration of the IMP, a plasma glucose value of <45 mg/dL (2.5 mmol/L) will be treated by an IV glucose solution. After administration of the IMP in the period from 8 minutes until 44 minutes after dosing, a plasma glucose value of <45 mg/dL (2.5 mmol/L) will be treated by an IV glucose solution, and if a plasma glucose value of ≥70 mg/dL (3.9 mmol/L) is not achieved within the 45 minutes after IMP administration, IV glucose infusion will also be initiated.

If the subject experiences symptoms of hypoglycemia, a plasma glucose measurement should be taken in order to classify the event (please refer to Section 7.4.2 for additional details).

7.2.3 Physical examination

The physical examination will be carried out at screening (Visit 1) and at the follow-up visit (Visit 3; see Table 3).

The physical examination includes examination of the following body systems: head, ears, eyes, nose, throat, including the thyroid gland; heart, lung, chest; abdomen; skin and mucosae; musculoskeletal system; nervous system; lymph node; other findings.

At the screening visit, any abnormality will be recorded and described in the eCRF, including the investigator's assessment of clinical significance ('abnormal, not clinically significant' or 'abnormal, clinically significant'). Clinically significant findings at the screening visit should be recorded as concomitant illness.

At subsequent visits, any clinically significant deterioration of a pre-existing condition as well as any new clinically significant findings will be recorded as AEs (see Section 7.2.1).

7.2.4 Vital signs

An examination of the following vital signs will be performed at screening (Visit 1), the dosing visit (Visit 2) and at the follow-up visit (Visit 3):

- Diastolic and systolic blood pressure (mmHg) will be measured after at least 5 minutes rest in a supine position. At Visit 1, blood pressure will be measured in both arms. The blood pressure from the arm with the higher systolic value is

transcribed into the eCRF and this arm should be used for all subsequent measurements of the subject's blood pressure in this trial.
- Pulse (beats per min) measured after at least 5 minutes rest in a supine position.
- Body temperature (°C).

At the dosing visit, measurements will be taken prior to the start of the insulin-induced hypoglycemic procedure (within 30 minutes) and at 30, 90, and 300 minutes after dosing (see Table 3). The actual time of the assessment should not deviate from the nominal time by more than ±10 minutes. In addition to the pre-specified assessments as listed in Table 3, blood pressure and pulse may be assessed at any time during the trial at the discretion of the investigator.

7.2.5 Electrocardiogram

A standard 12-lead ECG will be performed at the screening visit (Visit 1), the dosing visit (Visit 2; prior to the start of the insulin-induced hypoglycemic procedure [within 30 minutes]) and at 20, 35, 45, 60, and 300 minutes after dosing) and at the follow-up visit (Visit 3; see Table 3). The actual time of the assessment should not deviate from the nominal time by more than ±5 minutes.

ECG parameters (heart rate, PQ, QRS, QT, QTcB) and any abnormality will be recorded and described in the eCRF including the investigator's assessment of clinical significance ('abnormal, not clinically significant' or 'abnormal, clinically significant').

At subsequent visits, any clinically significant deterioration of a pre-existing condition as well as any new clinically significant findings will be recorded as AEs (see Section 7.2.1).

7.2.6 Local tolerability

Immediately prior to treatment administration, it should be verified that the injection site is normal. To ensure all injection site assessments are performed at the injection site, the site will be marked with a pen prior to injection. Assessment of local tolerability at the injection site will be performed at the dosing visit (Visit 2; at 30, 120 and 300 minutes after dosing), and at the follow-up visit (Visit 3) (see Table 3) and more frequently, if deemed necessary by the investigator. The actual time of the assessment should not deviate from the nominal time by more than ±10 minutes. In the eCRF, the time of assessment and any injection site reaction observed will be recorded. In case of an observation, the Local Tolerability form will be completed, as well as the (S)AE form.

The local tolerability at the injection site will be evaluated by means of the following assessments: spontaneous pain, pain on palpation, itching, redness, edema,

induration/infiltration, and other. Each of these assessments will be reported on a scale of 0 (none), 1 (mild), 2 (moderate), and 3 (severe). The evaluation and the actual time of the assessment will be recorded. The assessments will be performed by a trial physician or nurse.

Digital pictures will be taken of the injection site at the time of identification, and thereafter as often as judged necessary by the investigator. The pictures should include a subject identifier, visit number, time after dosing, and a ruler for scaling.

7.2.7 Clinical laboratory assessments

The safety parameters that will be assessed at the clinical laboratory are listed in Table 3. Routine clinical laboratory tests will be performed centrally. Samples for clinical laboratory parameters (biochemistry, hematology, coagulation) will be collected at screening (Visit 1), the dosing visit (Visit 2, prior to the start of the insulin-induced hypoglycemic procedure [within 30 minutes]) and at 30 and 300 minutes after dosing), and at the follow-up visit (Visit 3). The actual time of the assessment should not deviate from the nominal time by more than ±10 minutes. Samples for glycated hemoglobin (HbA_{1c}) will be collected at screening only (Visit 1). Samples for urinalysis will be collected at screening (Visit 1), at the dosing visit (Visit 2, prior to the start of the insulin-induced hypoglycemic procedure [within 120 minutes]) and at the follow-up visit (Visit 3). The following parameters will be assessed:

- Clinical biochemistry: sodium, potassium, calcium, glucose, urea, creatinine, total bilirubin, AST, ALT, gamma-glutamyltransferase, 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 (leukocytes).
- Coagulation: international normalized ratio, fibrinogen (at screening visit only).
- Urinalysis: pH, blood (leukocytes and erythrocytes), protein, glucose, ketones, nitrite.

Pregnancy tests will be performed at each visit for women of childbearing potential only. A serum pregnancy test will be performed at screening (Visit 1) and urine stick tests will be performed at the dosing visit (Visit 2, prior to the start of the insulin-induced hypoglycemic procedure) and the follow-up visit (Visit 3). Test sticks will be provided to the trial centers.

Alcohol breath tests and a urine drug screen will be performed at screening (Visit 1) and at the dosing visit (Visit 2, prior to the start of the insulin-induced hypoglycemic procedure). Equipment for the alcohol breath test and uring drug screen will be provided to the trial centers.

Re-assessment of laboratory parameters will be allowed only if handling issues, damaged samples, or hemolyzed samples have confounded the measurement results.

For further details of the clinical laboratory assessments, please refer to the laboratory manual.

7.2.8 Pregnancy

Female subjects 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 subjects must be instructed to notify the investigator immediately if their partner becomes pregnant or suspects pregnancy. The sponsor has a responsibility to monitor the outcome of all pregnancies reported during the clinical trial. During an information meeting at the trial center, the subject'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 immediately (within 24 hours), according to the procedure stated in Section 7.2.1.2. 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 follow 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 7.2.1.2. Any (S)AEs in the newborn must be reported on the (S)AE form.

The SAEs that must be reported include 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 until the age of 1 month.

7.2.9 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 subjects. During a subject's participation in the trial, the investigator should ensure that adequate medical care is provided to the subject for any AEs, including clinically significant laboratory values related to the trial. The investigator should inform the subject when medical care is needed for intercurrent illnesses of which the investigator becomes aware.

For further information on safety precautions for dasiglucagon and GlucaGen, please refer to the current version of the Investigator's Brochure (14) and the Summary of Product Characteristics for GlucaGen (13), respectively.

7.2.10 Safety committee

The internal Zealand Pharma Safety Committee is constituted to perform ongoing blinded 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 Safety Committee meetings, or on notification of significant findings, the Safety Committee will take appropriate measures to safeguard the subjects.

7.3 Demography, concomitant illness, medical history and concomitant medication

Demographics, body measurements, concomitant illness and medical history will be assessed only at screening (Visit 1). Concomitant medication will be assessed at screening (Visit 1), the dosing visit (Visit 2, prior to the start of the insulin-induced hypoglycemic procedure) and at the follow-up visit (Visit 3).

7.3.1 Demography and body measurements

Subject demographics and body measurements will include:

- Age
- Race, ethnicity
- Sex
- Height (meters or inch), without shoes
- Body weight (kg or lb), only wearing underwear and measured using standard scales
- Body mass index (kg/m^2) calculated based on height and body weight (body weight/height^2).

7.3.2 Concomitant illness and medical history

A concomitant illness is any illness that is present at the start of the trial (i.e. at the screening visit). Concomitant illnesses present at the start of the trial will be recorded in the eCRF at screening.

Medical history is an account of medical events that the subject has experienced in the past, including prior medications. Relevant medical conditions/illnesses in the past obtained by asking the subject or by inspecting his/her medical records will be recorded in the eCRF at screening. History of alcohol or drug abuse will also be recorded.

The information collected for concomitant illness and medical history should include diagnosis, date of onset, date of resolution or continuation.

Any change to a concomitant illness should be recorded during the trial, including end date, if applicable. A clinically significant worsening of a concomitant illness must be reported according to Section 7.2.1.

All previous and concomitant diseases will be coded with Medical Dictionary for Regulatory Activities (MedDRA), the version of which will be provided in the clinical trial report.

7.3.3 Diabetes diagnosis and current treatment

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

7.3.4 Concomitant medication

A concomitant medication is any medication, other than the trial products and current diabetes treatment (including insulin glulisine [Apidra®] for diabetes therapy wash-out), which is taken during the trial, including screening and follow-up periods.

Details of any concomitant medication must be recorded at trial entry (i.e. at screening). Any changes in concomitant medication must be recorded at each visit as they occur. The information collected for each concomitant medication includes (at a minimum) trade name or generic name, indication, start date and stop date or continuation. A change in medication due to an AE must be recorded and reported according to Section 7.2.1. If the change in medication influences the subject's eligibility to continue in the trial, the sponsor and trial monitor must be informed.

7.4 Other assessments

7.4.1 Immunogenicity

Antibodies against dasiglucagon/GlucaGen will be measured at the dosing visit (Visit 2) and at follow-up (Visit 3). At the dosing visit (Visit 2), samples will be collected prior to the start of the insulin-induced hypoglycemic procedure.

The clinical ADA assays, specific for dasiglucagon and GlucaGen, respectively, have been validated in accordance with existing guidelines and recommendations (17-21).

Confirmed positive anti-dasiglucagon antibody samples (treatment-induced or treatment-boosted) from anti-dasiglucagon antibody-positive subjects 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 neutralizing antibody analysis.

The in vitro neutralizing effect of the antibodies will be measured using an assay based on glucagon receptor transfected human embryonic kidney cells (20,22). The calculated sensitivity in previous studies was about 51.8 ng/mL. The assay was also validated for recombinant glucagon with similar results (21,23). In case of a positive result in the neutralizing antibody assay, a titer estimation will be performed. The cell-based neutralizing antibody analyses will be performed by a special laboratory, BioAgilytix, Durham, NC, USA.

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.

7.4.2 Plasma glucose measurements for safety

During insulin-induced hypoglycemia, plasma glucose levels will be monitored closely at site. After the start of insulin infusion, plasma glucose should be checked every 10 minutes while plasma glucose is above 110 md/dL, and every 5 minutes once plasma glucose is at or below 110 mg/dL and until after dosing when the subject's plasma glucose is ≥70mg/dL (3.9 mmol/L). Hereafter, plasma glucose should be checked every 30 minutes until 300 minutes (5 hours) after dosing. Plasma glucose concentrations will be measured using a US FDA-approved glucose analyzer (e.g., YSI 2300, Yellow Springs Instruments, Yellow Springs, OH).

At the discretion of the investigator, additional plasma glucose measurements can be taken at any time during the trial, for example when there is a suspicion (e.g. symptoms) of a hypoglycemic episode.

Plasma glucose measurements for safety should only be recorded in the eCRF if they are related to an AE (e.g. a hypoglycemic episode).

In case of persistent post-treatment hypoglycemia, subjects will receive rescue treatment with an IV glucose infusion (see Section 8.2.3.1 for details). Blood samples for PD and PK assessments should still be taken at the specified timepoints.

7.4.3 Plasma insulin measurements

Samples for insulin assessment will be collected at the dosing visit (Visit 2, pre-dose and at 30 and 60 minutes after dosing). The actual time of blood sampling for evaluation of plasma insulin should not deviate from the nominal time by more than ±1 minute. Pre-dose is defined as within 2 minutes prior to dosing.

8 TRIAL CONDUCT

8.1 Schedule of procedures

The schedule of procedures is provided in Table 3. Informed consent will be obtained prior to any trial-related procedures; see Section 10.8.

8.2 Procedures by visit

8.2.1 Visit 1 (screening, Day -30 to Day -3)

Visit 1 will take place between 3 and 30 days before Visit 2, Day -1 to Day 1 (dosing day).

Informed consent can be obtained prior to or at Visit 1, however it must in any case be obtained prior to any trial related procedures. During the screening visit, the following assessments will take place:

- Informed consent (obtain or check)
- Check of subject eligibility (inclusion/exclusion criteria)
- Demography
- Body measurements
- Medical history, diabetes diagnosis, current diabetes treatment
- Concomitant illnesses
- Concomitant medications
- History of alcohol/drug abuse
- Physical examination
- Vital signs
- 12-lead ECG
- AEs
- Biochemistry, hematology, coagulation, HbA_{1c}
- Serum pregnancy test (women of childbearing potential only)
- Urinalysis
- Urine drug screen
- Alcohol breath test

Table 3 Schedule of procedures

Visit number	V1	V2	V3
Trial day	**-3**	**-1 and 1**	**28**
Visit type	**Screening**	**Dosing**	**Follow-up**
Window	**-30 to -3**		**+5 days**
Subject related information/assessments			
Informed consent	x		
Inclusion/exclusion criteria	x	x[1,2]	
Demography	x		
Body measurements	x		
Medical history, diabetes diagnosis, and current diabetes treatment	x		
Concomitant illnesses	x		
Concomitant medications	x	x[1]	x
History of alcohol/drug abuse	x		
Randomization		x[1]	
Withdrawal criteria		x[1]	
Dosing day exclusion criteria		x[1]	
Insulin-induced hypoglycemia		x	
Safety assessments			
Physical examination	x		x
Vital signs	x	x[3]	x
12-lead ECG	x	x[4]	x
Local tolerability		x[5]	x
Adverse events	x	x	x
Laboratory			
Biochemistry, hematology, coagulation, HbA_{1c} (HbA_{1c} at Visit 1 only)	x	x[6]	x
Pregnancy test (women of childbearing potential only)	x[7]	x[1,8]	x[8]
Urinalysis	x	x[1]	x
Urine drug screen	x	x[1]	
Alcohol breath test	x	x[1]	
PK/Clinical efficacy			
Plasma dasiglucagon/GlucaGen		x[9]	
Plasma glucose		x[10]	
Other assessments			
Antibodies against dasiglucagon/GlucaGen		x[1]	x[11]
Plasma insulin		x[12]	
Trial material			
Administration of trial product (during hypoglycemic clamp procedure)		x	

[1]Prior to the start of the insulin-induced hypoglycemic procedure.
[2]Only check of dosing day exclusion criteria and changes between screening visit and Visit 2.
[3]Prior to the start of the insulin-induced hypoglycemic procedure (within 30 minutes), and at 30, 90 and 300 minutes after dosing. The actual time of the assessment should not deviate from the nominal time by more than ±10 minutes.

[4]Prior to the start of the insulin-induced hypoglycemic procedure (within 30 minutes), and at 20, 35, 45, 60 and 300 minutes after dosing. The actual time of the assessment should not deviate from the nominal time by more than ±5 minutes.
[5]Local tolerability assessed at 30, 120, and 300 minutes after dosing. The actual time of the assessment should not deviate from the nominal time by more than ±10 minutes.
[6]Prior to the start of the insulin-induced hypoglycemic procedure (within 30 minutes), and at 30 and 300 minutes after dosing. The actual time of the assessment should not deviate from the nominal time by more than ±10 minutes.
[7]Serum pregnancy test.
[8]Urine stick pregnancy test.
[9]Pre-dose, and at 15, 30, 35, 40, 50, 60, 90, and 120 minutes after dosing. The actual time of blood sampling should not deviate from the nominal time by more than ±1 minute. Pre-dose is defined as within 2 minutes prior to dosing.
[10]Pre-dose, and at 4, 6, 8, 10, 12, 15, 17, 20, 25, 30, 40, 50, 60, 75, 90 minutes after dosing. The actual time of blood sampling should not deviate from the nominal time by more than ±30 seconds until the 20 minute collection time point and by more than ±1 minute for the subsequent collection time points. Pre-dose is defined as within 2 minutes prior to dosing.
[11]Any subject that tests positive for ADA will be monitored until the ADA levels return to baseline levels.
[12]Pre-dose, and at 30 and 60 minutes after dosing. The actual time of blood sampling should not deviate from the nominal time by more than ±1 minute. Pre-dose is defined as within 2 minutes prior to dosing.
ADA = anti-drug antibodies; ECG = electrocardiogram; HbA_{1c} = glycated hemoglobin.

Eligible or potentially eligible subjects (laboratory results pending) will be provided with an Identification card (ID card), stating that the subject is participating in the trial and whom to contact (site address, investigator name and telephone number). The subjects should be instructed to return the ID card to the investigator at the last visit or to destroy the card after the last visit.

8.2.2 Instructions to subjects prior to the dosing visit (Visit 2)

At the screening visit, the investigator will inform the subject about the changes to his/her insulin therapy leading up to the start of the insulin-induced hypoglycemic procedure. The subject may be provided with insulin NPH in the wash-out period to cover the need of basal insulin, if deemed necessary by the investigator. The subject's current insulin therapy will be washed out as defined in Section 5.5.: 48 hours prior to dosing and during the dosing visit, treatment with insulin Degludec and insulin Glargine U300 are not allowed; 24 hours prior to dosing and during the dosing visit, treatment with other long-acting insulins (e.g., insulin Glargine U100 or insulin Detemir) is not allowed; 16 hours prior dosing and during the dosing visit treatment with insulin NPH is not allowed; 6 hours prior to dosing and during the dosing visit, treatment with any short acting (bolus) insulin, except insulin glulisine (Apidra®), is not allowed. The basal rate of insulin pumps (continuous SC insulin infusion) will be discontinued on the morning of the dosing day (if using insulin glulisine [Apidra®]) OR at least 6 hours prior to dosing (if using other insulins).

On the day prior to dosing (Day -1), the subjects will need to attend the clinical center and will be required to stay onsite overnight. On the morning of the dosing day (Day 1), patients are required to be in a fasting condition, defined as having consumed only water since 22:00 hours the night before. However, the subjects are allowed to consume small

amounts (up to 20 g) of carbohydrates to prevent hypoglycemia. The subjects must also not consume any alcohol within 24 hours prior to dosing (refer to Section 5.5 for all dosing day exclusion criteria).

8.2.3 Visit 2 (dosing visit)

Visit 2 will take place on Day -1 to Day 1.

The subjects will attend the clinical center the day prior to dosing (Day -1) and subject eligibility is rechecked (check of changes between the screening visit and Visit 2) and those subjects eligible to participate should stay onsite overnight. To target a glucose level around 90-110 mg/dL (5.0-6.1 mmol/L) the following morning, subjects may be administered insulin glulisine (Apidra®) at the investigator's discretion either by IV infusion, SC bolus or continuous SC insulin infusion. Dosing will take place the following morning (Day 1).

On Day 1 and prior to the start of the insulin-induced hypoglycemic procedure, those subjects eligible to participate will be randomized to treatment with dasiglucagon, placebo, or GlucaGen.

The following assessments will also take place:

- Document all changes in concomitant medication (prior to the start of the insulin-induced hypoglycemic procedure)
- Check of withdrawal criteria (prior to the start of the insulin-induced hypoglycemic procedure)
- Check of dosing day exclusion criteria (prior to the start of the insulin-induced hypoglycemic procedure)
- Vital signs (prior to the start [within 30 minutes] of the insulin-induced hypoglycemic procedure, and at 30, 90 and 300 minutes after dosing). 12-lead ECG (prior to the start [within 30 minutes] of the insulin-induced hypoglycemic procedure, and at 20, 35, 45, 60, and 300 minutes after dosing)
- Local tolerability (at 30, 120, and 300 minutes after dosing)
- AEs
- Biochemistry, hematology, coagulation (prior to the start of the insulin-induced hypoglycemic procedure [within 30 minutes], and at 30 and 300 minutes after dosing)
- Urine stick pregnancy test (women of childbearing potential only; prior to the start of the insulin-induced hypoglycemic procedure)
- Urinalysis (prior to the start of the insulin-induced hypoglycemic procedure [within 120 minutes])
- Urine drug screen (prior to the start of the insulin-induced hypoglycemic procedure)
- Alcohol breath test (prior to the start of the insulin-induced hypoglycemic procedure)

- Dasiglucagon/GlucaGen plasma concentrations:
  - Pre-dose, 15, 30, 35, 40, 50, 60, 90, and 120 minutes after dosing. The actual time of blood sampling for exposure should not deviate from the nominal time by more than ±1 minute. Pre-dose is defined as within 2 minutes prior to dosing.
- Plasma glucose concentrations:
  - Pre-dose, 4, 6, 8 ,10, 12, 15, 17, 20, 25, 30, 40, 50, 60, 75, and 90 minutes after dosing. The actual time for blood sampling for plasma glucose should not deviate from the nominal time by more than ±30 seconds until the 20 minute collection time point and by more than ±1 minute for the subsequent collection time points. Pre-dose is defined as within 2 minutes prior to dosing.
- Antibodies against dasiglucagon/GlucaGen (prior to the start of the insulin-induced hypoglycemic procedure).
- Plasma insulin concentrations:
  - Pre-dose, 30 and 60 minutes after dosing. The actual time of blood sampling for plasma insulin should not deviate from the nominal time by more than ±1 minute. Pre-dose is defined as within 2 minutes prior to dosing.

8.2.3.1 Hypoglycemic clamp procedure and administration of trial medication

The following procedure is based on precendent procedures for hypoglycemia induction in patients with T1DM [24, 25].

The treatment day (Visit 2, Day 1) will be conducted after an overnight fast of at least 8 hours, targeting a starting plasma glucose around 90 to 110 mg/dL (5.0-6.1 mmol/L).

Subjects who meet one or more of the dosing day exclusion criteria (Section 5.5) at the dosing visit will be excluded from the dosing visit, but the visit can be rescheduled 1-7 days later. The dosing visit can only be rescheduled once.

The subject's current insulin therapy will be washed out as defined in Section 5.5. For subjects using multiple daily injections, the date, time and the dose of the last basal insulin and the last short-acting insulin (except insulin glulisine [Apidra®]) administration prior to dosing will be captured. For subjects using an insulin pump, the time of discontinuation of the basal rate will be captured. Any use of insulin glulisine (Apidra®) in the last 5 hours prior to initiation of the hypoglycemia induction procedure will also be captured.

At approximately 08:00 hours, an infusion catheter will be inserted into each arm (forearm cephalic vein) for the manual glucose clamp procedure, with the glucose infusion in one arm and the insulin infusion in the opposite arm (if IV insulin glulisine [Apidra®] has been administered during the night the same infusion catheter can be used). A third catheter for blood sampling will be placed into a metacarpel vein for blood

sampling. This hand will be warmed (55-65ºC) to arterialize venous blood. If there are issues with blood sampling from the metacarpal vein for the purpose of glucose measurements, a new and more proximal IV access may be used at the discretion of the investigator.

Hypoglycemia will be gradually induced by a fast-acting IV insulin glulisine (Apidra®) infusion (15 U [100 U/mL] in 49 mL saline and 1 mL of subject's blood or plasma), initially at 150% of the subject's usual basal rate and can be increased or decreased over a range of 0% to 200% as judged necessary by the investigator, to achieve a controlled decline in plasma glucose, targeting a plasma glucose level of 55 mg/dL (3.1 mmol/L).

Plasma glucose concentrations will be measured using a US FDA-approved glucose analyzer (e.g., YSI 2300, Yellow Springs Instruments, Yellow Springs, OH). After the start of the insulin infusion, plasma glucose will be measured every 10 minutes while plasma glucose is above 110 mg/dL, and every 5 minutes once plasma glucose is at or below 110 mg/dL.

Once the glucose concentration declines to <60 mg/dL (3.3 mmol/L), the insulin infusion will be stopped, and 5 min later plasma glucose concentration will be measured at the glucose analyzer and blood samples for baseline assessment of plasma glucose, dasiglucagon/GlucaGen PK, and insulin PK will be collected. The samples are the baseline samples and should be collected within 2 minutes before IMP administration.

- If plasma glucose is ≥45 mg/dL and <60 mg/dL (2.5-3.3 mmol/L), study treatment (IMP) will be administered, defining time, t=0. The study treatment will be delivered in the abdomen, buttock, or thigh (according to stratification) via SC injection, with the subject lying in a lateral recumbent position.
- If plasma glucose is <45 mg/dL (2.5 mmol/L), IV glucose solution will be administered sufficient to raise plasma glucose to within the 45-60 mg/dL target range. The run-in period will be adequately extended (at least 30 min) until the above target is achieved and new baseline samples for plasma glucose, dasiglucagon/GlucaGen PK, and insulin PK will be collected. Glucose should not be infused within 10 minutes before IMP administration. If plasma glucose is not within target range after the second attempt, the subject should be rescheduled for a new treatment visit within 7 days (+ 2 days).

Administration of IMP should not occur earlier than 9:00 hours in the morning or later than 12:00 hours.

As shown in Table 4, serial blood samples for glucose will be collected at t=0, 4, 6, 8, 10, 12, 15, 17, 20, 25, 30, 40, 50, 60, 75 and 90 minutes post-dosing. Samples for assessing plasma dasiglucagon/GlucaGen concentration will be collected at t=0, 15, 30, 35, 40, 50,

60, 90, and 120 minutes. Samples for assessing plasma insulin concentration will be collected at t=0, 30, and 60 minutes.

Table 4 Post-treatment blood sampling times

Times	0	4	6	8	10	12	15	17	20	25	30	35	40	50	60	75	90	120
Plasma glucose	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y		Y	Y	Y	Y	Y	
PK Dasiglucagon/ GlucaGen	Y						Y				Y	Y	Y	Y	Y		Y	Y
PK Insulin	Y										Y				Y			

Refer to Section 7.2.7 for details of laboratory safety sampling and to Section 7.4.2 for details of blood glucose safety sampling.

When the t=90-minute blood sampling for plasma glucose has been collected the subjects are allowed to eat moderately. Drinking of water is allowed *ad libitum* during the entire procedure.

Hypoglycemia Rescue Provisions

During insulin-induced hypoglycemia, plasma glucose levels will be monitored closely at site for safety reasons as described in Section 7.4.2. Subjects should receive post-treatment rescue glucose infusion to ameliorate persistent hypoglycemia, as follows.

1. Glucose infusion should be initiated if a subject experiences severe alarming escalation of symptoms of hypoglycemia (e.g. symptoms suggesting a change in consciousness) at any time during the trial; glucose infusion should be initiated targeting a plasma glucose levels >70 mg/dL.
2. If plasma glucose is <45 mg/dL (2.5 mmol/L) between t=8 and t=44 minutes, rescue glucose infusion (1-2 mg/kg administered IV over about 5 seconds) should be initiated to maintain plasma glucose between 45 mg/dL and 55 mg/dL (2.5-3.1 mmol/L). Pause glucose infusion if plasma glucose is >55 mg/dL.
3. If plasma glucose is <70 mg/dL at t=45 minutes, rescue glucose infusion (2-3 mg/kg administered IV over about 5 seconds) should be initiated to maintain plasma glucose between 70 mg/dL and 80 mg/dL (3.9-4.4 mmol/L). Pause glucose infusion if plasma glucose is >75 mg/dL (4.2 mmol/L).

Subjects should remain in bed until completion of the test procedure 300 minutes after dosing (bathroom visits are allowed).

The IMP will be administered SC according to Section 6.3. The time of IMP administration will be recorded. At the timepoint when the insulin infusion is stopped, the total insulin dose which was required to induce hypoglycemia will be recorded.

AEs will be specifically recorded during the procedure at several timepoints.

The investigator must provide information to the subjects on how to resume their usual diabetes treatment.

The subject may be released from the clinical center if the investigator does not have any safety concerns based on the last safety plasma glucose value and the general condition of the subject. However, at the discretion of the investigator or on request of the subject, the subject may stay at the trial center for a longer period.

8.2.4 Visit 3 (follow-up visit)

Visit 3 will take place on Day 28 + 5 days. The subject does not need to be fasting.

At Visit 3, the following assessments will take place:

- Document all changes in concomitant medication
- Physical examination
- Vital signs
- 12-lead ECG
- Local tolerability
- AEs
- Biochemistry, hematology, coagulation
- Urine stick pregnancy test (women of childbearing potential only)
- Urinalysis
- Antibodies against dasiglucagon/GlucaGen.

After the follow-up visit the End of Trial form must be completed. Even if a subject is not able to attend the follow-up visit, the End of Trial form, the eCRF Accountability/Affirmation Statement form and the Drug Accountability form must be completed.

9 STATISTICAL METHODS

Before database lock and treatment unmasking, a separate statistical analysis plan (SAP) will be finalized, providing detailed methods for the analyses outlined below. Further analysis details may be added or refined in the SAP.

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

9.1 Determination of sample size

Due to requirements in the size of the safety database, the sample size is set to 78 subjects treated with dasiglucagon 0.6 mg. With a 2:1:1 randomization ratio for the three treatments, dasiglucagon 0.6 mg, placebo, and GlucaGen 1 mg, 78 subjects injected with dasiglucagon and 39 subjects with placebo will yield a power of 90% at a 5% two-sided significance level to detect a treatment group difference in recovery incidence within 20 minutes, assuming as low as 80% and as high as 50% recovery incidence for the dasiglucagon and placebo groups, respectively.

9.2 Trial subjects

9.2.1 Analysis samples

For presentation of data and reporting of the statistical analyses, the following analysis samples will be used, depending on the context:

- Safety analysis set (SAS): All randomized subjects who received at least one dose of trial medication.
- Full analysis set (FAS): All randomized subjects who received at least one dose of trial medication and contributed valid information for at least one post-dose endpoint.
- Per protocol (PP) set: All subjects of the FAS for whom no relevant protocol deviations were documented. This sample will primarily be used for sensitivity analysis.

The analysis of the primary endpoint and secondary endpoints will be based on the FAS. A secondary analysis of the primary endpoint will be based on the PP set. All safety analyses will be based upon the SAS.

The decision regarding whether a protocol deviation is relevant or not for the exclusion of subjects from the PP set will be made case-by-case in a data review meeting prior to treatment unmasking and database lock (see Section 9.2.3).

9.2.2 Disposition of subjects

Subject disposition will be tabulated including the number of screened subjects, screening failures, subjects exposed to trial product, subjects completing the trial and subjects in each analysis set.

Subjects withdrawn from the trial will be listed including the primary reason for withdrawal.

9.2.3 Protocol deviations

Before data are released for statistical analysis, a treatment-masked review of all data will take place to identify protocol deviations that may potentially affect the results. This review will be performed without revealing to which trial product the subjects were assigned. The masking of the trial products will be maintained for everyone involved in allocating subjects to the analysis sets until data are released for statistical analysis. Furthermore, spurious outliers will be evaluated. In addition, protocol deviations that may potentially affect the results will be identified and it will be evaluated if subjects and/or data should be excluded from the analysis. Protocol deviations will be classified as minor or major in a consistent way. Major deviations from the protocol may lead to the exclusion of a subject from the PP set.

Major protocol deviations may include deviations related to trial inclusion or exclusion criteria, conduct of the trial, subject management or subject assessment. Unless explicitly decided otherwise during the masked data review, the following will be considered major protocol deviations:

- Violation of one or more major inclusion/exclusion criteria
- Interruption of administration of trial product
- Significant deviation from time windows
- Incorrect treatment allocation
- Missing primary endpoint.

The violation of several major inclusion/exclusion criteria or the complete absence of efficacy data might lead to exclusion of the subject from FAS. In that case, the decision should be taken at the masked data review meeting, and the exclusion from efficacy analysis justified in the signed notes of the meeting.

Obviously erroneous data points may be excluded from the analyses or re-analyzed (in case of e.g. serum concentrations). The decision to re-analyze or exclude data points from the statistical analysis is the joint responsibility of the sponsor and the trial statistician.

The subjects or observations to be excluded and the reason for their exclusion will be documented and signed by those responsible prior to database release. The documentation will be stored together with the remaining trial documentation. The subjects and observations excluded from analysis sets, and the corresponding reasons, will be described in the clinical trial report.

9.3 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 timepoint will be included in the analysis.

Raw data listings and summary tables will be generated using the software SAS© version 9.4 or higher.

Continuous variables will be summarized using means, standard deviations, medians, coefficients of variation, and minimum and maximum values.

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

9.4 Demographics and baseline characteristics

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

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

9.5 Efficacy Analyses

For the confirmatory analyses, the primary and key secondary endpoints for the dasiglucagon 0.6 mg and placebo treated subjects will be compared. A hierarchical procedure will be applied for the control of multiplicity. The primary and secondary endpoints will be evaluated on the full analysis set. In the primary analysis, those subjects who require rescue IV glucose will be censored at the time to plasma glucose recovery. In a sensitivity analysis, the time to plasma glucose recovery will be analyzed without censoring the subjects who received rescue IV glucose.

9.5.1 Hierarchical testing procedure

For the confirmatory analyses, the following a priori defined hierarchical inferential test order will be applied for the control of the type 1 error rate across the planned multiple comparisons. The primary endpoint and key secondary endpoints will be inferentially evaluated within the FAS in the following order, where inference will proceed at the two-sided 0.05 criterion significance level until the first failure to reject the null hypothesis for a dasiglucagon versus placebo comparison:

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

The GlucaGen versus placebo comparisons will not be included in the inferential testing hierarchy, since the efficacy of GlucaGen is previously established, and these comparisons are intended to support the validity of the study for the dasiglucagon versus placebo comparisons.

Analogous supportive sensitivity analyses will be conducted in the PP set, but without inference intent.

9.5.2 Primary confirmatory endpoint

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

9.5.2.1 Primary analysis

The primary endpoint will be summarized using Kaplan-Meier (KM) estimates stratified by treatment group and injection site. The treatment group difference between dasiglucagon and placebo will be evaluated inferentially using a pairwise two-sided log-rank test.

In the primary analysis, those subjects who require rescue IV glucose will be censored at the time of plasma glucose recovery. This approach is based on the assumption that rescue IV glucose administration will shorten the time to recovery. This assumption implies that censoring at the time of recovey after rescue therapy is valid as a time to

recovery without rescue would be longer than the observed duration. If recovery has not occurred at 45 minutes after study drug injection, censoring will be applied irrespective of the use of rescue IV glucose.

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

The primary endpoint will additionally be analyzed using a Cox proportional hazards (CPH) time to event statistical model, if the hazards by treatment group can be assumed proportional using a graphical comparison of log-minus-log survival curves and no more than two-thirds of the recovery times are censored in each treatment group. The CPH model will be used for inferences, with treatment group and injection site modeled as categorical effects, and baseline plasma glucose modeled as a continuous covariate. The treatment group rate ratios, active versus placebo, will be estimated together with the 95% confidence intervals, and pairwise treatment group inferences (dasiglucagon vs placebo, GlucaGen vs placebo) will be evaluated using two-sided likelihood ratio tests.

9.5.3 Secondary endpoints

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

9.5.3.1 Confirmatory analysis

The key secondary incidence variables (plasma glucose recovery within 30 minutes, within 20 minutes, within 15 minutes, within 10 minutes, in hierarchical order for inference) will be analyzed using descriptive statistics (frequency and percentage) by treatment group. Inferential treatment group comparisons will be assessed via pairwise tests of independent binomial proportions (dasiglucagon versus placebo, GlucaGen versus placebo) using Fisher's exact test for each pairwise comparison.

The key secondary endpoints of plasma glucose CFB within 30 minutes, within 20 minutes, within 15 minutes, and within 10 minutes after study drug injection or at the time of rescue, will be analyzed with the plasma glucose CFB at rescue carried forward in those subjects who require rescue IV glucose before plasma glucose ≥20 mg/dL recovery. Each of these CFB variables will be analyzed using an Analysis of Covariance model, with treatment group modeled as a fixed effect and with the baseline plasma glucose modeled as a covariate. The dasiglucagon versus placebo treatment group difference will

be evaluated inferentially as a least squares means contrast, using a two-sided t-test at the 0.05 signficance level, first for the 30 minute CFB, and then sequentially for the 20 minute, 15 minute, and 10 minute variables, until the first failure to reject.

9.5.4 Secondary clinical efficacy (PD) endpoints

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

Secondary clinical efficacy endpoints will be assessed based on plasma concentration data from samples collected at the dosing visit (Visit 2).

Unless otherwise stated, the population base of analysis will be the FAS.

9.5.4.1 Analysis of secondary clinical efficacy (PD) endpoints

1. Time to first plasma glucose concentration ≥70 mg/dL from baseline. This time-to-event endpoint will be evaluated using a Kaplan-Meier approach, with treatment group as a stratification factor, analogous to that used for the primary endpoint analysis. Differences between the KM curves (dasiglucagon versus placebo, GlucaGen versus placebo) will be evaluated inferentially using pairwise two-sided log rank tests. If the ≥70 mg/dL endpoint is not met within 45 minutes post-dosing, the time of the last valid plasma glucose measurement up to 45 minutes will be the censoring time.
2. The AUC will be calculated as the baseline-adjusted area under the plasma glucose profile over time:
    a. $AUC_{0\text{-}30min}$: restricting the time window to the 0 to 30 minutes interval.
3. The log-transformed AUC endpoint will be analyzed using an analysis of covariance (ANCOVA) model with treatment as fixed effect and baseline plasma glucose modeled as a covariate. The least squares means treatment group differences will be back-transformed (anti-logged) for presentation as a ratio of the treatment group geometric means, with their corresponding 95% CI.

9.5.5 Exposure (PK) endpoints

- Plasma dasiglucagon and GlucaGen concentrations from time zero to 90 minutes: $AUC_{0\text{-}90min}$, C_{max}, and t_{max}.

9.5.5.1 Analysis of exposure (PK) endpoints

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

C_{max} will be determined as the maximum of all valid plasma dasiglucagon/GlucaGen concentrations.

T_{max} will be determined as the timepoint where the maximum of all valid plasma dasiglucagon/GlucaGen concentration measurements for each measurement series is observed.

The log-transformed PK endpoints AUC and C_{max} will be analyzed in the same way as the AUC endpoints.

As t_{max} is a highly discrete endpoint, Wilcoxon's rank sum test for unpaired observations will be used to assess differences between the two treatment groups.

9.6 Exploratory analyses

Exploratory analyses will include descriptive statistics and modeling analogous to that done for key secondary endpoints. However, treatment group comparisons will be summarized without inference intent.

- Plasma glucose concentration ≥70 mg/dL (3.9 mmol/L) or increase of ≥20 mg/dL (1.1 mmol/L) within 30 minutes after study drug injection without administration of rescue IV glucose.

- Plasma insulin response as area under the curve above baseline from time zero to 60 minutes, $AUC_{0\text{-}60\ min}$.

Plasma insulin concentrations measured pre-dose and at 30 and 60 minutes after dosing (see Table 3) will be presented individually. A summary table per timepoint will be provided. The $AUC_{0\text{-}60min}$ will be determined and a summary presented.

9.7 Safety analyses

9.7.1 Adverse events

AEs will be coded using the latest available version of the MedDRA, the version of which will be provided in the clinical trial report. AE summary tables will include the number and percentage of subjects who experienced AEs summarized by system organ class and preferred term.

An overall summary table will be provided showing the number and percentage of subjects with any:

- TEAE
- Severe TEAE
- Serious TEAE
- Drug-related TEAE
- Drug-related severe TEAE
- Drug-related serious TEAE
- TEAE leading to withdrawal
- TEAE with outcome death
- AESI

Refer to Section 7.2.1 for the definition of TEAEs.

9.7.2 Immunogenicity data

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

9.7.3 Clinical laboratory assessments

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

9.7.4 Other safety data

Incidence of rescue infusion of IV glucose during the hypoglycemic clamp procedure will be analyzed using descriptive statistics (frequency and percentage) by treatment group. Inferential treatment group comparisons will be assessed via pairwise tests of

independent binomial proportions (dasiglucagon versus placebo, GlucaGen versus placebo).

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

Vital signs, physical examination, 12-lead ECG and local tolerability data will be summarized using descriptive statistics.

9.8 Treatment compliance

Trained unblinded members of staff will perform all administrations of the IMP at the trial center. The administered doses will be recorded in the blinded Drug Accountability form in the eCRF.

PK assessments will support the surveillance of compliance with IMP administration.

9.9 Subject withdrawals and missing data

In the case of subject withdrawal, no imputation of values for PK or PD measurements will be done. Analyses will be done on valid cases only, i.e., no imputation techniques such as last observation carried forward will be applied. For the primary analysis in the FAS, missing values for the primary endpoint will be imputed by a conservative rule considering any missing value as a failure.

9.10 Interim analyses

No interim analyses are currently planned.

10 ETHICAL, LEGAL, AND ADMINISTRATIVE ASPECTS

10.1 Quality assurance

The sponsor or designee will conduct a site visit to verify the qualifications of each investigator, inspect the facilities, and inform the investigator of responsibilities and the procedures for ensuring adequate and correct documentation.

The investigator is required to prepare and maintain adequate and accurate case histories designed to record all observations and other data pertinent to the trial for each trial participant. All information recorded on the eCRFs for this trial must be consistent with the subjects' source documentation (i.e. medical records).

The investigator will permit trial-related monitoring, IRB/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 monitor or a representative of the sponsor will cross-check the data entered in the eCRFs with the source data at the trial center and observe the trial procedures in order to verify adherence to the trial protocol. Any queries will be resolved by the investigator or his/her delegate.

All of the clinical data will be captured via electronic data capture (EDC) using a web-based tool.

The investigator center 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 subjects. The investigator's data will be accessible from the investigator's center throughout the trial. The eCRFs must be kept current to reflect subject status at each phase during the course of the trial. The eCRF will not capture personalized data. The investigator must make a separate confidential record of personalized details (name and initials) on the subject identification and enrollment log. All changes to data are made by the investigator or his/her delegate through the EDC system.

It is the responsibility of the principal investigator of the respective center to ensure that all subject discontinuations or changes in trial or other medications entered on the subject's eCRF are also made on the subject's medical records.

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

10.2 Electronic case report forms

Remote data capture software will be used for data collection. Following training, trial staff will be given access to the software. Access to the software is restricted to staff participating in the trial and the extent of access will depend on the participants' user role in the trial.

The subjects enrolled into the trial will be identified in the database by subject number and trial identification code. The investigator or delegate will enter subject data into the eCRF promptly. All data is to be entered in English. Data recorded in the eCRFs will be accessible to the trial staff throughout the trial.

After data entry, systematic data validation will be performed and data entry discrepancies will be presented electronically directly to the center staff. Queries for discrepant data may be generated automatically by the software upon entry and/or generated manually by the trial monitor or the trial data manager. All queries, whether generated by the system or by trial staff, will be in electronic format.

All sections of the eCRF are to be electronically approved by the investigator or a medically qualified delegate after the data has been entered and all queries have been resolved. Changes to any eCRF page subsequent to the approval require a new approval signature.

All queries and changes/corrections to the data are documented in the eCRF.

10.3 Access to source data

During the course of the trial, a trial monitor will make site visits to review protocol compliance, compare eCRFs with individual subject’s medical records, assess drug accountability, and ensure that the trial is being conducted according to pertinent regulatory requirements.

Checking of the eCRFs for completeness and clarity, and cross-checking with source documents, will be required to monitor the progress of the trial. Moreover, regulatory authorities of certain countries, IRBs/IECs, and/or the sponsor may wish to carry out such source data checks and/or on-site audit inspections. Direct access to source data will be required for these inspections and audits; they will be carried out giving due consideration to data protection and medical confidentiality.

10.4 Source documentation

All source documents from which eCRF entries are derived should be placed in the subject's medical records. If data are to be entered directly into the eCRF this must be specified in a source data agreement prior to the start of the trial.

eCRF entries will be verified with source documentation. The review of medical records will be performed in a manner to ensure that subject confidentiality is maintained.

The trial monitor will check the eCRF for accuracy and completion and perform source data verification. The trial monitor will document source data verification of all reviewed sections of the eCRF.

10.5 Data processing

The trial is run as an EDC trial, i.e. all relevant data is entered by the centers directly into the clinical database. The eCRF is designed to capture all required information in compliance with GCP standards.

10.6 Archiving trial records

According to ICH guidelines, essential documents should be retained for a minimum of 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. However, these documents should be retained for a longer period if required by the applicable legal requirements.

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

10.7 Good clinical practice

The procedures set out in this trial protocol are designed to ensure that the sponsor and investigator abide by the principles of the GCP guidelines of the ICH (1), and of the Declaration of Helsinki (2008) (2). The trial also will be carried out in keeping with local legal requirements.

10.8 Informed consent

Before each subject is admitted to the trial, written informed consent will be obtained from the subject according to the regulatory and legal requirements of the participating country. This consent form must be dated and retained by the investigator as part of the trial records. The investigator will not undertake any investigation specifically required only for the clinical trial until valid consent has been obtained. The terms of the consent and when it was obtained must also be documented in the eCRF.

The investigator will explain to each subject orally and in writing (subject information sheet) the nature, significance, risks and implications of the trial before inclusion. In particular, the subjects 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.
- That his/her medical records may be examined by authorized monitors or clinical quality assurance auditors appointed by the sponsor, by appropriate IRB/IEC members, and by inspectors from regulatory authorities.

All subjects will receive a copy of the subject information sheet and a copy of their signed and dated informed consent form, both of which will be in the subject's local language.

If a protocol amendment is required, the informed consent form may need to be revised to reflect the changes to the protocol. If the consent form is revised, it must be reviewed and approved by the appropriate IEC/IRB, and signed by all subjects subsequently enrolled in the trial as well as those currently enrolled in the trial.

10.9 Protocol approval and amendment

Before the start of the trial, the trial protocol and/or other relevant documents will be approved by the IEC/IRB/competent authorities, in accordance with local legal requirements. The sponsor must ensure that all ethical and legal requirements have been met before the first subject is enrolled in the trial.

This protocol is to be followed exactly. To alter the protocol, amendments must be written, receive approval from the appropriate personnel, and receive IRB/IEC/competent authority approval prior to implementation (if appropriate).

Administrative changes (not affecting the subject benefit/risk ratio) may be made without the need for a formal amendment. All amendments will be distributed to all protocol recipients, with appropriate instructions.

10.10 Duration of the trial

The maximum duration of the trial for each subject will be up to 63 days (including up to 30 days for screening and up to 33 days until the follow-up visit).

The trial will be closed when all subjects have completed Visit 3.

10.11 Premature termination of the trial

If the investigator, the sponsor (e.g. safety committee), or the safety medical monitor becomes aware of conditions or events that suggest a possible hazard to subjects if the trial continues, the trial may be terminated after appropriate consultation between the relevant parties. The trial may also be terminated early at the sponsor's discretion in the absence of such a finding.

Conditions that may warrant termination include, but are not limited to:

- The discovery of an unexpected, significant, or unacceptable risk to the subjects enrolled in the trial
- Failure to enroll subjects at an acceptable rate.
- A decision on the part of the sponsor to suspend or discontinue development of the drug.

The trial can be terminated prematurely by the sponsor at an individual center if:

- The center cannot comply with the requirements of the protocol.
- It is not possible for the center to comply with GCP standards.

10.12 Confidentiality

All trial findings and documents will be regarded as confidential. The investigator and members of his/her research team must not disclose such information without prior written approval from the sponsor.

The anonymity of participating subjects must be maintained. Subjects will be identified on eCRFs submitted to the sponsor by their subject number. On the SAE reports and all other source documents, the subject will be identified via subject number. Documents not

to be submitted to the sponsor that identify the subject (e.g. the signed informed consent form) must be maintained in confidence by the investigator.

10.13 Other ethical and regulatory issues

If a significant safety issue is identified, either from an individual case report or review of aggregate data, then the sponsor will issue prompt notification to all parties: regulatory authorities, investigators and IRB/IECs.

A significant safety issue is one that has a significant impact on the course of the clinical trial or program (including the potential for suspension of the trial program or amendments to protocols) or warrants immediate update of informed consent.

10.14 Liability and insurance

The sponsor will take out reasonable third-party liability insurance cover in accordance with all local legal requirements. The civil liability of the investigator, the persons instructed by him and the hospital, practice or institute in which they are employed and the liability of the sponsor with respect to financial loss due to personal injury and other damage that may arise as a result of the carrying out of this trial are governed by the applicable law.

The sponsor will arrange for liability insurance if subjects should be injured due to the participation in the trial and provided that the sponsor is legally liable for that.

Excluded from the insurance cover are injuries to health and deteriorations of illnesses already in existence which would have occurred or continued to exist even if the subject had not taken part in the clinical trial.

The insurance cover is jeopardized if the subject fails to report immediately to the investigator or responsible physician any injury to health, which might have resulted from participation in the clinical trial, or if he/she undergoes any other medical treatment without their consent before the clinical trial has been completely finished in so far as the individual subject is concerned.

Any injury to health, which might have occurred as a result of participation in the clinical trial must be reported by the subject to the investigator without delay. The investigator is obliged to make such a report in any case.

10.15 Publication policy

By signing the trial protocol, the investigator agrees with the use of results of the trial for the purposes of national and international registration, publication and information for medical and pharmaceutical professionals. If necessary, the authorities will be notified of the investigator's name, address, qualifications and extent of involvement.

A clinical trial report will be prepared and reviewed by the sponsor in co-operation with the coordinating investigator. The coordinatoing investigator will be appointed by Zealand Pharma to review and sign the clinical trial report on behalf of all participating investigators. A summary of the clinical trial report (according to CPMP/ICH137/95) should be sent to the regulatory authorities and IRB/IEC according to the relevant guidelines.

According to the Declaration of Helsinki (2) investigators and sponsors 'have ethical obligations with regard to the publication and dissemination of the results of research'.

The trial design and results may be published as one or more original research manuscripts/abstracts and presented at a scientific meeting. 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 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. Authorship on any publication(s) resulting from this clinical trial will be assigned according to the recommendations of the International Committee of Medicinal Journal Editors (26).

Participating subjects will not be identified by name in any published reports about the clinical trial.

The sponsor is responsible for trial registration at ClinicalTrials.gov (www.clinicaltrials.gov) according to FDA requirements, as well as the European Medicines Agency's Clinical Trials Database (EudraCT).

11 REFERENCE LIST

1. ICH Harmonised Tripartite Guideline. International Conference on Harmonisation. ICH Harmonised Tripartite Guideline. Integrated addendum to ICH E6(R1): Guideline for Good Clinical Practice E6 (R2), Step 4. 09-Nov-2016. Available from: URL: http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E6/E6_R2__Step_4.pdf
2. World Medical Association. Declaration of Helsinki. Ethical Principles for Medical Research Involving Human Subjects. 52nd WMA General Assembly, Edinburgh, Scotland, October 2000. Last amended with Note of Clarification on Paragraph 29 by the WMA General Assembly, Washington 2002, and Note of Clarification on Paragraph 30 by the WMA General assembly, Tokyo 2004
3. 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 May;36:1384-95
4. Cryer PE. The barrier of hypoglycemia in diabetes. Diabetes 2008;57:3169-76
5. American Diabetes Association Position Statement. Standards of medical care in diabetes - 2017. Diabetes Care 2017;40(Supplement 1):S1-S135
6. Davies MJ, Gagliardino JJ, Gray LJ, Khunti K, Mohan V, Hughes R. Real-world factors affecting adherence to insulin therapy in patients with Type 1 or Type 2 diabetes mellitus: a systematic review. Diabetic Medicine 2013;30:512-24
7. International Hypoglycaemia Study Group. Glucose concentrations of less than 3.0 mmol/L (54 mg/dL) should be reported in clinical trials: a joint position statement of the American Diabetes Association and the European Association for the Study of Diabetes. Diabetes Care 2017;40(1):155-57
8. Christensen M, Bagger JI, Vilsbøll T, Knop FK. The alpha-cell as target for type 2 diabetes therapy. Rev Diabet Stud 2011;8:369-81
9. Lund A, Bagger JI, Christensen M, Knop FK, Vilsbøll T. Glucagon and type 2 diabetes: the return of the alpha cell. Curr Diab Rep 2014;14:555
10. Habegger KM, Heppner KM, Geary N, Bartness TJ, DiMarchi R, Tschöp MH. The metabolic actions of glucagon revisited. Nat Rev Endocrinol 2010;6:689-97
11. Onoue S, Ohshima K, Debari K, Koh K, Shioda S, Iwasa S, et al. Mishandling of the therapeutic peptide glucagon generates cytotoxic amyloidogenic fibrils. Pharm Res 2004;21:1274-83

12. Fosgerau K, Hoffmann T. Peptide therapeutics: current status and future directions. Drug Discovery Today 2015;20:122-8
13. Novo Nordisk Limited. SPC for GlucaGen Hypokit 1 mg. 2015. http://www.medicines.org.uk/emc/medicine/4258/SPC/GlucaGen+Hypokit+1+mg - Accessed 13 Mar 2017 .
14. Zealand Pharma A/S. Dasiglucagon Investigator's Brochure, Edition 3, 12 Dec 2016.
15. The Task Force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). 2013 ESH/ESC guidelines for the management of arterial hypertension. European Heart Journal 2013;34:2159-219
16. Levey AS, Coresh J, Greene T, Stevens LA, Zhang Y, Hendriksen BA, et al. Using standardized serum creatinine values in the modification of diet in renal disease study equation for estimating glomerular filtration rate. Annals of Internal Medicine 2006;145:247-54
17. Food and Drug Administration (FDA). Guidance for Industry on Bioanalytical Method Validation. Food and Drug Administration (FDA) 2001
18. Viswanathan CT, Bansal S, Booth B, DeStefano AJ, Rose MJ, Sailstad J, et al. Quantitative bioanalytical methods validation and implementation: best practices for chromatographic and ligand binding assays. Pharm Res 2007 Oct;24:1962-73
19. Shankar G, Devanarayan V, Amaravadi L, Barrett YC, Bowsher 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;48:1267-81
20. Zealand Pharma report SS. Commissioning and validation of a method for the validation of anti-ZP4207 antibodies in human serum. 2014
21. Zealand Pharma report SS. Commissioning and validation of a method for the validation of anti-glucagon antibodies in human serum. 2014
22. Bioagilytix Labs. Validation of an assay to detect neutralizing antibodies to ZP4207 by measuring cyclic adenosine monophosphate (cAMP) in a glucagon receptor transfected cell line, BAL-14-206-004-REP. 2016. Report No.: Zealand Pharma, study 15-039
23. 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. 2016. Report No.: Zealand Pharma, Study 15-040

24. Amiel SA, Tamborlane WV, Simonson DC, et al. Defective glucose counterregulation after strict glycemic control of insulin-dependent diabetes mellitus. N Eng J Med 1987; 316: 1376-83.

25. Rickels MR, Ruedy KJ, Foster NC, et al. Intranasal glucagon for treatment of insulin-induced hypoglycemia in adults with type 1 diabetes: A randomized crossover noninferiority study. Diabetes Care 2016; 39: 264-70.

26. ICMJE. Recommendations for the conduct, reporting, editing, and publication of scholarly work in medical journals. Updated December 2016

12 APPENDICES

12.1 List of trial personnel

Sponsor **Clinical Trial Manager**	[redacted] Zealand Pharma Smedeland 36 2600 Glostrup, Denmark Phone: [redacted]
Medical Officer	[redacted] Zealand Pharma Smedeland 36 2600 Glostrup, Denmark Phone: [redacted]
Contract Research Organization	Chiltern International (Ltd) 171 Bath Road Slough Berkshire SL1 4AA United Kingdom Phone: +44 (0) 175351 2000
Project Manager	[redacted] Chiltern International (Ltd) 171 Bath Road Slough Berkshire SL1 4AA United Kingdom Phone: [redacted]
Senior Medical Officer and Safety medical monitor	[redacted] Chiltern International kft Canada Square Office House Ganz u

	12-14, 4 emelet 1027 Budapest Hungary Phone: [redacted]
Pharmacovigilance unit Responsible for Serious Adverse Event (SAE) Management and 24-hour SAE reporting	[redacted] PharmaLex Agern Allé 24 2970 Hørsholm, Denmark Phone: [redacted] (8 a.m. to 4 p.m.) Phone: (outside 8 a.m. to 4 p.m.) Fax: email: PV-nordic@pharmalex.com
Central laboratory	MLM Laboratory Dohrweg 63 41066 Mönchengladbach Germany
Special laboratory (GlucaGen PK, insulin PK)	MLM Laboratory Dohrweg 63 41066 Mönchengladbach Germany
Special laboratory (dasiglucagon PK, dasiglucagon ADA, GlucaGen ADA)	York Bioanalytical Solutions (YBS) Cedar House Northminster Business Park Northfield Lane York, YO26 6QR, United Kingdom
Special laboratory (neutralizing antibodies)	BioAgilytix 2300 Englert Drive Durham, NC, 27713, USA

A list of all investigators, IECs and IRBs will be provided in a separate document and in the clinical trial report.

CLINICAL TRIAL PROTOCOL

A phase 3, randomized, double-blind, parallel trial to confirm the clinical efficacy and safety of dasiglucagon in the rescue treatment of hypoglycemia in subjects with type 1 diabetes mellitus (T1DM) compared to placebo and with reference to GlucaGen®

Sponsor:	Zealand Pharma A/S
Sponsor Protocol No.:	ZP4207-16137
EudraCT No.:	2017-002449-31
IND No:	127866
Trial Drug Name:	Dasiglucagon* injection
Date of Protocol:	31 Aug 2017

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

*Dasiglucagon is the proposed international nonproprietary name for ZP4207.

Declaration of sponsor or responsible medical officer

Title: A phase 3, randomized, double-blind, parallel trial to confirm the clinical efficacy and safety of dasiglucagon in the rescue treatment of hypoglycemia in subjects with type 1 diabetes mellitus (T1DM) compared to placebo and with reference to GlucaGen®

This trial protocol 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 Declaration of Helsinki and the guidelines on Good Clinical Practice (GCP) (1).

[redacted] [redacted]

Date

Title Clinical Trial Manager
Institution Zealand Pharma
Smedeland 36
2600 Glostrup, Denmark
Phone: [redacted]

[redacted] [redacted]

Date

Title Medical Officer
Institution Zealand Pharma
Smedeland 36
2600 Glostrup, Denmark
Phone: [redacted]

Declaration of the coordinating investigator

Title: A phase 3, randomized, double-blind, parallel trial to confirm the clinical efficacy and safety of dasiglucagon in the rescue treatment of hypoglycemia in subjects with type 1 diabetes mellitus (T1DM) compared to placebo and with reference to GlucaGen®

I have read this protocol. I approve this document and I agree that it contains all necessary details for carrying out the trial as described. I will conduct this trial in accordance with the design and specific provision of this protocol and will make a reasonable effort to complete the trial within the time designated. I will provide copies of this protocol and access to all information furnished by Zealand Pharma A/S to trial personnel under my supervision. I will discuss this material with them to ensure they are fully informed about the trial product and trial procedures. I will let them know that this information is confidential and proprietary to Zealand Pharma A/S and that it may not be further disclosed to third parties. I understand that the trial may be terminated or enrollment suspended at any time by Zealand Pharma A/S, with or without cause, or by me if it becomes necessary to protect the best interests of the trial subjects.

I agree that the trial will be carried out in accordance with GCP (1), with the Declaration of Helsinki (with amendments) (2) and with the laws and regulations of the countries in which the trial takes place.

_____ _____

Name Date
Title
Institution
Phone: +
Fax: +

Declaration of the investigator

Title: A phase 3, randomized, double-blind, parallel trial to confirm the clinical efficacy and safety of dasiglucagon in the rescue treatment of hypoglycemia in subjects with type 1 diabetes mellitus (T1DM) compared to placebo and with reference to GlucaGen®

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 this trial protocol, Investigator's Brochure, Case Report Forms, 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 subjects.

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

_____ _____

Signature Date

_____

Name (block letters)

_____

Title (block letters)

_____

Institution (block letters)

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List of abbreviations and definitions of terms

ADA	Anti-drug antibody
AE	Adverse event
AESI	Adverse event of special interest
ALT	Alanine aminotransferase
ANCOVA	Analysis of covariance
AST	Aspartate aminotransferase
AUC	Area under the concentration-time curve
$AUC_{0\text{-inf}}$	AUC from time zero to infinity
AUE	Area under the effect curve
CFB	Changes from baseline
CI	Confidence interval
C_{max}	Maximum plasma concentration
CPH	Cox proportional hazards
CRO	Contract research organization
ECG	Electrocardiogram
eCRF	Electronic case report form
EDC	Electronic data capture
EU	European Union
EudraCT	European Medicines Agency's Clinical Trials Database
FAS	Full analysis set
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HbA_{1c}	Glycated hemoglobin
ICH	International Conference on Harmonization
ID card	Identification card
IEC	Independent Ethics Committee
IM	Intramuscular(ly)
IMP	Investigational medicinal product
IRB	Institutional Review Board
IV	Intravenous(ly)

IWRS	Interactive Web Response System
MedDRA	Medical Dictionary for Regulatory Activities
NPH	Neutral protamine Hagedorn
PD	Pharmacodynamics
PK	Pharmacokinetics
PP	Per protocol
SAE	Serious adverse event
SAP	Statistical analysis plan
SAS	Safety analysis set
SC	Subcutaneous(ly)
SUSAR	Serious unexpected suspected adverse reaction
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
TEAE	Treatment-emergent adverse event
t_{max}	Time to the maximum plasma concentration
ULN	Upper limit of the normal range
US	United States of America

1 SYNOPSIS

Name of sponsor: Zealand Pharma A/S	**Trial ID:** ZP4207-16137
Title of the trial: A phase 3, randomized, double-blind, parallel trial to confirm the clinical efficacy and safety of dasiglucagon in the rescue treatment of hypoglycemia in subjects with type 1 diabetes mellitus (T1DM) compared to placebo and with reference to GlucaGen®	
Trial design: The trial is a global, multicenter, randomized, parallel-group, double-blind, clinical trial confirming the efficacy and safety of dasiglucagon for insulin-induced hypoglycemia in subjects with type 1 diabetes mellitus. The subjects will be randomized 2:1:1 to receive a single fixed subcutaneous 0.6 mg dose of dasiglucagon (hereinafter dasiglucagon), placebo for dasiglucagon (hereinafter referred to as placebo), or a 1 mg dose of GlucaGen® (hereafter referred to as GlucaGen) and followed for at least 28 days after treatment.	
Clinical phase of development: Phase 3	
Trial centers: This trial will be conducted at 4 to 6 sites in the United States of America, Canada, and Europe.	
Planned trial start (first subject first visit): Q4/2017	**Planned trial end (last subject last visit):** Q3/2018
Trial population: Male and female adult subjects with type 1 diabetes mellitus treated with insulin for at least one year	
Key objectives: **Primary objective:** • To demonstrate superiority of dasiglucagon compared to placebo following a single subcutaneous 0.6 mg dose administered to subjects with type 1 diabetes mellitus with insulin-induced hypoglycemia. **Secondary objective:** • To compare the glycemic response observed after dasiglucagon with that of GlucaGen.	
Key endpoints: **Primary endpoint:** • Time to plasma glucose recovery. Plasma glucose recovery is defined as first increase in plasma glucose of ≥20 mg/dL (1.1 mmol/L) from baseline during the hypoglycemic clamp procedure without administration of rescue intravenous (IV) glucose. **Key secondary endpoints:** • Plasma glucose recovery within 30 minutes, within 20 minutes, within 15 minutes, and within 10 minutes after study drug injection without administration of rescue IV glucose. • Plasma glucose changes from baseline within 30 minutes, within 20 minutes, within 15 minutes, and within 10 minutes after study drug injection or at the time of rescue. **Clinical efficacy (Pharmacodynamic) endpoints:** • Time to first plasma glucose concentration of ≥70 mg/dL (3.9 mmol/L) without administration of rescue IV glucose.	

- Plasma glucose response as area under the curve above baseline from time zero to 30 minutes, $AUC_{0\text{-}30min}$.

Exposure (Pharmacokinetic) endpoints:

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

Safety endpoints:

- Adverse events, clinical laboratory assessments (biochemistry, hematology, coagulation, urinalysis), vital signs, physical examination, electrocardiogram, and local tolerability.
- Administration of rescue infusion of IV glucose during the hypoglycemic clamp procedure.
- Time to first rescue infusion of IV glucose during the hypoglycemic clamp procedure.

Immunogenicity endpoint:

- Occurrence of anti-drug antibodies

Exploratory endpoints:

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

Key inclusion criteria:

- Male and female subjects with type 1 diabetes mellitus treated with insulin for at least one year, diagnostic criteria as defined by the American Diabetes Association.
- Stable insulin treatment 30 days prior to screening, defined as no more than a 10-unit daily variation in total daily insulin dose.
- Hemoglobin A_{1c} <10%.
- Aged between 18 and 75 years, both inclusive.

Key exclusion criteria:

- Previously treated with dasiglucagon.
- Known or suspected allergy to trial product(s) or related products.
- History of anaphylaxis or symptoms of severe systemic allergy (such as angioedema).
- Previous participation (randomization) in this trial.

Sample size:
Approximately 156 subjects are intended to complete the trial, with 78 subjects randomized to the dasiglucagon group and 39 subjects randomized to each of the placebo and GlucaGen groups.

Investigational medicinal product:
Test product: dasiglucagon liquid formulation in pre-filled syringes.
Comparator products: Placebo and GlucaGen® lyophilized powder.

Duration of treatment:
Subjects will be randomized 2:1:1 to receive a single fixed subcutaneous dose of 0.6 mg dasiglucagon, placebo, or 1 mg GlucaGen, and followed for at least 28 days after receiving treatment.

Assessments:
The plasma glucose profile for evaluation of the primary and secondary clinical efficacy (pharmacodynamic) endpoints will be assessed based on plasma concentration data from samples collected at the dosing visit.
The exposure to trial medication (dasiglucagon, placebo, or GlucaGen) for evaluation of pharmacokinetics will also be assessed based on plasma concentration data.

Statistical methods:
Due to requirements in the size of the safety database, the sample size is set to 78 subjects treated with dasiglucagon 0.6 mg. With a 2:1:1 randomization ratio for the three treatments, dasiglucagon 0.6 mg, placebo, and GlucaGen 1 mg, 78 subjects injected with dasiglucagon and 39 subjects with placebo will yield a power of 90% at a 5% two-sided significance level to detect a treatment group difference in recovery incidence within 20 minutes, assuming as low as 80% and as high as 50% recovery incidence for the dasiglucagon and placebo groups, respectively.
For the confirmatory analyses, the primary and key secondary endpoints for the dasiglucagon 0.6 mg and placebo treated subjects will be compared. A hierarchical procedure will be applied for the control of multiplicity in statistical inferences. The primary and secondary endpoints will be evaluated on the Full Analysis Set sample. The statistical inference comparisons with placebo will be conducted as superiority tests. The comparisons of dasiglucagon versus GlucaGen will be summarized descriptively.
The primary endpoint will be summarized using Kaplan-Meier (KM) estimates stratified by treatment group and injection site. The treatment group difference between dasiglucagon and placebo will be evaluated inferentially using a pairwise two-sided log-rank test.
In the primary analysis, those subjects who require rescue IV glucose will be censored at the time of plasma glucose recovery. This approach is based on the assumption that rescue IV glucose administration will shorten the time to recovery. This assumption implies that censoring at the time of recovery after rescue therapy is valid as a time to recovery without rescue would be longer than the observed duration. If recovery has not occurred at 45 minutes after study drug injection, censoring will be applied irrespective of the use of rescue IV glucose.
In sensitivity analyses, the time to plasma glucose recovery will be analyzed 1) without censoring at the time of recovery for those subjects who require rescue IV glucose before 45 minutes and 2) with censoring at the time of administration of rescue IV glucose before 45 minutes.
The primary endpoint will additionally be analyzed using a Cox proportional hazards (CPH) time to event statistical model, if the hazards by treatment group can be assumed proportional using a graphical comparison of log-minus-log survival curves and no more than two-thirds of the recovery times are censored in each treatment group. The CPH model will be used for inferences, with treatment group and injection site modeled as categorical effects, and baseline plasma glucose modeled as a continuous covariate. The treatment group rate ratios, active versus placebo, will be estimated together with the 95% confidence intervals, and pairwise treatment group inferences (dasiglucagon vs placebo, GlucaGen vs placebo) will be evaluated using two-sided likelihood ratio tests.
The key secondary incidence variables (plasma glucose recovery within 30 minutes, within 20 minutes, within 15 minutes, within 10 minutes, in hierarchical order for inference) will be analyzed using descriptive statistics (frequency and percentage) by treatment group. Inferential treatment group comparisons will be assessed via pairwise tests of independent binomial proportions (dasiglucagon versus placebo, GlucaGen versus placebo) using Fisher's exact test for each pairwise comparison.
The key secondary endpoints of plasma glucose changes from baseline (CFB) within 30 minutes, within 20 minutes, within 15 minutes, and within 10 minutes after study drug injection or at the time of rescue, will

be analyzed with the plasma glucose CFB at rescue carried forward in those subjects who require rescue IV glucose before plasma glucose ≥20 mg/dL recovery. Each of these CFB variables will be analyzed using an Analysis of Covariance model, with treatment group modeled as a fixed effect and with the baseline plasma glucose modeled as a covariate. The dasiglucagon versus placebo treatment group difference will be evaluated inferentially as a least squares means contrast, using a two-sided t-test at the 0.05 significance level, first for the 30 minute CFB, and then sequentially for the 20 minute, 15 minute, and 10 minute variables, until the first failure to reject.

The continuous clinical efficacy variables, the exploratory variables, and the pharmacokinetics variables will each be summarized descriptively by treatment group. The clinical efficacy variables will be analyzed analogous to the plasma glucose CFB variables.

The safety analyses will include by-treatment-group descriptive summaries of vital sign measurements, laboratory measures (including immunogenicity incidence), physical examination assessments, rescue IV glucose (incidence and amount of glucose infused), and adverse events. The number and percentage of subjects reporting specific events, such as nausea and vomiting, will be presented by body system and preferred term.

Further details will be included in the Statistical Analysis Plan, to be completed before database lock and treatment unmasking.

2 INTRODUCTION

2.1 Background

Zealand Pharma A/S (Zealand Pharma) is developing dasiglucagon, a physically and chemically stable peptide analog of human glucagon, in a ready-to-use liquid formulation for the acute treatment of severe hypoglycemia in patients with insulin-treated diabetes mellitus. Like native glucagon, dasiglucagon is comprised of 29 amino acids, but with 7 substitutions which improve its physical and chemical stability in aqueous media. These amino acid substitutions make dasiglucagon suitable for a liquid formulation, while providing similar efficacy and safety as compared with recombinant glucagon in the clinical setting of acute treatment of severe hypoglycemia.

2.1.1 Hypoglycemia

Hypoglycemia in patients with diabetes is defined as episodes of an abnormally low plasma glucose concentration (3). This is a common, unpredictable, and potentially dangerous side effect of treatment of diabetes mellitus, especially with 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 (4).

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 (5).

The incidence of hypoglycemic events or even the fear of hypoglycemia influences patients' adherence to prescribed treatment regimens for diabetes mellitus (6). This leads to inadequate glycemic control, which in turn may lead to an increased risk of diabetic complications (5). Serious clinically significant hypoglycemia is now defined as plasma glucose <54 mg/dL (3.0 mmol/L), while the plasma glucose alert value is defined as <70 mg/dL (3.9 mmol/L) (7). When plasma glucose falls below these values, some kind of treatment strategy is needed.

2.1.2 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) (8,9). 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 (10). Insulin decreases blood glucose levels and cases of hypoglycemia can be reversed by glucagon. Therefore, glucagon is indicated for the treatment of severe hypoglycemia.

Besides intravenous (IV) glucose administration, an injectable form of glucagon is given as first aid in cases of severe hypoglycemia, when the patient is unconscious or for other reasons cannot take glucose orally. The approved glucagon dose for an adult is 1 mg, given by intramuscular (IM), IV, or subcutaneous (SC) injection, which quickly raises blood glucose levels. As current marketed recombinant glucagon is highly unstable when dissolved in solution, the injectable form has to be reconstituted prior to use in a 9-step procedure that requires a sterile diluent to be injected into a vial containing lyophilized powdered glucagon. When dissolved in a fluid state, glucagon can form amyloid fibrils (11), or tightly woven chains of proteins made up of the individual glucagon peptides. The reconstitution process makes the use of marketed glucagon products cumbersome (12), and a more patient-friendly formulation is needed. Currently, the Food and Drug Administration (FDA) approved instructions for commercially available glucagon allow only for immediate usage of glucagon after the powder is reconstituted in aqueous solution (13). Therefore, a glucagon analog with enhanced biophysical stability may represent a leap forward in terms of convenient therapeutic applications.

2.1.3 Dasiglucagon

Dasiglucagon (ZP4207) is a stable peptide analog of human glucagon, available in a ready-to-use liquid formulation. Dasiglucagon (hereinafter referred to as dasiglucagon) 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® (hereafter referred to as GlucaGen). Dasiglucagon exhibits improved physical and chemical stability and is available in an aqueous solution at neutral pH (14).

Three clinical trials have been completed with dasiglucagon: a first-in-human dose trial in healthy volunteers and subjects with T1DM (ZP4207-14013), a multiple-dose dose-escalation trial (ZP4207-15007) evaluating the safety, tolerability, pharmacokinetics (PK) and pharmacodynamics (PD) of dasiglucagon in healthy volunteers, and a phase 2 crossover trial to assess the PK and PD of a single dose of an optimized formulation of dasiglucagon administered SC in subjects with T1DM (ZP4207-15126) (14).

2.1.3.1 Pharmacokinetics and pharmacodynamics of dasiglucagon

The results of the phase 1 and 2 clinical trials confirmed dose-proportionality for dasiglucagon PK, 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 (t_{max}) was 35 minutes for dasiglucagon compared with 20 minutes for GlucaGen.

At all dose levels in the phase 2 trial, all subjects achieved a plasma glucose level of at least 70 mg/dL (3.9 mmol/L) as well as an increase in plasma glucose by at least 20 mg/dL (1.1 mmol/L) within 30 minutes after dosing. The PD responses to 0.6 mg of dasiglucagon and 1 mg of GlucaGen were comparable.

2.1.3.2 Safety of dasiglucagon

The safety data for dasiglucagon do not give rise to any safety concerns. No new signals were observed, 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 mg (4 with dasiglucagon and 1 with GlucaGen). This is not considered a safety concern; however, hemodynamic changes after dosing will be considered an adverse event of special interest (AESI).

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's Brochure (14).

2.2 Trial rationale

The aim of the current trial is to confirm the superiority of dasiglucagon for the treatment of insulin-induced hypoglycemia in subjects with T1DM as compared to placebo for dasiglucagon (hereinafter placebo) and to compare the clinical efficacy and safety of dasiglucagon with reference to GlucaGen. A randomized, controlled trial design was used.

See Section 4.2 for justification of the design of this trial.

2.3 Risk-benefit assessment

Non-clinical experience

The nonclinical development program did not reveal any safety findings that would prohibit administration of dasiglucagon to humans. None of the safety pharmacology studies, repeated dose toxicity studies or genotoxicity studies revealed any significant toxicity findings relevant to the therapeutic use of dasiglucagon.

Clinical experience

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 IMP 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 extent in the 3 clinical studies conducted to date with dasiglucagon. As with every novel drug substance, new and as yet unknown side effects may also 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 low immunogenic potential.

Overall, dasiglucagon is judged to be a low-risk molecule, based upon the available clinical data. Administration of dasiglucagon may be associated with a risk of allergic reactions similar to those observed for other therapeutic peptides or proteins. Subjects 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 centers.

With the exception of medical examinations, a subject 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.

The development program including 141 subjects exposed to dasiglucagon to date has demonstrated that administration of dasiglucagon is efficacious and well tolerated, with a safety profile that does not give rise to specific safety concerns. Two phase 1 and one phase 2 clinical trials have been conducted to investigate the safety, tolerability, PK and PD of dasiglucagon after single and multiple dosing to healthy volunteers and subjects with T1DM under insulin-induced hypoglycemic conditions. Dasiglucagon has proven to have relevant clinical effects in the acute severe hypoglycemia rescue setting and may be an effective and reliable emergency treatment for severe hypoglycemia. Overall, the anticipated benefits for subjects entering the ZP4207-16137 trial are considered to justify the risks.

3 TRIAL OBJECTIVES AND ENDPOINTS

3.1 Primary objective

- To demonstrate superiority of dasiglucagon compared to placebo following a single subcutaneous 0.6 mg dose administered to subjects with type 1 diabetes mellitus with insulin-induced hypoglycemia.

3.2 Secondary objectives

- To compare the glycemic response observed after dasiglucagon with that of GlucaGen.

3.3 Primary endpoint

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

3.4 Key secondary endpoints

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

3.5 Other secondary endpoints

- Clinical efficacy (PD) endpoints:
  - Time to first plasma glucose concentration ≥70 mg/dL (3.9 mmol/L) without administration of rescue IV glucose.
  - Plasma glucose response as area under the curve (AUC) above baseline from time zero to 30 minutes, $AUC_{0\text{-}30min}$.
- Exposure (PK) endpoints:
  - Area under the drug concentration curve from time zero to 90 minutes, $AUC_{0\text{-}90min}$.
  - Maximum plasma drug concentration (C_{max}).
  - Time to maximum plasma drug concentration (t_{max}).

- Safety endpoints:
  - Adverse events, clinical laboratory assessments (biochemistry, hematology, coagulation, urinalysis), vital signs, physical examination, electrocardiogram (ECG), and local tolerability.
  - Administration of rescue infusion of IV glucose during the hypoglycemic clamp procedure.
  - Time to first rescue infusion of IV glucose during the hypoglycemic clamp procedure.
- Immunogenicity endpoint:
  - Occurrence of anti-drug antibodies

3.6 Exploratory endpoints

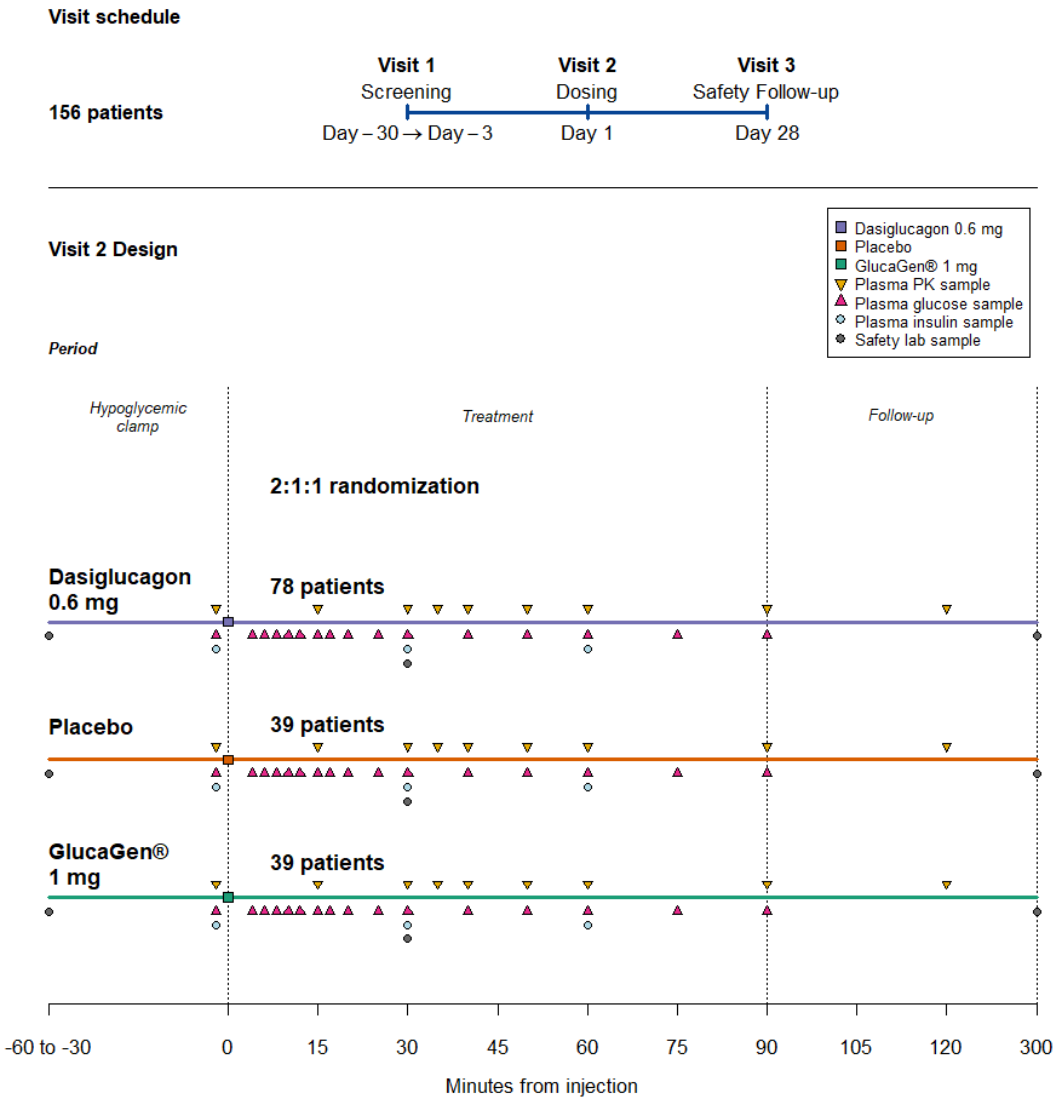
- Plasma glucose concentration ≥70 mg/dL (3.9 mmol/L) or increase of ≥20 mg/dL (1.1 mmol/L) within 30 minutes after study drug injection without administration of rescue IV glucose.
- Plasma insulin response as area under the curve above baseline from time zero to 60 minutes, $AUC_{0\text{-}60\ min}$.

4 OVERALL DESIGN AND PLAN OF THE TRIAL

4.1 Overview

This trial is a global, multicenter, randomized, parallel, double-blind, clinical trial confirming the efficacy and safety of dasiglucagon for insulin-induced hypoglycemia in subjects with T1DM. The subjects will be randomized 2:1:1 to receive a single fixed SC 0.6 mg dose of dasiglucagon, placebo, or a 1 mg dose of GlucaGen and followed for at least 28 days after receiving treatment. A total of 156 subjects with T1DM are expected to complete the treatment visit. The trial will be conducted in the European Union (EU) and North America. See Figure 1 for an overview of the trial design.

Figure 1 Trial design



4.2 Justification of the trial design

4.2.1 Justification for design and parameters

In order to avoid bias in subject selection and in the evaluation of clinical assessments, subjects will be randomly assigned 2:1:1 to either dasiglucagon, placebo, or GlucaGen and the trial will be conducted in a double-blinded manner. The randomized parallel treatment design with administration of fixed SC doses of dasiglucagon, placebo, or GlucaGen to subjects with T1DM and insulin-induced hypoglycemia allows for direct comparison of the clinical efficacy of the treatments.

The trial is double-blind to increase trial validity and to reduce bias during evaluation of the treatments. However, since the trial medications are not identical in appearance, the handling, preparation and administration of trial medication will be done by unblinded trial personnel who will not be involved in any other trial procedures or assessments. See Section 6.6 for more information about which assessments are blinded and which are not, with reasons.

A superiority trial design is used because the aim is to show that treatment with dasiglucagon is an effective treatment compared to placebo. The secondary objective was chosen to allow a comparison between treatment with dasiglucagon and an established comparator treatment for severe hypoglycemia, GlucaGen.

Administration of glucagon is intended to quickly raise blood glucose levels in subjects with T1DM with insulin-induced hypoglycemia. Therefore, in order to assess the clinical efficacy of dasiglucagon as compared to placebo and GlucaGen following a single SC dose, the primary endpoint and secondary efficacy endpoints involve the measurement of plasma glucose concentrations at different timepoints.

4.2.2 Justification for drug, route, dosage and treatment duration

Dasiglucagon and GlucaGen will be administered as 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 approved dose for treatment of severe hypoglycemia. Data from the studies conducted to date with dasiglucagon, including the phase 2 trial in subjects with T1DM, have been used to establish that 0.6 mg of dasiglucagon is an effective dose and also represents a therapeutically equivalent dose to 1 mg of GlucaGen (see also Section 6.1).

Dasiglucagon, placebo, and GlucaGen will be administered in the abdomen, buttocks, or thigh by SC injection, as this is one of the intended routes of administration for dasiglucagon, besides IM and IV.

Subjects will be followed for at least 28 days after dosing in order to perform an adequate immunogenicity evaluation of treatment.

5 TRIAL POPULATION

5.1 Rationale for trial population

Dasiglucagon is in development for the treatment of severe hypoglycemia in insulin-dependent patients with diabetes mellitus. In the present trial, subjects with T1DM are included in the evaluation of efficacy and safety of dasiglucagon under hypoglycemic conditions as this is part of the intended target population. Subjects with T1DM are selected to avoid the endogenous glucagon counter-regulatory response to insulin-induced hypoglycemia that is present in patients with T2DM. The inclusion and exclusion criteria are set to include a trial population that represents the general population of subjects with T1DM.

5.2 Planned sample size and number of trial centers

A total number of 156 subjects with T1DM are expected to complete the treatment visit (Visit 2). To qualify as completed, the subject must be dosed and have blood drawn for the PK and PD assessments described in the protocol. It is expected that up to 176 subjects will be randomized to have 156 subjects completing Visit 2. Completion of 156 subjects (78 subjects in the dasiglucagon group and 39 subjects in each of the placebo and GlucaGen groups) would be expected to provide adequate power for the primary efficacy evaluation, as described in Section 9.1.

The planned date for first subject first visit is expected to take place in Q4, 2017 and the planned date for last subject last visit is expected to take place in Q3, 2018.

This trial is planned to be conducted at 4 to 6 trial sites in the United States of America [US], Canada, and Europe.

5.3 Inclusion criteria

Subjects will be entered into this trial only if they meet 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 subject).
2. Female or male subjects with T1DM for at least 1 year, diagnostic criteria as defined by the American Diabetes Association (3).
3. Treated with insulin for T1DM for at least 1 year and with stable insulin treatment (defined as no more than a 10-unit daily variation in total daily insulin dose) 30 days prior to screening
4. Hemoglobin A_{1c} <10%.

5. Aged between 18 and 75 years, both inclusive.
6. A female subject 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 and until last follow-up visit. Additionally, if the participant is using systemic contraceptives, she must use an additional form of acceptable contraception. An acceptable method of contraception includes one of the following:
        i. Abstinence from heterosexual intercourse;
        ii. Systemic contraceptives (birth control pills, injectable/implant/ insertable hormonal birth control products, transdermal patch);
        iii. Intrauterine device (with and without hormones); or
        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).
7. A male subject must meet the following criteria: Surgically sterilized or willing to refrain from sexual intercourse from screening and until last follow-up visit or, if sexually active, uses condom and partner practices contraception during the trial from screening and until last follow-up visit.

5.4 Exclusion criteria

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

1. Previously treated with dasiglucagon (previously referred to as ZP4207).
2. Known or suspected allergy to trial product(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, are actively attempting to get pregnant, or are lactating.
6. History of hypoglycemic events associated with seizures in the last year prior to screening.
7. History of severe hypoglycemia (defined as plasma glucose <54 mg/dL [3.0 mmol/L]) in the last month prior to screening.
8. Receipt of any investigational drug within 3 months prior to screening.
9. Active malignancy within the last 5 years.
10. Congestive heart failure, New York Heart Association class II-IV.
11. Inadequately treated blood pressure, defined as systolic ≥160 mmHg or diastolic ≥90 mmHg) at screening (15).

12. Current bleeding disorder, including anti-coagulant treatment.
13. Known presence or history of pheochromocytoma (i.e. adrenal gland tumor) or insulinoma (i.e. insulin secreting pancreas tumor).
14. Use of a daily systemic beta-blocker drug, indomethacin, warfarin or anticholinergic drugs in the previous 28 days before Day 1 of this trial.
15. Aspartate aminotransferase (AST) or alanine aminotransferase (ALT) >2.5 × the upper limit of the normal range (ULN), bilirubin >1.5 × ULN, estimated glomerular filtration rate <30 mL/min/1.73 m^2 according to the Modification of Diet in Renal Disease study definition (16), or altered electrolyte values of clinical relevance for cardiac conduction, as judged by the investigator.
16. Clinically significant abnormal ECG at screening as judged by the investigator.
17. Clinically significant illness within 4 weeks before screening, as judged by the investigator.
18. Donation of blood or plasma in the past month, or in excess of 500 mL within 12 weeks prior to screening.
19. Surgery or trauma with significant blood loss within the last 2 months prior to screening.
20. 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.
21. Subjects with mental incapacity or language barriers which 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.
22. Any condition interfering with trial participation or evaluation or that could be hazardous to the subject.
23. The use of prescription or non-prescription medications known to cause QT prolongation.

5.5 Dosing day exclusion criteria

Subjects who meet one or more of the following dosing day exclusion criteria at the dosing visit will be excluded from the dosing visit, but the visit can be rescheduled 1-7 days later. The dosing visit can only be rescheduled once.

1. Atypically strenuous exercise within 4 days prior to dosing, as judged by the investigator. Exercise during the trial should follow subject's typical routine, and should not exceed a near maximum intensity for more than 20 minutes per day, or moderate intensity for more than 90 minutes per day.
2. Clinically significant illness within 4 weeks before dosing, as judged by the investigator.

3. Consumption of alcohol within 24 hours prior to dosing visit, or positive results from an alcohol breath test.
4. Not fasting from 22:00 hours the evening prior to dosing, apart from water. Small amounts of carbohydrates (up to 20 g) to prevent hypoglycemia are allowed.
5. The use of any non-prescribed systemic or topical medication, except routine vitamins and occasional use (as judged by the investigator) of acetylsalicylic acid and paracetamol within 2 weeks prior to dosing. Treatment with insulin, including analogs, is allowed.
6. Use of any basal insulin within 16 hours prior to dosing.
7. Use of any short acting (bolus) insulin within 6 hours prior to dosing, except insulin glulisine (Apidra®).
8. Changes in medical history or concomitant medication resulting in fulfillment of clinical exclusion criteria, as judged by the investigator.
9. Plasma glucose value <50 mg/dL (2.8 mmol/L) within the last 24 hours or plasma glucose value <60 mg/dL (3.3 mmol/L) within the last 5 hours prior to dosing.

5.6 Premature treatment discontinuation and withdrawal

Participation in the trial is strictly voluntary. A subject 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 subject at any time if the investigator deems it in the subject's best interest. The reason and circumstances for withdrawal will be documented in the electronic case report form (eCRF).

5.6.1 Possible reasons for treatment visit discontinuation

A subject will be discontinued from treatment if the following applies:

- Withdrawal of consent by subject.
- If a protocol deviation occurs which, in the clinical judgement of the investigator, can invalidate the trial or endpoints or can interfere pharmacokinetically or pharmacodynamically with the trial product, the subject will be discontinued by the investigator.
- Adverse events occur which are considered unacceptable by the subject or the investigator.

If discontinuation occurs following administration of trial medication, every effort should be made to have the subject return and participate in the complete follow-up visit on Day 28 (see Table 3) to avoid missing data.

If trial participation is terminated due to an AE possibly related to any of the trial medications or trial examinations, the subject 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.

A total of 156 subjects must complete the treatment visit (Visit 2). To qualify as completed, the subject must be dosed and have blood drawn for the PK and PD assessments described in the protocol.

5.6.2 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 subjects within the agreed time frame.
- The center does not respond to trial management requests.
- Repeat protocol violations.

5.6.3 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 subjects.
- New scientific knowledge becomes known that makes the objectives of the trial no longer feasible/valid.
- Unsatisfactory enrolment of subjects.

5.7 Subject identification and randomization

Subjects who have given written informed consent and meet all inclusion and none of the exclusion criteria will be randomized in a 2:1:1 ratio to either 0.6 mg dasiglucagon (n=78), placebo (n=39), or 1 mg GlucaGen (n=39) via an Interactive Web Response System (IWRS). Randomization will continue until 156 subjects have completed Visit 2.

Subjects 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 subject is taking, the subject code can be broken by the investigator, preferably after consultation with the medical monitor. Emergency code breaks can be performed using the IWRS.

6 TRIAL DRUG

6.1 Identity

The following trial drugs will be administered:

- Dasiglucagon, liquid formulation, 1 mg/mL, 0.6 mL delivered in a prefilled syringe.
- Placebo, liquid formulation, 0.6 mL delivered in a prefilled syringe.
- Recombinant glucagon hydrochloride, 1 mg for reconstitution (GlucaGen®, Novo Nordisk) in 1 mL sterile water.

The description of the three trial drugs is provided in Table 1. Dasiglucagon is a stable peptide analog of human glucagon in a ready-to-use liquid formulation for treatment of severe hypoglycemia in insulin-dependent patients with diabetes mellitus. 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.

Table 1 Description of trial drugs

	Test product	Placebo Product	Comparator product
Name	Dasiglucagon	Placebo	GlucaGen®
Active substance	Dasiglucagon	N/A	Recombinant glucagon hydrochloride
Formulation	Liquid formulation, 0.6 mL	Liquid formulation, 0.6 mL	Powder and solvent for reconstitution as 1 mL solution for injection
Strength	1 mg/mL	N/A	1 mg
Device	Single use pre-filled syringe	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	Zealand Pharma A/S, Glostrup (Copenhagen), Denmark	Novo Nordisk A/S, Bagsværd, Denmark

	Test product	Placebo Product	Comparator product
Storage requirements	Store between 2 and 8°C	Store between 2 and 8°C	Store between 2 and 8°C

The quantities of ingredients for dasiglucagon and placebo are provided in Table 2.

Table 2 Quantities of ingredients in dasiglucagon and placebo injection

Component	Amount per mL (dasiglucagon)	Amount per mL (placebo)	Function
Dasiglucagon*	1.0 mg	N/A	[redacted]
Sodium chloride	10.23 mg	10.23 mg	
Trometamol/Tromethamine	6.06 mg	6.06 mg	
Water for injection	To make 1 mL	To make 1 mL	
Sodium hydroxide	q.s.	q.s.	
Hydrochloric acid	q.s.	q.s.	

*The quantity of drug substance to be used is calculated according to net peptide content and purity.
q.s. = quantum sufficit (quantity required).

6.2 Treatment assignment and randomization

Subjects successfully completing screening and who fulfill entry eligibility and randomization criteria will be randomized to one of three treatment groups in a ratio of 2:1:1:

- Test treatment: Dasiglucagon, liquid formulation, 1 mg/mL, 0.6 mL delivered in a prefilled syringe.
- Placebo treatment: Placebo, liquid formulation, 0.6 mL delivered in a prefilled syringe.
- Reference treatment: Recombinant glucagon hydrochloride, 1 mg for reconstitution.

Randomization will be performed using a fixed-block randomization scheme. The randomization scheme will be generated prior to the initiation of the study by an independent statistician/programmer who will not be a member of the study team; all investigators will not be aware of the block size of the randomization scheme. Randomization will be stratified by treatment group and by injection site (abdomen, buttocks, or thigh) and controlled via the IWRS.

Subjects will be randomized to study treatment using an interactive, automated system which has been validated for the intended use under the International Society of Pharmaceutical Engineers Good Automated Manufacturing Process guidelines, 21CFR Part 11 (FDA regulation for Electronic Records and Electronic Signatures) and the International Conference on Harmonization (ICH) Guidance E6 for Industry on Good Clinical Practice (GCP).

6.3 Administration

Dasiglucagon, placebo, and GlucaGen will be administered by SC injection in the abdomen, buttocks, or thigh.

An unblinded person (appropriately trained) authorized to prepare the dose and administer the treatment in accordance with the randomization will prepare the treatment required for each subject on each dosing day. The dose will be administered by the unblinded, trained and qualified person. The content of the syringe has to be checked for clarity and absence of bubbles. To ensure proper administration, the unblinded person should administer the SC injection at a 90 degree angle by grasping skin between the thumb and first finger. The injection should be given within 5 seconds.

Syringes will be discarded after dose administration. Used GlucaGen vials will be stored in a lockable box (separated from unused vials) at ambient temperature.

6.4 Packaging and labelling

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

The study drug label will describe the storage conditions for study drug. The labels will supply no information about the subjects. Each treatment kit (pre-filled syringe/vial for reconstitution) will have a unique Dispensing Unit Number for drug allocation, drug accountability, and traceability purposes.

Labelling will be performed according to Annex 13 of the Good Manufacturing Practice guidelines of the European Commission, ICH GCP guidelines, and local law.

6.5 Storage of study drugs

The investigator must ensure the availability of proper storage conditions. All study drug supplies provided for this study will be stored in a secure area with restricted access at the study site.

The temperature should be monitored by recording the actual, minimum, and maximum temperatures using a calibrated thermometer or thermocouple, or by continuous recording using a qualified temperature monitoring system. The temperature should be evaluated and documented at least on working days on a temperature log. This log must be included in the Investigator Site File upon study termination.

Dasiglucagon and placebo must be stored in a refrigerator (at a temperature of 2–8°C), and should be handled in accordance with guidelines from the sponsor. GlucaGen must also be stored in a refrigerator (at a temperature of 2–8°C), and should be handled in accordance with the Summary of Product Characteristics (13).

The unblinded person responsible for study drug handling must contact the unblinded monitor in case of temperature deviations outside the acceptable range.

Please see the Pharmacy Manual for additional information on handling study drug.

6.6 Blinding and breaking the blind

This is a double-blind trial. As the trial products are not identical in appearance, dasiglucagon and placebo being available as a liquid formulation and GlucaGen as a powder for reconstitution, unblinded trial personnel will be responsible for handling, preparing (according to the prescription from the IWRS), and administering the trial medication, as well as for keeping the records strictly confidential and accessible only to unblinded staff until after the database has been locked. To maintain double-blind conditions, all trial assessments at the trial center will be done by blinded trial personnel not involved in the administration of trial medications. However, exposure assessments and anti-drug antibody (ADA) assessments will be performed by unblinded personnel at the special laboratories, to ensure that dasiglucagon, placebo, or GlucaGen administration is matched with the applicable bioanalytical assay.

Treatment assignment will be kept strictly confidential and accessible only to authorized persons until after the time of unblinding. Codes with treatment assignment will, however, be readily available in the IWRS to the blinded personnel in case of an emergency.

The code for a particular subject may be broken in a medical emergency if knowing the identity of the treatment allocation would influence the treatment of the subject. The emergency code break can be performed using the IWRS. Whenever a code is broken, the person breaking the code must record the time, date and reason as well as his/her initials in the source documents. The breaking of blinded codes in case of medical emergency for one subject should not unblind the trial personnel to the treatment information of other subjects. The person performing the unblinding should inform as few people as possible

about the result of the unblinding. All persons unblinded for a specific subject should be documented.

If the trial center needs to break the code, the medical monitor should, if at all possible, be contacted prior to breaking the code and the monitor must be notified within 24 hours after the code has been broken.

The pharmacovigilance unit (safety contract research organization [CRO]; see the list of trial personnel in Section 12.1) will be able to break the code in case of a serious unexpected suspected adverse reaction (SUSAR).

The central and specialty laboratories will be provided with a copy of the randomization list.

6.7 Drug accountability

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 for destruction. This does not apply to the used syringes as they will be discarded after dose administration. Destruction must not take place until approved by the Sponsor.

6.8 Treatment compliance

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

PK assessments will support the surveillance of compliance with IMP administration.

6.9 Prior and concomitant medications

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

Subjects using any new concomitant medication resulting in fulfillment of a dosing day exclusion criterion (see Section 5.5) will be excluded from the dosing visit, but can be rescheduled to one of the following days (1–7 days later). The dosing visit can only be rescheduled once.

6.9.1 Prohibited medications

The use of anti-coagulant treatments and medications (prescription and non-prescription) that are known to cause QT prolongation are prohibited during the course of the trial.

Within 28 days prior to dosing, the use of daily systemic beta-blockers, indomethacin, warfarin, and anticholinergic drugs is prohibited.

Within 2 weeks prior to dosing, the use of any non-prescribed systemic or topical medication (with the exception of vitamins and the occasional use of acetylsalicylic acid and paracetamol) is prohibited.

Within 16 hours prior to dosing, the use of any basal insulin is prohibited.

Within 6 hours prior to dosing, the use of any short acting (bolus) insulin, except insulin glulisine (Apidra®) is prohibited.

During the insulin-induced hypoglycemic procedure, continuous SC insulin infusion must be stopped.

7 PARAMETERS AND METHODS OF ASSESSMENT

Overall, approximately 180 mL of blood will be drawn from each subject for PK, PD, ADA, and safety laboratory assessments.

7.1 Efficacy parameters

A description of the sample handling and sample processing at the site will be included in the laboratory manuals. Validation documentation for the assays must be available prior to sample analyses. A bioanalytical report for each analysis of trial PD and PK samples will be prepared.

7.1.1 Pharmacodynamic measurements

The plasma glucose profile for evaluation of the primary and secondary clinical efficacy endpoints will be assessed based on plasma concentration data ($AUC_{0\text{-}30min}$) from samples collected at the dosing visit (Visit 2). The samples will be sent to the clinical laboratory and analyzed using a sensitive and validated assay for glucose measurements.

Samples will be collected pre-dose, and at 4, 6, 8, 10, 12, 15, 17, 20, 25, 30, 40, 50, 60, 75, and 90 minutes after dosing (see the schedule of procedures in Table 3). The actual time of blood sampling for evaluation of plasma glucose should not deviate from the nominal time by more than ±30 seconds until the 20 minute collection time point and by more than ±1 minute for the subsequent collection time points. Pre-dose is defined as within 2 minutes prior to dosing.

7.1.2 Pharmacokinetic measurements

The exposure to trial medication (dasiglucagon or GlucaGen) for evaluation of PK will be assessed based on plasma concentration data ($AUC_{0\text{-}90\ min}$, C_{max}, t_{max}) from samples collected at the dosing visit (Visit 2).

Samples (including back-up samples) will be collected pre-dose, and at 15, 30, 35, 40, 50, 60, 90, and 120 minutes after dosing (see the schedule of procedures in Table 3). The actual time of blood sampling for exposure to trial medication should not deviate from the nominal time by more than ±1 minute. Pre-dose is defined as within 2 minutes prior to dosing.

7.2 Safety parameters

7.2.1 Adverse events

The investigator is responsible for the detection and documentation of events meeting the criteria and definition of an AE or serious adverse event (SAE), as provided in this protocol. During the trial, the investigator or center staff will be responsible for reporting AEs and SAEs, as detailed in this section of the protocol.

Based on the investigator's clinical judgment it will be determined whether an AE is related to treatment and of sufficient severity to require the subject's removal from treatment or from the trial. A subject may also voluntarily withdraw from treatment due to what he or she perceives as an intolerable AE. If either of these situations arises, the subject should be under medical supervision until symptoms cease or the condition becomes stable.

7.2.1.1 Definitions

Adverse event

An AE is any untoward medical occurrence in a trial subject given an IMP which does not necessarily have a causal relationship with the treatment. An AE can therefore be any unfavorable and/or 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.

In this trial, only treatment-emergent adverse events (TEAEs) will be collected and reported. TEAEs are events that occur from the first trial-related activity after the subject has signed the informed consent form until the end of the post-treatment follow-up period.

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 7.2.2).
- Injection site reactions (see Section 7.2.6).

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

- Pre-planned procedure, unless the condition for which the procedure was planned has worsened from the first trial related activity after the subject 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

An 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 events may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above.

Adverse drug reaction

All noxious and unintended responses to an investigational product related to any dose should be considered adverse drug reactions. The phrase responses to an investigational product means that a causal relationship between an investigational product and an adverse event is at least a reasonable possibility, i.e. the relationship cannot be ruled out.

Suspected unexpected serious adverse reactions (SUSARs)

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. dasiglucagon Investigator's Brochure or package leaflet/Summary of Product Characteristics for GlucaGen).

Adverse event of special interest

An AESI 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 AESIs:

- Post-dose clinical signs, or measured vital signs, indicating a clinically significant drop in blood pressure including signs of orthostatic hypotension, vasovagal responses or bradycardia.
- Post-dose 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 AE 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 AE 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 subject.

Severe: A type of AE 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 an SAE may not necessarily 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 subject 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 subject signed the informed consent.
Recovering/resolving:	The condition is improving and the subject is expected to recover from the event. This term is only applicable if the subject has completed the trial.
Recovered/resolved with sequelae:	The subject 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 subject has not improved and the symptoms are unchanged.
Fatal:	This term is only applicable if the subject died from a condition related to the reported AE. Outcomes of other reported AEs in a subject 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 subject is lost to follow-up.

7.2.1.2 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 subject has signed the informed consent until the end of the post-treatment follow-up period. At each contact with the center (visit or telephone, excluding visits where the subject is not seeing the investigator or his/her staff [e.g. visits to the laboratory]) the subject must be asked about AEs. All AEs, either observed by the investigator or reported by the subject, 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.

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 AESIs, the AESI 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 during IMP administration 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 AESI, the investigator must tick the AESI box on the AE form and complete the AESI form. The AESI 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 all AESIs to the sponsor's responsible pharmacovigilance unit (Safety CRO; see the list of trial personnel in Section 12.1) immediately (within 24 hours) after obtaining knowledge about the event.

The investigator must report initial information electronically (e.g. in PDF format) on all SAEs to the sponsor's responsible pharmacovigilance unit (Safety CRO; see the list of trial personnel in Section 12.1) immediately (within 24 hours) after obtaining knowledge about the event. 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 (see Section 7.2.1.1) that occur in this trial to the Competent Authorities and Institutional Review Boards (IRBs)/Independent Ethics Committees (IECs) in accordance with the local requirements in force and ICH guideline for GCP. The trial monitor must be informed accordingly.

7.2.1.3 Follow-up of adverse events

All AEs that are ongoing at the end of the subject's participation in the trial will be followed-up until the event is resolved or reaches a satisfactory outcome as deemed by the investigator or until the last visit of the last subject enrolled in the trial, whichever occurs first.

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 an SAE.

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

The sponsor and/or CROs acting on behalf of the sponsor can upgrade a non-serious AE to an SAE. In these situations the investigator will be informed and asked to fill out an SAE form and forward to the Safety CRO immediately (within 24 hours).

7.2.1.4 Clinical laboratory abnormalities and other abnormal assessments as adverse events or serious adverse events

Abnormal laboratory findings (e.g. biochemistry, hematology, urinalysis) or other abnormal assessments (e.g. vital signs) that are judged by the investigator as **clinically significant** will be recorded as AEs or SAEs if they meet the definition of an AE or SAE. Clinically significant abnormal laboratory findings or other abnormal assessments that are detected during the trial or are present at baseline and significantly worsen following the start of the trial will be reported as AEs or SAEs. However, clinically significant abnormal laboratory findings or other abnormal assessments that are associated with the disease being studied, unless judged by the investigator as more severe than expected for the subject's condition, or that are present or detected at the start of the trial and do not worsen, will not be reported as AEs or SAEs.

The investigator will exercise his or her medical and scientific judgment in deciding whether an abnormal laboratory finding or other abnormal assessment is clinically significant.

7.2.2 Hypoglycemia

Hypoglycemia will be regarded as an AE and recorded and documented on an AE form (and SAE form, if applicable).

Hypoglycemia is defined as a decline in plasma glucose to below 70 mg/dL (3.9 mmol/L). However, in the time period from initiation of the hypoglycemic clamp procedure (see Section 8.2.3.1) until 45 minutes after dosing, hypoglycemia is defined as a decline in plasma glucose to below 45 mg/dL (2.5 mmol/L).

During the dosing visit, prior to administration of the IMP, a plasma glucose value of <45 mg/dL (2.5 mmol/L) will be treated by an IV glucose solution. After administration of the IMP in the period from 8 minutes until 44 minutes after dosing, a plasma glucose value of <45 mg/dL (2.5 mmol/L) will be treated by an IV glucose solution, and if a plasma glucose value of ≥70 mg/dL (3.9 mmol/L) is not achieved within the 45 minutes after IMP administration, IV glucose infusion will also be initiated.

If the subject experiences symptoms of hypoglycemia, a plasma glucose measurement should be taken in order to classify the event (please refer to Section 7.4.2 for additional details).

7.2.3 Physical examination

The physical examination will be carried out at screening (Visit 1) and at the follow-up visit (Visit 3; see Table 3).

The physical examination includes examination of the following body systems: head, ears, eyes, nose, throat, including the thyroid gland; heart, lung, chest; abdomen; skin and mucosae; musculoskeletal system; nervous system; lymph node; other findings.

At the screening visit, any abnormality will be recorded and described in the eCRF, including the investigator's assessment of clinical significance ('abnormal, not clinically significant' or 'abnormal, clinically significant'). Clinically significant findings at the screening visit should be recorded as concomitant illness.

At subsequent visits, any clinically significant deterioration of a pre-existing condition as well as any new clinically significant findings will be recorded as AEs (see Section 7.2.1).

7.2.4 Vital signs

An examination of the following vital signs will be performed at screening (Visit 1), the dosing visit (Visit 2) and at the follow-up visit (Visit 3):

- Diastolic and systolic blood pressure (mmHg) will be measured after at least 5 minutes rest in a supine position. At Visit 1, blood pressure will be measured in both arms. The blood pressure from the arm with the higher systolic value is

transcribed into the eCRF and this arm should be used for all subsequent measurements of the subject's blood pressure in this trial.
- Pulse (beats per min) measured after at least 5 minutes rest in a supine position.
- Body temperature (°C).

At the dosing visit, measurements will be taken prior to the start of the insulin-induced hypoglycemic procedure (within 30 minutes) and at 30, 90, and 300 minutes after dosing (see Table 3). The actual time of the assessment should not deviate from the nominal time by more than ±10 minutes. In addition to the pre-specified assessments as listed in Table 3, blood pressure and pulse may be assessed at any time during the trial at the discretion of the investigator.

7.2.5 Electrocardiogram

A standard 12-lead ECG will be performed at the screening visit (Visit 1), the dosing visit (Visit 2; prior to the start of the insulin-induced hypoglycemic procedure [within 30 minutes]) and at 20, 35, 45, 60, and 300 minutes after dosing) and at the follow-up visit (Visit 3; see Table 3). The actual time of the assessment should not deviate from the nominal time by more than ±5 minutes.

ECG parameters (heart rate, PQ, QRS, QT, QTcB) and any abnormality will be recorded and described in the eCRF including the investigator's assessment of clinical significance ('abnormal, not clinically significant' or 'abnormal, clinically significant').

At subsequent visits, any clinically significant deterioration of a pre-existing condition as well as any new clinically significant findings will be recorded as AEs (see Section 7.2.1).

7.2.6 Local tolerability

Immediately prior to treatment administration, it should be verified that the injection site is normal. To ensure all injection site assessments are performed at the injection site, the site will be marked with a pen prior to injection. Assessment of local tolerability at the injection site will be performed at the dosing visit (Visit 2; at 30, 120 and 300 minutes after dosing), and at the follow-up visit (Visit 3) (see Table 3) and more frequently, if deemed necessary by the investigator. The actual time of the assessment should not deviate from the nominal time by more than ±10 minutes. In the eCRF, the time of assessment and any injection site reaction observed will be recorded. In case of an observation, the Local Tolerability form will be completed, as well as the (S)AE form.

The local tolerability at the injection site will be evaluated by means of the following assessments: spontaneous pain, pain on palpation, itching, redness, edema,

induration/infiltration, and other. Each of these assessments will be reported on a scale of 0 (none), 1 (mild), 2 (moderate), and 3 (severe). The evaluation and the actual time of the assessment will be recorded. The assessments will be performed by a trial physician or nurse.

Digital pictures will be taken of the injection site at the time of identification, and thereafter as often as judged necessary by the investigator. The pictures should include a subject identifier, visit number, time after dosing, and a ruler for scaling.

7.2.7 Clinical laboratory assessments

The safety parameters that will be assessed at the clinical laboratory are listed in Table 3. Routine clinical laboratory tests will be performed centrally. Samples for clinical laboratory parameters (biochemistry, hematology, coagulation) will be collected at screening (Visit 1), the dosing visit (Visit 2, prior to the start of the insulin-induced hypoglycemic procedure [within 30 minutes]) and at 30 and 300 minutes after dosing), and at the follow-up visit (Visit 3). The actual time of the assessment should not deviate from the nominal time by more than ±10 minutes. Samples for glycated hemoglobin (HbA_{1c}) will be collected at screening only (Visit 1). Samples for urinalysis will be collected at screening (Visit 1), at the dosing visit (Visit 2, prior to the start of the insulin-induced hypoglycemic procedure [within 120 minutes]) and at the follow-up visit (Visit 3). The following parameters will be assessed:

- Clinical biochemistry: sodium, potassium, calcium, glucose, urea, creatinine, total bilirubin, AST, ALT, gamma-glutamyltransferase, 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 (leukocytes).
- Coagulation: international normalized ratio, fibrinogen (at screening visit only).
- Urinalysis: pH, blood (leukocytes and erythrocytes), protein, glucose, ketones, nitrite.

Pregnancy tests will be performed at each visit for women of childbearing potential only. A serum pregnancy test will be performed at screening (Visit 1) and urine stick tests will be performed at the dosing visit (Visit 2, prior to the start of the insulin-induced hypoglycemic procedure) and the follow-up visit (Visit 3). Test sticks will be provided to the trial centers.

Alcohol breath tests and a urine drug screen will be performed at screening (Visit 1) and at the dosing visit (Visit 2, prior to the start of the insulin-induced hypoglycemic procedure). Equipment for the alcohol breath test and uring drug screen will be provided to the trial centers.

Re-assessment of laboratory parameters will be allowed only if handling issues, damaged samples, or hemolyzed samples have confounded the measurement results.

For further details of the clinical laboratory assessments, please refer to the laboratory manual.

7.2.8 Pregnancy

Female subjects 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 subjects must be instructed to notify the investigator immediately if their partner becomes pregnant or suspects pregnancy. The sponsor has a responsibility to monitor the outcome of all pregnancies reported during the clinical trial. During an information meeting at the trial center, the subject'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 immediately (within 24 hours), according to the procedure stated in Section 7.2.1.2. 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 follow 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 7.2.1.2. Any (S)AEs in the newborn must be reported on the (S)AE form.

The SAEs that must be reported include 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 until the age of 1 month.

7.2.9 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 subjects. During a subject's participation in the trial, the investigator should ensure that adequate medical care is provided to the subject for any AEs, including clinically significant laboratory values related to the trial. The investigator should inform the subject when medical care is needed for intercurrent illnesses of which the investigator becomes aware.

For further information on safety precautions for dasiglucagon and GlucaGen, please refer to the current version of the Investigator's Brochure (14) and the Summary of Product Characteristics for GlucaGen (13), respectively.

7.2.10 Safety committee

The internal Zealand Pharma Safety Committee is constituted to perform ongoing blinded 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 Safety Committee meetings, or on notification of significant findings, the Safety Committee will take appropriate measures to safeguard the subjects.

7.3 Demography, concomitant illness, medical history and concomitant medication

Demographics, body measurements, concomitant illness and medical history will be assessed only at screening (Visit 1). Concomitant medication will be assessed at screening (Visit 1), the dosing visit (Visit 2, prior to the start of the insulin-induced hypoglycemic procedure) and at the follow-up visit (Visit 3).

7.3.1 Demography and body measurements

Subject demographics and body measurements will include:

- Age
- Race, ethnicity
- Sex
- Height (meters or inch), without shoes
- Body weight (kg or lb), only wearing underwear and measured using standard scales
- Body mass index (kg/m^2) calculated based on height and body weight (body weight/height^2).

7.3.2 Concomitant illness and medical history

A concomitant illness is any illness that is present at the start of the trial (i.e. at the screening visit). Concomitant illnesses present at the start of the trial will be recorded in the eCRF at screening.

Medical history is an account of medical events that the subject has experienced in the past, including prior medications. Relevant medical conditions/illnesses in the past obtained by asking the subject or by inspecting his/her medical records will be recorded in the eCRF at screening. History of alcohol or drug abuse will also be recorded.

The information collected for concomitant illness and medical history should include diagnosis, date of onset, date of resolution or continuation.

Any change to a concomitant illness should be recorded during the trial, including end date, if applicable. A clinically significant worsening of a concomitant illness must be reported according to Section 7.2.1.

All previous and concomitant diseases will be coded with Medical Dictionary for Regulatory Activities (MedDRA), the version of which will be provided in the clinical trial report.

7.3.3 Diabetes diagnosis and current treatment

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

7.3.4 Concomitant medication

A concomitant medication is any medication, other than the trial products and current diabetes treatment (including insulin glulisine [Apidra®] for diabetes therapy wash-out), which is taken during the trial, including screening and follow-up periods.

Details of any concomitant medication must be recorded at trial entry (i.e. at screening). Any changes in concomitant medication must be recorded at each visit as they occur. The information collected for each concomitant medication includes (at a minimum) trade name or generic name, indication, start date and stop date or continuation. A change in medication due to an AE must be recorded and reported according to Section 7.2.1. If the change in medication influences the subject's eligibility to continue in the trial, the sponsor and trial monitor must be informed.

7.4 Other assessments

7.4.1 Immunogenicity

Antibodies against dasiglucagon/GlucaGen will be measured at the dosing visit (Visit 2) and at follow-up (Visit 3). At the dosing visit (Visit 2), samples will be collected prior to the start of the insulin-induced hypoglycemic procedure.

The clinical ADA assays, specific for dasiglucagon and GlucaGen, respectively, have been validated in accordance with existing guidelines and recommendations (17-21).

Confirmed positive anti-dasiglucagon antibody samples (treatment-induced or treatment-boosted) from anti-dasiglucagon antibody-positive subjects 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 neutralizing antibody analysis.

The in vitro neutralizing effect of the antibodies will be measured using an assay based on glucagon receptor transfected human embryonic kidney cells (20,22). The calculated sensitivity in previous studies was about 51.8 ng/mL. The assay was also validated for recombinant glucagon with similar results (21,23). In case of a positive result in the neutralizing antibody assay, a titer estimation will be performed. The cell-based neutralizing antibody analyses will be performed by a special laboratory, BioAgilytix, Durham, NC, USA.

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.

7.4.2 Plasma glucose measurements for safety

During insulin-induced hypoglycemia, plasma glucose levels will be monitored closely at site. After the start of insulin infusion, plasma glucose should be checked every

10 minutes while plasma glucose is above 110 md/dL, and every 5 minutes once plasma glucose is at or below 110 mg/dL and until after dosing when the subject's plasma glucose is ≥70mg/dL (3.9 mmol/L). Hereafter, plasma glucose should be checked every 30 minutes until 300 minutes (5 hours) after dosing. Plasma glucose concentrations will be measured using a US FDA-approved glucose analyzer (e.g., YSI 2300, Yellow Springs Instruments, Yellow Springs, OH).

At the discretion of the investigator, additional plasma glucose measurements can be taken at any time during the trial, for example when there is a suspicion (e.g. symptoms) of a hypoglycemic episode.

Plasma glucose measurements for safety should only be recorded in the eCRF if they are related to an AE (e.g. a hypoglycemic episode).

In case of persistent post-treatment hypoglycemia, subjects will receive rescue treatment with an IV glucose infusion (see Section 8.2.3.1 for details). Blood samples for PD and PK assessments should still be taken at the specified timepoints.

7.4.3 Plasma insulin measurements

Samples for insulin assessment will be collected at the dosing visit (Visit 2, pre-dose and at 30 and 60 minutes after dosing). The actual time of blood sampling for evaluation of plasma insulin should not deviate from the nominal time by more than ±1 minute. Pre-dose is defined as within 2 minutes prior to dosing.

8 TRIAL CONDUCT

8.1 Schedule of procedures

The schedule of procedures is provided in Table 3. Informed consent will be obtained prior to any trial-related procedures; see Section 10.8.

8.2 Procedures by visit

8.2.1 Visit 1 (screening, Day -30 to Day -3)

Visit 1 will take place between 3 and 30 days before Visit 2, Day -1 to Day 1 (dosing day).

Informed consent can be obtained prior to or at Visit 1, however it must in any case be obtained prior to any trial related procedures. During the screening visit, the following assessments will take place:

- Informed consent (obtain or check)
- Check of subject eligibility (inclusion/exclusion criteria)
- Demography
- Body measurements
- Medical history, diabetes diagnosis, current diabetes treatment
- Concomitant illnesses
- Concomitant medications
- History of alcohol/drug abuse
- Physical examination
- Vital signs
- 12-lead ECG
- AEs
- Biochemistry, hematology, coagulation, HbA_{1c}
- Serum pregnancy test (women of childbearing potential only)
- Urinalysis
- Urine drug screen
- Alcohol breath test

Table 3 Schedule of procedures

Visit number	V1	V2	V3
Trial day	**-3**	**-1 and 1**	**28**
Visit type	**Screening**	**Dosing**	**Follow-up**
Window	**-30 to -3**		**+5 days**
Subject related information/assessments			
Informed consent	x		
Inclusion/exclusion criteria	x	x[1,2]	
Demography	x		
Body measurements	x		
Medical history, diabetes diagnosis, and current diabetes treatment	x		
Concomitant illnesses	x		
Concomitant medications	x	x[1]	x
History of alcohol/drug abuse	x		
Randomization		x[1]	
Withdrawal criteria		x[1]	
Dosing day exclusion criteria		x[1]	
Insulin-induced hypoglycemia		x	
Safety assessments			
Physical examination	x		x
Vital signs	x	x[3]	x
12-lead ECG	x	x[4]	x
Local tolerability		x[5]	x
Adverse events	x	x	x
Laboratory			
Biochemistry, hematology, coagulation, HbA_{1c} (HbA_{1c} at Visit 1 only)	x	x[6]	x
Pregnancy test (women of childbearing potential only)	x[7]	x[1,8]	x[8]
Urinalysis	x	x[1]	x
Urine drug screen	x	x[1]	
Alcohol breath test	x	x[1]	
PK/Clinical efficacy			
Plasma dasiglucagon/GlucaGen		x[9]	
Plasma glucose		x[10]	
Other assessments			
Antibodies against dasiglucagon/GlucaGen		x[1]	x[11]
Plasma insulin		x[12]	
Trial material			
Administration of trial product (during hypoglycemic clamp procedure)		x	

[1]Prior to the start of the insulin-induced hypoglycemic procedure.
[2]Only check of dosing day exclusion criteria and changes between screening visit and Visit 2.
[3]Prior to the start of the insulin-induced hypoglycemic procedure (within 30 minutes), and at 30, 90 and 300 minutes after dosing. The actual time of the assessment should not deviate from the nominal time by more than ±10 minutes.

[4]Prior to the start of the insulin-induced hypoglycemic procedure (within 30 minutes), and at 20, 35, 45, 60 and 300 minutes after dosing. The actual time of the assessment should not deviate from the nominal time by more than ±5 minutes.
[5]Local tolerability assessed at 30, 120, and 300 minutes after dosing. The actual time of the assessment should not deviate from the nominal time by more than ±10 minutes.
[6]Prior to the start of the insulin-induced hypoglycemic procedure (within 30 minutes), and at 30 and 300 minutes after dosing. The actual time of the assessment should not deviate from the nominal time by more than ±10 minutes.
[7]Serum pregnancy test.
[8]Urine stick pregnancy test.
[9]Pre-dose, and at 15, 30, 35, 40, 50, 60, 90, and 120 minutes after dosing. The actual time of blood sampling should not deviate from the nominal time by more than ±1 minute. Pre-dose is defined as within 2 minutes prior to dosing.
[10]Pre-dose, and at 4, 6, 8, 10, 12, 15, 17, 20, 25, 30, 40, 50, 60, 75, 90 minutes after dosing. The actual time of blood sampling should not deviate from the nominal time by more than ±30 seconds until the 20 minute collection time point and by more than ±1 minute for the subsequent collection time points. Pre-dose is defined as within 2 minutes prior to dosing.
[11]Any subject that tests positive for ADA will be monitored until the ADA levels return to baseline levels.
[12]Pre-dose, and at 30 and 60 minutes after dosing. The actual time of blood sampling should not deviate from the nominal time by more than ±1 minute. Pre-dose is defined as within 2 minutes prior to dosing.
ADA = anti-drug antibodies; ECG = electrocardiogram; HbA_{1c} = glycated hemoglobin.

Eligible or potentially eligible subjects (laboratory results pending) will be provided with an Identification card (ID card), stating that the subject is participating in the trial and whom to contact (site address, investigator name and telephone number). The subjects should be instructed to return the ID card to the investigator at the last visit or to destroy the card after the last visit.

8.2.2 Instructions to subjects prior to the dosing visit (Visit 2)

At the screening visit, the investigator will inform the subject about the changes to his/her insulin therapy leading up to the start of the insulin-induced hypoglycemic procedure. The subject's current insulin therapy will be washed out as defined in Section 5.5: 16 hours prior to dosing and during the dosing visit, treatment with any basal insulin is not allowed; 6 hours prior to dosing and during the dosing visit, treatment with any short acting (bolus) insulin, except insulin glulisine (Apidra®), is not allowed. The basal rate of insulin pumps (continuous SC insulin infusion) will be discontinued on the morning of the dosing day OR 6 hours prior to dosing.

On the day prior to dosing (Day -1), the subjects will need to attend the clinical center and will be required to stay onsite overnight. On the morning of the dosing day (Day 1), patients are required to be in a fasting condition, defined as having consumed only water since 22:00 hours the night before. However, the subjects are allowed to consume small amounts (up to 20 g) of carbohydrates to prevent hypoglycemia. The subjects must also not consume any alcohol within 24 hours prior to dosing (refer to Section 5.5 for all dosing day exclusion criteria).

8.2.3 Visit 2 (dosing visit)

Visit 2 will take place on Day -1 to Day 1.

The subjects will attend the clinical center the day prior to dosing (Day -1) and should stay onsite overnight. Dosing will take place the following morning (Day 1).

On Day 1 and prior to the start of the insulin-induced hypoglycemic procedure, subject eligibility is rechecked (check of changes between the screening visit and Visit 2) and those subjects eligible to participate will be randomized to treatment with dasiglucagon, placebo, or GlucaGen.

The following assessments will also take place:

- Document all changes in concomitant medication (prior to the start of the insulin-induced hypoglycemic procedure)
- Check of withdrawal criteria (prior to the start of the insulin-induced hypoglycemic procedure)
- Check of dosing day exclusion criteria (prior to the start of the insulin-induced hypoglycemic procedure)
- Vital signs (prior to the start [within 30 minutes] of the insulin-induced hypoglycemic procedure, and at 30, 90 and 300 minutes after dosing). Blood pressure should be measured first in sitting and then in standing.
- 12-lead ECG (prior to the start [within 30 minutes] of the insulin-induced hypoglycemic procedure, and at 20, 35, 45, 60, and 300 minutes after dosing)
- Local tolerability (at 30, 120, and 300 minutes after dosing)
- AEs
- Biochemistry, hematology, coagulation (prior to the start of the insulin-induced hypoglycemic procedure [within 30 minutes], and at 30 and 300 minutes after dosing)
- Urine stick pregnancy test (women of childbearing potential only; prior to the start of the insulin-induced hypoglycemic procedure)
- Urinalysis (prior to the start of the insulin-induced hypoglycemic procedure [within 120 minutes])
- Urine drug screen (prior to the start of the insulin-induced hypoglycemic procedure)
- Alcohol breath test (prior to the start of the insulin-induced hypoglycemic procedure)
- Dasiglucagon/GlucaGen plasma concentrations:
  - Pre-dose, 15, 30, 35, 40, 50, 60, 90, and 120 minutes after dosing. The actual time of blood sampling for exposure should not deviate from the nominal time by more than ±1 minute. Pre-dose is defined as within 2 minutes prior to dosing.
- Plasma glucose concentrations:
  - Pre-dose, 4, 6, 8 ,10, 12, 15, 17, 20, 25, 30, 40, 50, 60, 75, and 90 minutes after dosing. The actual time for blood sampling for plasma glucose should not deviate

from the nominal time by more than ±30 seconds until the 20 minute collection time point and by more than ±1 minute for the subsequent collection time points. Pre-dose is defined as within 2 minutes prior to dosing.

- Antibodies against dasiglucagon/GlucaGen (prior to the start of the insulin-induced hypoglycemic procedure).
- Plasma insulin concentrations:
  - Pre-dose, 30 and 60 minutes after dosing. The actual time of blood sampling for plasma insulin should not deviate from the nominal time by more than ±1 minute. Pre-dose is defined as within 2 minutes prior to dosing.

8.2.3.1 Hypoglycemic clamp procedure and administration of trial medication

The following procedure is based on precendent procedures for hypoglycemia induction in patients with T1DM [24, 25].

The treatment day (Visit 2, Day 1) will be conducted after an overnight fast of at least 8 hours, targeting a starting plasma glucose around 90 to 110 mg/dL (5.0-6.1 mmol/L).

Subjects who meet one or more of the dosing day exclusion criteria (Section 5.5) at the dosing visit will be excluded from the dosing visit, but the visit can be rescheduled 1-7 days later. The dosing visit can only be rescheduled once.

The subject's current insulin therapy will be washed out as defined in Section 5.5. For subjects using multiple daily injections, the date, time and the dose of the last basal insulin administration prior to dosing will be captured.

At approximately 08:00 hours, an infusion catheter will be inserted into each arm (forearm cephalic vein) for the manual glucose clamp procedure, with the glucose infusion in one arm and the insulin infusion in the opposite arm. A third catheter for blood sampling will be placed into a metacarpel vein for blood sampling. This hand will be warmed (55-65ºC) to arterialize venous blood.

Hypoglycemia will be gradually induced by a fast-acting IV insulin glulisine (Apidra®) infusion (15 U [100 U/mL] in 49 mL saline and 1 mL of subject's blood or plasma), initially at 150% of the subject's usual basal rate and can be increased or decreased over a range of 75% to 200% as judged necessary by the investigator, to achieve a controlled decline in plasma glucose, targeting a plasma glucose level of 55 mg/dL (3.1 mmol/L).

Plasma glucose concentrations will be measured using a US FDA-approved glucose analyzer (e.g., YSI 2300, Yellow Springs Instruments, Yellow Springs, OH). After the start of the insulin infusion, plasma glucose will be measured every 10 minutes while

plasma glucose is above 110 mg/dL, and every 5 minutes once plasma glucose is at or below 110 mg/dL.

Once the glucose concentration declines to <60 mg/dL (3.3 mmol/L), the insulin infusion will be stopped, and 5 min later plasma glucose concentration will be measured at the glucose analyzer and blood samples for baseline assessment of plasma glucose, dasiglucagon/GlucaGen PK, and insulin PK will be collected. The samples are the baseline samples and should be collected within 2 minutes before IMP administration.

- If plasma glucose is >45 mg/dL and <60 mg/dL (2.5-3.3 mmol/L), study treatment (IMP) will be administered, defining time, t=0. The study treatment will be delivered in the abdomen, buttock, or thigh (according to stratification) via SC injection, with the subject lying in a lateral recumbent position.
- If plasma glucose is <45 mg/dL (2.5 mmol/L), IV glucose solution will be administered sufficient to raise plasma glucose to within the 45-60 mg/dL target range. The run-in period will be adequately extended (at least 30 min) until the above target is achieved and new baseline samples for plasma glucose, dasiglucagon/GlucaGen PK, and insulin PK will be collected. Glucose should not be infused within 10 minutes before IMP administration. If plasma glucose is not within target range after the second attempt, the subject should be rescheduled for a new treatment visit within 7 days (+ 2 days).

Administration of IMP should not occur earlier than 9:00 hours in the morning or later than 12:00 hours.

As shown in Table 4, serial blood samples for glucose will be collected at t=0, 4, 6, 8, 10, 12, 15, 17, 20, 25, 30, 40, 50, 60, 75 and 90 minutes post-dosing. Samples for assessing plasma dasiglucagon/GlucaGen concentration will be collected at t=0, 15, 30, 35, 40, 50, 60, 90, and 120 minutes. Samples for assessing plasma insulin concentration will be collected at t=0, 30, and 60 minutes.

Table 4 Post-treatment blood sampling times

Times	0	4	6	8	10	12	15	17	20	25	30	35	40	50	60	75	90	120
Plasma glucose	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y		Y	Y	Y	Y	Y	
PK Dasiglucagon/ GlucaGen	Y						Y				Y	Y	Y	Y	Y		Y	Y
PK Insulin	Y										Y				Y			

Refer to Section 7.2.7 for details of laboratory safety sampling and to Section 7.4.2 for details of blood glucose safety sampling.

When the t=90-minute blood sampling for plasma glucose has been collected the subjects are allowed to eat moderately. Drinking of water is allowed *ad libitum* during the entire procedure.

Hypoglycemia Rescue Provisions

During insulin-induced hypoglycemia, plasma glucose levels will be monitored closely at site for safety reasons as described in Section 7.4.2. Subjects should receive post-treatment rescue glucose infusion to ameliorate persistent hypoglycemia, as follows.

1. Glucose infusion should be initiated if a subject experiences severe alarming escalation of symptoms of hypoglycemia (e.g. symptoms suggesting a change in consciousness) at any time during the trial; glucose infusion should be initiated targeting a plasma glucose levels >70 mg/dL.
2. If plasma glucose is <45 mg/dL (2.5 mmol/L) between t=8 and t=44 minutes, rescue glucose infusion (1-2 mg/kg administered IV over about 5 seconds) should be initiated to maintain plasma glucose between 45 mg/dL and 55 mg/dL (2.5-3.1 mmol/L). Pause glucose infusion if plasma glucose is >55 mg/dL.
3. If plasma glucose is <70 mg/dL at t=45 minutes, rescue glucose infusion (2-3 mg/kg administered IV over about 5 seconds) should be initiated to maintain plasma glucose between 70 mg/dL and 80 mg/dL (3.9-4.4 mmol/L). Pause glucose infusion if plasma glucose is >75 mg/dL (4.2 mmol/L).

Subjects should remain in bed until completion of the test procedure 300 minutes after dosing (bathroom visits are allowed).

The IMP will be administered SC according to Section 6.3. The time of IMP administration will be recorded. At the timepoint when the insulin infusion is stopped, the total insulin dose which was required to induce hypoglycemia will be recorded.

AEs will be specifically recorded during the procedure at several timepoints.

The investigator must provide information to the subjects on how to resume their usual diabetes treatment.

The subject may be released from the clinical center if the investigator does not have any safety concerns based on the last safety plasma glucose value and the general condition of the subject. However, at the discretion of the investigator or on request of the subject, the subject may stay at the trial center for a longer period.

8.2.4 Visit 3 (follow-up visit)

Visit 3 will take place on Day 28 + 5 days. The subject does not need to be fasting.

At Visit 3, the following assessments will take place:

- Document all changes in concomitant medication
- Physical examination
- Vital signs
- 12-lead ECG
- Local tolerability
- AEs
- Biochemistry, hematology, coagulation
- Urine stick pregnancy test (women of childbearing potential only)
- Urinalysis
- Antibodies against dasiglucagon/GlucaGen.

After the follow-up visit the End of Trial form must be completed. Even if a subject is not able to attend the follow-up visit, the End of Trial form, the eCRF Accountability/Affirmation Statement form and the Drug Accountability form must be completed.

9 STATISTICAL METHODS

Before database lock and treatment unmasking, a separate statistical analysis plan (SAP) will be finalized, providing detailed methods for the analyses outlined below. Further analysis details may be added or refined in the SAP.

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

9.1 Determination of sample size

Due to requirements in the size of the safety database, the sample size is set to 78 subjects treated with dasiglucagon 0.6 mg. With a 2:1:1 randomization ratio for the three treatments, dasiglucagon 0.6 mg, placebo, and GlucaGen 1 mg, 78 subjects injected with dasiglucagon and 39 subjects with placebo will yield a power of 90% at a 5% two-sided significance level to detect a treatment group difference in recovery incidence within 20 minutes, assuming as low as 80% and as high as 50% recovery incidence for the dasiglucagon and placebo groups, respectively.

9.2 Trial subjects

9.2.1 Analysis samples

For presentation of data and reporting of the statistical analyses, the following analysis samples will be used, depending on the context:

- Safety analysis set (SAS): All randomized subjects who received at least one dose of trial medication.
- Full analysis set (FAS): All randomized subjects who received at least one dose of trial medication and contributed valid information for at least one post-dose endpoint.
- Per protocol (PP) set: All subjects of the FAS for whom no relevant protocol deviations were documented. This sample will primarily be used for sensitivity analysis.

The analysis of the primary endpoint and secondary endpoints will be based on the FAS. A secondary analysis of the primary endpoint will be based on the PP set. All safety analyses will be based upon the SAS.

The decision regarding whether a protocol deviation is relevant or not for the exclusion of subjects from the PP set will be made case-by-case in a data review meeting prior to treatment unmasking and database lock (see Section 9.2.3).

9.2.2 Disposition of subjects

Subject disposition will be tabulated including the number of screened subjects, screening failures, subjects exposed to trial product, subjects completing the trial and subjects in each analysis set.

Subjects withdrawn from the trial will be listed including the primary reason for withdrawal.

9.2.3 Protocol deviations

Before data are released for statistical analysis, a treatment-masked review of all data will take place to identify protocol deviations that may potentially affect the results. This review will be performed without revealing to which trial product the subjects were assigned. The masking of the trial products will be maintained for everyone involved in allocating subjects to the analysis sets until data are released for statistical analysis. Furthermore, spurious outliers will be evaluated. In addition, protocol deviations that may potentially affect the results will be identified and it will be evaluated if subjects and/or data should be excluded from the analysis. Protocol deviations will be classified as minor or major in a consistent way. Major deviations from the protocol may lead to the exclusion of a subject from the PP set.

Major protocol deviations may include deviations related to trial inclusion or exclusion criteria, conduct of the trial, subject management or subject assessment. Unless explicitly decided otherwise during the masked data review, the following will be considered major protocol deviations:

- Violation of one or more major inclusion/exclusion criteria
- Interruption of administration of trial product
- Significant deviation from time windows
- Incorrect treatment allocation
- Missing primary endpoint.

The violation of several major inclusion/exclusion criteria or the complete absence of efficacy data might lead to exclusion of the subject from FAS. In that case, the decision should be taken at the masked data review meeting, and the exclusion from efficacy analysis justified in the signed notes of the meeting.

Obviously erroneous data points may be excluded from the analyses or re-analyzed (in case of e.g. serum concentrations). The decision to re-analyze or exclude data points from the statistical analysis is the joint responsibility of the sponsor and the trial statistician.

The subjects or observations to be excluded and the reason for their exclusion will be documented and signed by those responsible prior to database release. The documentation will be stored together with the remaining trial documentation. The subjects and observations excluded from analysis sets, and the corresponding reasons, will be described in the clinical trial report.

9.3 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 timepoint will be included in the analysis.

Raw data listings and summary tables will be generated using the software SAS© version 9.4 or higher.

Continuous variables will be summarized using means, standard deviations, medians, coefficients of variation, and minimum and maximum values.

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

9.4 Demographics and baseline characteristics

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

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

9.5 Efficacy Analyses

For the confirmatory analyses, the primary and key secondary endpoints for the dasiglucagon 0.6 mg and placebo treated subjects will be compared. A hierarchical procedure will be applied for the control of multiplicity. The primary and secondary endpoints will be evaluated on the full analysis set. In the primary analysis, those subjects who require rescue IV glucose will be censored at the time to plasma glucose recovery. In a sensitivity analysis, the time to plasma glucose recovery will be analyzed without censoring the subjects who received rescue IV glucose.

9.5.1 Hierarchical testing procedure

For the confirmatory analyses, the following a priori defined hierarchical inferential test order will be applied for the control of the type 1 error rate across the planned multiple comparisons. The primary endpoint and key secondary endpoints will be inferentially evaluated within the FAS in the following order, where inference will proceed at the two-sided 0.05 criterion significance level until the first failure to reject the null hypothesis for a dasiglucagon versus placebo comparison:

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

The GlucaGen versus placebo comparisons will not be included in the inferential testing hierarchy, since the efficacy of GlucaGen is previously established, and these comparisons are intended to support the validity of the study for the dasiglucagon versus placebo comparisons.

Analogous supportive sensitivity analyses will be conducted in the PP set, but without inference intent.

9.5.2 Primary confirmatory endpoint

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

9.5.2.1 Primary analysis

The primary endpoint will be summarized using Kaplan-Meier (KM) estimates stratified by treatment group and injection site. The treatment group difference between dasiglucagon and placebo will be evaluated inferentially using a pairwise two-sided log-rank test.

In the primary analysis, those subjects who require rescue IV glucose will be censored at the time of plasma glucose recovery. This approach is based on the assumption that rescue IV glucose administration will shorten the time to recovery. This assumption implies that censoring at the time of recovey after rescue therapy is valid as a time to

recovery without rescue would be longer than the observed duration. If recovery has not occurred at 45 minutes after study drug injection, censoring will be applied irrespective of the use of rescue IV glucose.

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

The primary endpoint will additionally be analyzed using a Cox proportional hazards (CPH) time to event statistical model, if the hazards by treatment group can be assumed proportional using a graphical comparison of log-minus-log survival curves and no more than two-thirds of the recovery times are censored in each treatment group. The CPH model will be used for inferences, with treatment group and injection site modeled as categorical effects, and baseline plasma glucose modeled as a continuous covariate. The treatment group rate ratios, active versus placebo, will be estimated together with the 95% confidence intervals, and pairwise treatment group inferences (dasiglucagon vs placebo, GlucaGen vs placebo) will be evaluated using two-sided likelihood ratio tests.

9.5.3 Secondary endpoints

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

9.5.3.1 Confirmatory analysis

The key secondary incidence variables (plasma glucose recovery within 30 minutes, within 20 minutes, within 15 minutes, within 10 minutes, in hierarchical order for inference) will be analyzed using descriptive statistics (frequency and percentage) by treatment group. Inferential treatment group comparisons will be assessed via pairwise tests of independent binomial proportions (dasiglucagon versus placebo, GlucaGen versus placebo) using Fisher's exact test for each pairwise comparison.

The key secondary endpoints of plasma glucose CFB within 30 minutes, within 20 minutes, within 15 minutes, and within 10 minutes after study drug injection or at the time of rescue, will be analyzed with the plasma glucose CFB at rescue carried forward in those subjects who require rescue IV glucose before plasma glucose ≥20 mg/dL recovery. Each of these CFB variables will be analyzed using an Analysis of Covariance model, with treatment group modeled as a fixed effect and with the baseline plasma glucose modeled as a covariate. The dasiglucagon versus placebo treatment group difference will

be evaluated inferentially as a least squares means contrast, using a two-sided t-test at the 0.05 signficance level, first for the 30 minute CFB, and then sequentially for the 20 minute, 15 minute, and 10 minute variables, until the first failure to reject.

9.5.4 Secondary clinical efficacy (PD) endpoints

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

Secondary clinical efficacy endpoints will be assessed based on plasma concentration data from samples collected at the dosing visit (Visit 2).

Unless otherwise stated, the population base of analysis will be the FAS.

9.5.4.1 Analysis of secondary clinical efficacy (PD) endpoints

1. Time to first plasma glucose concentration ≥70 mg/dL from baseline. This time-to-event endpoint will be evaluated using a Kaplan-Meier approach, with treatment group as a stratification factor, analogous to that used for the primary endpoint analysis. Differences between the KM curves (dasiglucagon versus placebo, GlucaGen versus placebo) will be evaluated inferentially using pairwise two-sided log rank tests. If the ≥70 mg/dL endpoint is not met within 45 minutes post-dosing, the time of the last valid plasma glucose measurement up to 45 minutes will be the censoring time.
2. The AUC will be calculated as the baseline-adjusted area under the plasma glucose profile over time:
    a. $AUC_{0\text{-}30min}$: restricting the time window to the 0 to 30 minutes interval.
3. The log-transformed AUC endpoint will be analyzed using an analysis of covariance (ANCOVA) model with treatment as fixed effect and baseline plasma glucose modeled as a covariate. The least squares means treatment group differences will be back-transformed (anti-logged) for presentation as a ratio of the treatment group geometric means, with their corresponding 95% CI.

9.5.5 Exposure (PK) endpoints

- Plasma dasiglucagon and GlucaGen concentrations from time zero to 90 minutes: $AUC_{0\text{-}90min}$, C_{max}, and t_{max}.

9.5.5.1 Analysis of exposure (PK) endpoints

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

C_{max} will be determined as the maximum of all valid plasma dasiglucagon/GlucaGen concentrations.

T_{max} will be determined as the timepoint where the maximum of all valid plasma dasiglucagon/GlucaGen concentration measurements for each measurement series is observed.

The log-transformed PK endpoints AUC and C_{max} will be analyzed in the same way as the AUC endpoints.

As t_{max} is a highly discrete endpoint, Wilcoxon's rank sum test for unpaired observations will be used to assess differences between the two treatment groups.

9.6 Exploratory analyses

Exploratory analyses will include descriptive statistics and modeling analogous to that done for key secondary endpoints. However, treatment group comparisons will be summarized without inference intent.

- Plasma glucose concentration ≥70 mg/dL (3.9 mmol/L) or increase of ≥20 mg/dL (1.1 mmol/L) within 30 minutes after study drug injection without administration of rescue IV glucose.

- Plasma insulin response as area under the curve above baseline from time zero to 60 minutes, $AUC_{0\text{-}60\ min}$.

Plasma insulin concentrations measured pre-dose and at 30 and 60 minutes after dosing (see Table 3) will be presented individually. A summary table per timepoint will be provided. The $AUC_{0\text{-}60min}$ will be determined and a summary presented.

9.7 Safety analyses

9.7.1 Adverse events

AEs will be coded using the latest available version of the MedDRA, the version of which will be provided in the clinical trial report. AE summary tables will include the number and percentage of subjects who experienced AEs summarized by system organ class and preferred term.

An overall summary table will be provided showing the number and percentage of subjects with any:

- TEAE
- Severe TEAE
- Serious TEAE
- Drug-related TEAE
- Drug-related severe TEAE
- Drug-related serious TEAE
- TEAE leading to withdrawal
- TEAE with outcome death
- AESI

Refer to Section 7.2.1 for the definition of TEAEs.

9.7.2 Immunogenicity data

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

9.7.3 Clinical laboratory assessments

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

9.7.4 Other safety data

Incidence of rescue infusion of IV glucose during the hypoglycemic clamp procedure will be analyzed using descriptive statistics (frequency and percentage) by treatment group. Inferential treatment group comparisons will be assessed via pairwise tests of

independent binomial proportions (dasiglucagon versus placebo, GlucaGen versus placebo).

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

Vital signs, physical examination, 12-lead ECG and local tolerability data will be summarized using descriptive statistics.

9.8 Treatment compliance

Trained unblinded members of staff will perform all administrations of the IMP at the trial center. The administered doses will be recorded in the blinded Drug Accountability form in the eCRF.

PK assessments will support the surveillance of compliance with IMP administration.

9.9 Subject withdrawals and missing data

In the case of subject withdrawal, no imputation of values for PK or PD measurements will be done. Analyses will be done on valid cases only, i.e., no imputation techniques such as last observation carried forward will be applied. For the primary analysis in the FAS, missing values for the primary endpoint will be imputed by a conservative rule considering any missing value as a failure.

9.10 Interim analyses

No interim analyses are currently planned.

10 ETHICAL, LEGAL, AND ADMINISTRATIVE ASPECTS

10.1 Quality assurance

The sponsor or designee will conduct a site visit to verify the qualifications of each investigator, inspect the facilities, and inform the investigator of responsibilities and the procedures for ensuring adequate and correct documentation.

The investigator is required to prepare and maintain adequate and accurate case histories designed to record all observations and other data pertinent to the trial for each trial participant. All information recorded on the eCRFs for this trial must be consistent with the subjects' source documentation (i.e. medical records).

The investigator will permit trial-related monitoring, IRB/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 monitor or a representative of the sponsor will cross-check the data entered in the eCRFs with the source data at the trial center and observe the trial procedures in order to verify adherence to the trial protocol. Any queries will be resolved by the investigator or his/her delegate.

All of the clinical data will be captured via electronic data capture (EDC) using a web-based tool.

The investigator center 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 subjects. The investigator's data will be accessible from the investigator's center throughout the trial. The eCRFs must be kept current to reflect subject status at each phase during the course of the trial. The eCRF will not capture personalized data. The investigator must make a separate confidential record of personalized details (name and initials) on the subject identification and enrollment log. All changes to data are made by the investigator or his/her delegate through the EDC system.

It is the responsibility of the principal investigator of the respective center to ensure that all subject discontinuations or changes in trial or other medications entered on the subject's eCRF are also made on the subject's medical records.

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

10.2 Electronic case report forms

Remote data capture software will be used for data collection. Following training, trial staff will be given access to the software. Access to the software is restricted to staff participating in the trial and the extent of access will depend on the participants' user role in the trial.

The subjects enrolled into the trial will be identified in the database by subject number and trial identification code. The investigator or delegate will enter subject data into the eCRF promptly. All data is to be entered in English. Data recorded in the eCRFs will be accessible to the trial staff throughout the trial.

After data entry, systematic data validation will be performed and data entry discrepancies will be presented electronically directly to the center staff. Queries for discrepant data may be generated automatically by the software upon entry and/or generated manually by the trial monitor or the trial data manager. All queries, whether generated by the system or by trial staff, will be in electronic format.

All sections of the eCRF are to be electronically approved by the investigator or a medically qualified delegate after the data has been entered and all queries have been resolved. Changes to any eCRF page subsequent to the approval require a new approval signature.

All queries and changes/corrections to the data are documented in the eCRF.

10.3 Access to source data

During the course of the trial, a trial monitor will make site visits to review protocol compliance, compare eCRFs with individual subject’s medical records, assess drug accountability, and ensure that the trial is being conducted according to pertinent regulatory requirements.

Checking of the eCRFs for completeness and clarity, and cross-checking with source documents, will be required to monitor the progress of the trial. Moreover, regulatory authorities of certain countries, IRBs/IECs, and/or the sponsor may wish to carry out such source data checks and/or on-site audit inspections. Direct access to source data will be required for these inspections and audits; they will be carried out giving due consideration to data protection and medical confidentiality.

10.4 Source documentation

All source documents from which eCRF entries are derived should be placed in the subject's medical records. If data are to be entered directly into the eCRF this must be specified in a source data agreement prior to the start of the trial.

eCRF entries will be verified with source documentation. The review of medical records will be performed in a manner to ensure that subject confidentiality is maintained.

The trial monitor will check the eCRF for accuracy and completion and perform source data verification. The trial monitor will document source data verification of all reviewed sections of the eCRF.

10.5 Data processing

The trial is run as an EDC trial, i.e. all relevant data is entered by the centers directly into the clinical database. The eCRF is designed to capture all required information in compliance with GCP standards.

10.6 Archiving trial records

According to ICH guidelines, essential documents should be retained for a minimum of 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. However, these documents should be retained for a longer period if required by the applicable legal requirements.

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

10.7 Good clinical practice

The procedures set out in this trial protocol are designed to ensure that the sponsor and investigator abide by the principles of the GCP guidelines of the ICH (1), and of the Declaration of Helsinki (2008) (2). The trial also will be carried out in keeping with local legal requirements.

10.8 Informed consent

Before each subject is admitted to the trial, written informed consent will be obtained from the subject according to the regulatory and legal requirements of the participating country. This consent form must be dated and retained by the investigator as part of the trial records. The investigator will not undertake any investigation specifically required only for the clinical trial until valid consent has been obtained. The terms of the consent and when it was obtained must also be documented in the eCRF.

The investigator will explain to each subject orally and in writing (subject information sheet) the nature, significance, risks and implications of the trial before inclusion. In particular, the subjects 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.
- That his/her medical records may be examined by authorized monitors or clinical quality assurance auditors appointed by the sponsor, by appropriate IRB/IEC members, and by inspectors from regulatory authorities.

All subjects will receive a copy of the subject information sheet and a copy of their signed and dated informed consent form, both of which will be in the subject's local language.

If a protocol amendment is required, the informed consent form may need to be revised to reflect the changes to the protocol. If the consent form is revised, it must be reviewed and approved by the appropriate IEC/IRB, and signed by all subjects subsequently enrolled in the trial as well as those currently enrolled in the trial.

10.9 Protocol approval and amendment

Before the start of the trial, the trial protocol and/or other relevant documents will be approved by the IEC/IRB/competent authorities, in accordance with local legal requirements. The sponsor must ensure that all ethical and legal requirements have been met before the first subject is enrolled in the trial.

This protocol is to be followed exactly. To alter the protocol, amendments must be written, receive approval from the appropriate personnel, and receive IRB/IEC/competent authority approval prior to implementation (if appropriate).

Administrative changes (not affecting the subject benefit/risk ratio) may be made without the need for a formal amendment. All amendments will be distributed to all protocol recipients, with appropriate instructions.

10.10 Duration of the trial

The maximum duration of the trial for each subject will be up to 63 days (including up to 30 days for screening and up to 33 days until the follow-up visit).

The trial will be closed when all subjects have completed Visit 3.

10.11 Premature termination of the trial

If the investigator, the sponsor (e.g. safety committee), or the safety medical monitor becomes aware of conditions or events that suggest a possible hazard to subjects if the trial continues, the trial may be terminated after appropriate consultation between the relevant parties. The trial may also be terminated early at the sponsor's discretion in the absence of such a finding.

Conditions that may warrant termination include, but are not limited to:

- The discovery of an unexpected, significant, or unacceptable risk to the subjects enrolled in the trial
- Failure to enroll subjects at an acceptable rate.
- A decision on the part of the sponsor to suspend or discontinue development of the drug.

The trial can be terminated prematurely by the sponsor at an individual center if:

- The center cannot comply with the requirements of the protocol.
- It is not possible for the center to comply with GCP standards.

10.12 Confidentiality

All trial findings and documents will be regarded as confidential. The investigator and members of his/her research team must not disclose such information without prior written approval from the sponsor.

The anonymity of participating subjects must be maintained. Subjects will be identified on eCRFs submitted to the sponsor by their subject number. On the SAE reports and all other source documents, the subject will be identified via subject number. Documents not to be submitted to the sponsor that identify the subject (e.g. the signed informed consent form) must be maintained in confidence by the investigator.

10.13 Other ethical and regulatory issues

If a significant safety issue is identified, either from an individual case report or review of aggregate data, then the sponsor will issue prompt notification to all parties: regulatory authorities, investigators and IRB/IECs.

A significant safety issue is one that has a significant impact on the course of the clinical trial or program (including the potential for suspension of the trial program or amendments to protocols) or warrants immediate update of informed consent.

10.14 Liability and insurance

The sponsor will take out reasonable third-party liability insurance cover in accordance with all local legal requirements. The civil liability of the investigator, the persons instructed by him and the hospital, practice or institute in which they are employed and the liability of the sponsor with respect to financial loss due to personal injury and other damage that may arise as a result of the carrying out of this trial are governed by the applicable law.

The sponsor will arrange for liability insurance if subjects should be injured due to the participation in the trial and provided that the sponsor is legally liable for that.

Excluded from the insurance cover are injuries to health and deteriorations of illnesses already in existence which would have occurred or continued to exist even if the subject had not taken part in the clinical trial.

The insurance cover is jeopardized if the subject fails to report immediately to the investigator or responsible physician any injury to health, which might have resulted from participation in the clinical trial, or if he/she undergoes any other medical treatment without their consent before the clinical trial has been completely finished in so far as the individual subject is concerned.

Any injury to health, which might have occurred as a result of participation in the clinical trial must be reported by the subject to the investigator without delay. The investigator is obliged to make such a report in any case.

10.15 Publication policy

By signing the trial protocol, the investigator agrees with the use of results of the trial for the purposes of national and international registration, publication and information for medical and pharmaceutical professionals. If necessary, the authorities will be notified of the investigator's name, address, qualifications and extent of involvement.

A clinical trial report will be prepared and reviewed by the sponsor in co-operation with the coordinating investigator. The coordinatoing investigator will be appointed by Zealand Pharma to review and sign the clinical trial report on behalf of all participating investigators. A summary of the clinical trial report (according to CPMP/ICH137/95) should be sent to the regulatory authorities and IRB/IEC according to the relevant guidelines.

According to the Declaration of Helsinki (2) investigators and sponsors 'have ethical obligations with regard to the publication and dissemination of the results of research'.

The trial design and results may be published as one or more original research manuscripts/abstracts and presented at a scientific meeting. 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 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. Authorship on any publication(s) resulting from this clinical trial will be assigned according to the recommendations of the International Committee of Medicinal Journal Editors (26).

Participating subjects will not be identified by name in any published reports about the clinical trial.

The sponsor is responsible for trial registration at ClinicalTrials.gov (www.clinicaltrials.gov) according to FDA requirements, as well as the European Medicines Agency's Clinical Trials Database (EudraCT).

11 REFERENCE LIST

1. ICH Harmonised Tripartite Guideline. International Conference on Harmonisation. ICH Harmonised Tripartite Guideline. Integrated addendum to ICH E6(R1): Guideline for Good Clinical Practice E6 (R2), Step 4. 09-Nov-2016. Available from: URL: http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E6/E6_R2__Step_4.pdf
2. World Medical Association. Declaration of Helsinki. Ethical Principles for Medical Research Involving Human Subjects. 52nd WMA General Assembly, Edinburgh, Scotland, October 2000. Last amended with Note of Clarification on Paragraph 29 by the WMA General Assembly, Washington 2002, and Note of Clarification on Paragraph 30 by the WMA General assembly, Tokyo 2004
3. 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 May;36:1384-95
4. Cryer PE. The barrier of hypoglycemia in diabetes. Diabetes 2008;57:3169-76
5. American Diabetes Association Position Statement. Standards of medical care in diabetes - 2017. Diabetes Care 2017;40(Supplement 1):S1-S135
6. Davies MJ, Gagliardino JJ, Gray LJ, Khunti K, Mohan V, Hughes R. Real-world factors affecting adherence to insulin therapy in patients with Type 1 or Type 2 diabetes mellitus: a systematic review. Diabetic Medicine 2013;30:512-24
7. International Hypoglycaemia Study Group. Glucose concentrations of less than 3.0 mmol/L (54 mg/dL) should be reported in clinical trials: a joint position statement of the American Diabetes Association and the European Association for the Study of Diabetes. Diabetes Care 2017;40(1):155-57
8. Christensen M, Bagger JI, Vilsbøll T, Knop FK. The alpha-cell as target for type 2 diabetes therapy. Rev Diabet Stud 2011;8:369-81
9. Lund A, Bagger JI, Christensen M, Knop FK, Vilsbøll T. Glucagon and type 2 diabetes: the return of the alpha cell. Curr Diab Rep 2014;14:555
10. Habegger KM, Heppner KM, Geary N, Bartness TJ, DiMarchi R, Tschöp MH. The metabolic actions of glucagon revisited. Nat Rev Endocrinol 2010;6:689-97
11. Onoue S, Ohshima K, Debari K, Koh K, Shioda S, Iwasa S, et al. Mishandling of the therapeutic peptide glucagon generates cytotoxic amyloidogenic fibrils. Pharm Res 2004;21:1274-83

12. Fosgerau K, Hoffmann T. Peptide therapeutics: current status and future directions. Drug Discovery Today 2015;20:122-8
13. Novo Nordisk Limited. SPC for GlucaGen Hypokit 1 mg. 2015. http://www.medicines.org.uk/emc/medicine/4258/SPC/GlucaGen+Hypokit+1+mg - Accessed 13 Mar 2017 .
14. Zealand Pharma A/S. Dasiglucagon Investigator's Brochure, Edition 3, 12 Dec 2016.
15. The Task Force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). 2013 ESH/ESC guidelines for the management of arterial hypertension. European Heart Journal 2013;34:2159-219
16. Levey AS, Coresh J, Greene T, Stevens LA, Zhang Y, Hendriksen BA, et al. Using standardized serum creatinine values in the modification of diet in renal disease study equation for estimating glomerular filtration rate. Annals of Internal Medicine 2006;145:247-54
17. Food and Drug Administration (FDA). Guidance for Industry on Bioanalytical Method Validation. Food and Drug Administration (FDA) 2001
18. Viswanathan CT, Bansal S, Booth B, DeStefano AJ, Rose MJ, Sailstad J, et al. Quantitative bioanalytical methods validation and implementation: best practices for chromatographic and ligand binding assays. Pharm Res 2007 Oct;24:1962-73
19. Shankar G, Devanarayan V, Amaravadi L, Barrett YC, Bowsher 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;48:1267-81
20. Zealand Pharma report SS. Commissioning and validation of a method for the validation of anti-ZP4207 antibodies in human serum. 2014
21. Zealand Pharma report SS. Commissioning and validation of a method for the validation of anti-glucagon antibodies in human serum. 2014
22. Bioagilytix Labs. Validation of an assay to detect neutralizing antibodies to ZP4207 by measuring cyclic adenosine monophosphate (cAMP) in a glucagon receptor transfected cell line, BAL-14-206-004-REP. 2016. Report No.: Zealand Pharma, study 15-039
23. 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. 2016. Report No.: Zealand Pharma, Study 15-040

24. Amiel SA, Tamborlane WV, Simonson DC, et al. Defective glucose counterregulation after strict glycemic control of insulin-dependent diabetes mellitus. N Eng J Med 1987; 316: 1376-83.

25. Rickels MR, Ruedy KJ, Foster NC, et al. Intranasal glucagon for treatment of insulin-induced hypoglycemia in adults with type 1 diabetes: A randomized crossover noninferiority study. Diabetes Care 2016; 39: 264-70.

26. ICMJE. Recommendations for the conduct, reporting, editing, and publication of scholarly work in medical journals. Updated December 2016

12 APPENDICES

12.1 List of trial personnel

Sponsor **Clinical Trial Manager**	[redacted] Zealand Pharma Smedeland 36 2600 Glostrup, Denmark Phone: [redacted]
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	12-14, 4 emelet 1027 Budapest Hungary Phone: [redacted]
Pharmacovigilance unit Responsible for Serious Adverse Event (SAE) Management and 24-hour SAE reporting	[redacted] PharmaLex Agern Allé 24 2970 Hørsholm, Denmark Phone: [redacted] (8 a.m. to 4 p.m.) Phone: [redacted] (outside 8 a.m. to 4 p.m.) Fax: email: PV-nordic@pharmalex.com
Central laboratory	MLM Laboratory Dohrweg 63 41066 Mönchengladbach Germany
Special laboratory (GlucaGen PK, insulin PK)	MLM Laboratory Dohrweg 63 41066 Mönchengladbach Germany
Special laboratory (dasiglucagon PK, dasiglucagon ADA, GlucaGen ADA)	York Bioanalytical Solutions (YBS) Cedar House Northminster Business Park Northfield Lane York, YO26 6QR, United Kingdom
Special laboratory (neutralizing antibodies)	BioAgilytix 2300 Englert Drive Durham, NC, 27713, USA

A list of all investigators, IECs and IRBs will be provided in a separate document and in the clinical trial report.

CLINICAL TRIAL PROTOCOL

A phase 3, randomized, double-blind, parallel trial to confirm the clinical efficacy and safety of dasiglucagon in the rescue treatment of hypoglycemia in subjects with type 1 diabetes mellitus (T1DM) compared to placebo and with reference to GlucaGen®

Sponsor:	Zealand Pharma A/S
Sponsor Protocol No.:	ZP4207-16137
EudraCT No.:	2017-002449-31
IND No:	127866
Trial Drug Name:	Dasiglucagon* injection
Date of Protocol:	29 Aug 2017

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

*Dasiglucagon is the proposed international nonproprietary name for ZP4207.

Declaration of sponsor or responsible medical officer

Title: A phase 3, randomized, double-blind, parallel trial to confirm the clinical efficacy and safety of dasiglucagon in the rescue treatment of hypoglycemia in subjects with type 1 diabetes mellitus (T1DM) compared to placebo and with reference to GlucaGen®

This trial protocol 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 Declaration of Helsinki and the guidelines on Good Clinical Practice (GCP) (1).

[redacted]

Date

Title Clinical Trial Manager
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2600 Glostrup, Denmark
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[redacted]

Date

Title Medical Officer
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Declaration of the coordinating investigator

Title: A phase 3, randomized, double-blind, parallel trial to confirm the clinical efficacy and safety of dasiglucagon in the rescue treatment of hypoglycemia in subjects with type 1 diabetes mellitus (T1DM) compared to placebo and with reference to GlucaGen®

I have read this protocol. I approve this document and I agree that it contains all necessary details for carrying out the trial as described. I will conduct this trial in accordance with the design and specific provision of this protocol and will make a reasonable effort to complete the trial within the time designated. I will provide copies of this protocol and access to all information furnished by Zealand Pharma A/S to trial personnel under my supervision. I will discuss this material with them to ensure they are fully informed about the trial product and trial procedures. I will let them know that this information is confidential and proprietary to Zealand Pharma A/S and that it may not be further disclosed to third parties. I understand that the trial may be terminated or enrollment suspended at any time by Zealand Pharma A/S, with or without cause, or by me if it becomes necessary to protect the best interests of the trial subjects.

I agree that the trial will be carried out in accordance with GCP (1), with the Declaration of Helsinki (with amendments) (2) and with the laws and regulations of the countries in which the trial takes place.

_____ _____

Name Date
Title
Institution
Phone: +
Fax: +

Declaration of the investigator

Title: A phase 3, randomized, double-blind, parallel trial to confirm the clinical efficacy and safety of dasiglucagon in the rescue treatment of hypoglycemia in subjects with type 1 diabetes mellitus (T1DM) compared to placebo and with reference to GlucaGen®

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 this trial protocol, Investigator's Brochure, Case Report Forms, 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 subjects.

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

_____ _____

Signature Date

_____

Name (block letters)

_____

Title (block letters)

_____

Institution (block letters)

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DECLARATION OF THE INVESTIGATOR 4

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List of abbreviations and definitions of terms

ADA	Anti-drug antibody
AE	Adverse event
AESI	Adverse event of special interest
ALT	Alanine aminotransferase
ANCOVA	Analysis of covariance
AST	Aspartate aminotransferase
AUC	Area under the concentration-time curve
$AUC_{0\text{-inf}}$	AUC from time zero to infinity
AUE	Area under the effect curve
CI	Confidence interval
C_{max}	Maximum plasma concentration
CPH	Cox proportional hazards
CRO	Contract research organization
ECG	Electrocardiogram
eCRF	Electronic case report form
EDC	Electronic data capture
EU	European Union
EudraCT	European Medicines Agency's Clinical Trials Database
FAS	Full analysis set
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HbA_{1c}	Glycated hemoglobin
ICH	International Conference on Harmonization
ID card	Identification card
IEC	Independent Ethics Committee
IM	Intramuscular(ly)
IMP	Investigational medicinal product
IRB	Institutional Review Board
IV	Intravenous(ly)
IWRS	Interactive Web Response System

MedDRA	Medical Dictionary for Regulatory Activities
NPH	Neutral protamine Hagedorn
PD	Pharmacodynamics
PK	Pharmacokinetics
PP	Per protocol
SAE	Serious adverse event
SAP	Statistical analysis plan
SAS	Safety analysis set
SC	Subcutaneous(ly)
SUSAR	Serious unexpected suspected adverse reaction
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
TEAE	Treatment-emergent adverse event
t_{max}	Time to the maximum plasma concentration
ULN	Upper limit of the normal range
US	United States of America

1 SYNOPSIS

Name of sponsor: Zealand Pharma A/S	**Trial ID:** ZP4207-16137
Title of the trial: A phase 3, randomized, double-blind, parallel trial to confirm the clinical efficacy and safety of dasiglucagon in the rescue treatment of hypoglycemia in subjects with type 1 diabetes mellitus (T1DM) compared to placebo and with reference to GlucaGen®	
Trial design: The trial is a global, multicenter, randomized, parallel-group, double-blind, clinical trial confirming the efficacy and safety of dasiglucagon for insulin-induced hypoglycemia in subjects with type 1 diabetes mellitus. The subjects will be randomized 2:1:1 to receive a single fixed subcutaneous 0.6 mg dose of dasiglucagon (hereinafter dasiglucagon), placebo for dasiglucagon (hereinafter referred to as placebo), or a 1 mg dose of GlucaGen® (hereafter referred to as GlucaGen) and followed for at least 28 days after treatment.	
Clinical phase of development: Phase 3	
Trial centers: This trial will be conducted at 4 to 6 sites in the United States of America, Canada, and Europe.	
Planned trial start (first subject first visit): Q4/2017	**Planned trial end (last subject last visit):** Q3/2018
Trial population: Male and female adult subjects with type 1 diabetes mellitus treated with insulin for at least one year	
Key objectives: **Primary objective:** • To demonstrate superiority of dasiglucagon compared to placebo following a single subcutaneous 0.6 mg dose administered to subjects with type 1 diabetes mellitus with insulin-induced hypoglycemia. **Secondary objective:** • To compare the glycemic response observed after dasiglucagon with that of GlucaGen.	
Key endpoints: **Primary endpoint:** • Time to plasma glucose ≥20 mg/dL recovery, defined as first increase in plasma glucose of ≥20 mg/dL (1.1 mmol/L) from baseline during the hypoglycemic clamp procedure. In the primary analysis, those subjects who require rescue intravenous (IV) glucose will be censored at the time to plasma glucose recovery. In a sensitivity analysis, the time to plasma glucose recovery will be analyzed without censoring those subjects who require rescue IV glucose before 45 minutes. **Key secondary endpoints:** • Plasma glucose changes from baseline within 30 minutes, within 20 minutes, within 15 minutes, and within 10 minutes after study drug injection or at the time of rescue. • Incidences of plasma glucose recovery (achieving a plasma glucose concentration increase of ≥20 mg/dL [1.1 mmol/L]) within 30 minutes, within 20 minutes, within 15 minutes, and within 10 minutes after study drug injection.	

Clinical efficacy (Pharmacodynamic) endpoints:

- Time to first plasma glucose concentration of ≥70 mg/dL (3.9 mmol/L).
- Plasma glucose response as area under the curve above baseline from time zero to 30 minutes, $AUC_{0\text{-}30min}$.

Exposure (Pharmacokinetic) endpoints:

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

Safety endpoints:

- Adverse events, clinical laboratory assessments (biochemistry, hematology, coagulation, urinalysis), vital signs, physical examination, electrocardiogram, and local tolerability.
- Incidence of rescue infusion of IV glucose during the hypoglycemic clamp procedure.
- Time to first rescue infusion of IV glucose during the hypoglycemic clamp procedure.

Immunogenicity endpoint:

- Incidence of anti-drug antibodies

Exploratory endpoint:

- Incidence of plasma glucose recovery (achieving a plasma glucose concentration ≥70 mg/dL [3.9 mmol/L] or an increase of ≥20 mg/dL [1.1 mmol/L]) within 30 minutes after study drug injection.
- Plasma insulin response as area under the curve above baseline from time zero to 60 minutes, $AUC_{0\text{-}60\ min}$.

Key inclusion criteria:

- Male and female subjects with type 1 diabetes mellitus treated with insulin for at least one year, diagnostic criteria as defined by the American Diabetes Association.
- Stable insulin treatment 30 days prior to screening, defined as no more than a 10-unit daily variation in total daily insulin dose.
- Hemoglobin A_{1c} <10%.
- Aged between 18 and 75 years, both inclusive.

Key exclusion criteria:

- Previously treated with dasiglucagon.
- Known or suspected allergy to trial product(s) or related products.
- History of anaphylaxis or symptoms of severe systemic allergy (such as angioedema).
- Previous participation (randomization) in this trial.

Sample size:
Approximately 156 subjects are intended to complete the trial, with 78 subjects randomized to the dasiglucagon group and 39 subjects randomized to each of the placebo and GlucaGen groups.

Investigational medicinal product:
Test product: dasiglucagon liquid formulation in pre-filled syringes.
Comparator products: Placebo and GlucaGen® lyophilized powder.

Duration of treatment: Subjects will be randomized 2:1:1 to receive a single fixed subcutaneous dose of 0.6 mg dasiglucagon, placebo, or 1 mg GlucaGen, and followed for at least 28 days after receiving treatment.
Assessments: The plasma glucose profile for evaluation of the primary and secondary clinical efficacy (pharmacodynamic) endpoints will be assessed based on plasma concentration data from samples collected at the dosing visit. The exposure to trial medication (dasiglucagon, placebo, or GlucaGen) for evaluation of pharmacokinetics will also be assessed based on plasma concentration data.
Statistical methods: Due to requirements in the size of the safety database, the sample size is set to 78 subjects treated with dasiglucagon 0.6 mg. With a 2:1:1 randomization ratio for the three treatments, dasiglucagon 0.6 mg, placebo, and GlucaGen 1 mg, 78 subjects injected with dasiglucagon and 39 subjects with placebo will yield a power of 90% at a 5% two-sided significance level to detect a treatment group difference in recovery incidence within 20 minutes, assuming as low as 80% and as high as 50% recovery incidence for the dasiglucagon and placebo groups, respectively. For the confirmatory analyses, the primary and key secondary endpoints for the dasiglucagon 0.6 mg and placebo treated subjects will be compared. A hierarchical procedure will be applied for the control of multiplicity in statistical inferences. The primary and secondary endpoints will be evaluated on the Full Analysis Set sample. The statistical inference comparisons with placebo will be conducted as superiority tests. The comparisons of dasiglucagon versus GlucaGen will be summarized descriptively. The primary endpoint of time to plasma glucose ≥20 mg/dL recovery, is defined as first increase from baseline in plasma glucose of ≥20 mg/dL (1.1 mmol/L) during the hypoglycemic clamp procedure. If the hazards by treatment group can be assumed proportional using a graphical comparison of the Nelson-Aalen estimates, a Cox proportional hazards (CPH) model will be used for inferences, with treatment group and injection site modeled as categorical effects, and baseline plasma glucose modeled as a continuous covariate. The treatment group rate ratios, active versus placebo, will be estimated together with the 95% confidence intervals, and pairwise treatment group inferences (dasiglucagon vs placebo, GlucaGen vs placebo) will be evaluated using two-sided likelihood ratio tests. If the PH assumption for treatment groups is not met, the primary endpoint will be analyzed using a Kaplan-Meier (KM) time to event statistical model, with treatment group and injection site as stratification factors. Treatment group differences between the KM curves (dasiglucagon vs placebo, GlucaGen vs placebo) will be evaluated inferentially using pairwise two-sided log-rank tests. In the primary analysis, subjects who require rescue IV glucose will be censored at the time to plasma glucose ≥20 mg/dL recovery. In a sensitivity analysis, the time to plasma glucose ≥20 mg/dL recovery will be analyzed without censoring the subjects who received rescue IV glucose before 45 minutes. The key secondary endpoints of plasma glucose changes from baseline (CFB) within 30 minutes, within 20 minutes, within 15 minutes, and within 10 minutes after study drug injection or at the time of rescue, will be analyzed with the plasma glucose CFB at rescue carried forward in those subjects who require rescue IV glucose before plasma glucose ≥20 mg/dL recovery. Each of these CFB variables will be analyzed using an Analysis of Covariance model, with treatment group modeled as a fixed effect and with the baseline plasma glucose modeled as a covariate. The dasiglucagon versus placebo treatment group

difference will be evaluated inferentially as a least squares means contrast, using a two-sided t-test at the 0.05 significance level, first for the 30 minute CFB, and then sequentially for the 20 minute, 15 minute, and 10 minute variables, until the first failure to reject.

The key secondary incidence variables (plasma glucose recovery within 30 minutes, within 20 minutes, within 15 minutes, within 10 minutes, in hierarchical order for inference) will be analyzed using descriptive statistics (frequency and percentage) by treatment group. Inferential treatment group comparisons will be assessed via pairwise tests of independent binomial proportions (dasiglucagon vs placebo, GlucaGen vs placebo).

The continuous clinical efficacy variables, the exploratory variables, and the pharmacokinetics variables will each be summarized descriptively by treatment group. The clinical efficacy variables will be analyzed analogous to the plasma glucose CFB variables.

The safety analyses will include by-treatment-group descriptive summaries of vital sign measurements, laboratory measures (including immunogenicity incidence), physical examination assessments, rescue IV glucose (incidence and amount of glucose infused), and adverse events. The number and percentage of subjects reporting specific events, such as nausea and vomiting, will be presented by body system and preferred term.

Further details will be included in the Statistical Analysis Plan, to be completed before database lock and treatment unmasking.

2 INTRODUCTION

2.1 Background

Zealand Pharma A/S (Zealand Pharma) is developing dasiglucagon, a physically and chemically stable peptide analog of human glucagon, in a ready-to-use liquid formulation for the acute treatment of severe hypoglycemia in patients with insulin-treated diabetes mellitus. Like native glucagon, dasiglucagon is comprised of 29 amino acids, but with 7 substitutions which improve its physical and chemical stability in aqueous media. These amino acid substitutions make dasiglucagon suitable for a liquid formulation, while providing similar efficacy and safety as compared with recombinant glucagon in the clinical setting of acute treatment of severe hypoglycemia.

2.1.1 Hypoglycemia

Hypoglycemia in patients with diabetes is defined as episodes of an abnormally low plasma glucose concentration (3). This is a common, unpredictable, and potentially dangerous side effect of treatment of diabetes mellitus, especially with 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 (4).

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 (5).

The incidence of hypoglycemic events or even the fear of hypoglycemia influences patients' adherence to prescribed treatment regimens for diabetes mellitus (6). This leads to inadequate glycemic control, which in turn may lead to an increased risk of diabetic complications (5). Serious clinically significant hypoglycemia is now defined as plasma glucose <54 mg/dL (3.0 mmol/L), while the plasma glucose alert value is defined as <70 mg/dL (3.9 mmol/L) (7). When plasma glucose falls below these values, some kind of treatment strategy is needed.

2.1.2 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) (8,9). 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 (10). Insulin decreases blood glucose levels and cases of hypoglycemia can be reversed by glucagon. Therefore, glucagon is indicated for the treatment of severe hypoglycemia.

Besides intravenous (IV) glucose administration, an injectable form of glucagon is given as first aid in cases of severe hypoglycemia, when the patient is unconscious or for other reasons cannot take glucose orally. The approved glucagon dose for an adult is 1 mg, given by intramuscular (IM), IV, or subcutaneous (SC) injection, which quickly raises blood glucose levels. As current marketed recombinant glucagon is highly unstable when dissolved in solution, the injectable form has to be reconstituted prior to use in a 9-step procedure that requires a sterile diluent to be injected into a vial containing lyophilized powdered glucagon. When dissolved in a fluid state, glucagon can form amyloid fibrils (11), or tightly woven chains of proteins made up of the individual glucagon peptides. The reconstitution process makes the use of marketed glucagon products cumbersome (12), and a more patient-friendly formulation is needed. Currently, the Food and Drug Administration (FDA) approved instructions for commercially available glucagon allow only for immediate usage of glucagon after the powder is reconstituted in aqueous solution (13). Therefore, a glucagon analog with enhanced biophysical stability may represent a leap forward in terms of convenient therapeutic applications.

2.1.3 Dasiglucagon

Dasiglucagon (ZP4207) is a stable peptide analog of human glucagon, available in a ready-to-use liquid formulation. Dasiglucagon (hereinafter referred to as dasiglucagon) 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® (hereafter referred to as GlucaGen). Dasiglucagon exhibits improved physical and chemical stability and is available in an aqueous solution at neutral pH (14).

Three clinical trials have been completed with dasiglucagon: a first-in-human dose trial in healthy volunteers and subjects with T1DM (ZP4207-14013), a multiple-dose dose-escalation trial (ZP4207-15007) evaluating the safety, tolerability, pharmacokinetics (PK) and pharmacodynamics (PD) of dasiglucagon in healthy volunteers, and a phase 2 crossover trial to assess the PK and PD of a single dose of an optimized formulation of dasiglucagon administered SC in subjects with T1DM (ZP4207-15126) (14).

2.1.3.1 Pharmacokinetics and pharmacodynamics of dasiglucagon

The results of the phase 1 and 2 clinical trials confirmed dose-proportionality for dasiglucagon PK, 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 (t_{max}) was 35 minutes for dasiglucagon compared with 20 minutes for GlucaGen.

At all dose levels in the phase 2 trial, all subjects achieved a plasma glucose level of at least 70 mg/dL (3.9 mmol/L) as well as an increase in plasma glucose by at least 20 mg/dL (1.1 mmol/L) within 30 minutes after dosing. The PD responses to 0.6 mg of dasiglucagon and 1 mg of GlucaGen were comparable.

2.1.3.2 Safety of dasiglucagon

The safety data for dasiglucagon do not give rise to any safety concerns. No new signals were observed, 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 mg (4 with dasiglucagon and 1 with GlucaGen). This is not considered a safety concern; however, hemodynamic changes after dosing will be considered an adverse event of special interest (AESI).

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's Brochure (14).

2.2 Trial rationale

The aim of the current trial is to confirm the superiority of dasiglucagon for the treatment of insulin-induced hypoglycemia in subjects with T1DM as compared to placebo for dasiglucagon (hereinafter placebo) and to compare the clinical efficacy and safety of dasiglucagon with reference to GlucaGen. A randomized, controlled trial design was used.

See Section 4.2 for justification of the design of this trial.

2.3 Risk-benefit assessment

Non-clinical experience

The nonclinical development program did not reveal any safety findings that would prohibit administration of dasiglucagon to humans. None of the safety pharmacology studies, repeated dose toxicity studies or genotoxicity studies revealed any significant toxicity findings relevant to the therapeutic use of dasiglucagon.

Clinical experience

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 IMP 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 extent in the 3 clinical studies conducted to date with dasiglucagon. As with every novel drug substance, new and as yet unknown side effects may also 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 low immunogenic potential.

Overall, dasiglucagon is judged to be a low-risk molecule, based upon the available clinical data. Administration of dasiglucagon may be associated with a risk of allergic reactions similar to those observed for other therapeutic peptides or proteins. Subjects 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 centers.

With the exception of medical examinations, a subject 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.

The development program including 141 subjects exposed to dasiglucagon to date has demonstrated that administration of dasiglucagon is efficacious and well tolerated, with a safety profile that does not give rise to specific safety concerns. Two phase 1 and one phase 2 clinical trials have been conducted to investigate the safety, tolerability, PK and PD of dasiglucagon after single and multiple dosing to healthy volunteers and subjects with T1DM under insulin-induced hypoglycemic conditions. Dasiglucagon has proven to have relevant clinical effects in the acute severe hypoglycemia rescue setting and may be an effective and reliable emergency treatment for severe hypoglycemia. Overall, the anticipated benefits for subjects entering the ZP4207-16137 trial are considered to justify the risks.

3 TRIAL OBJECTIVES AND ENDPOINTS

3.1 Primary objective

- To demonstrate superiority of dasiglucagon compared to placebo following a single subcutaneous 0.6 mg dose administered to subjects with type 1 diabetes mellitus with insulin-induced hypoglycemia.

3.2 Secondary objectives

- To compare the glycemic response observed after dasiglucagon with that of GlucaGen.

3.3 Primary endpoint

- Time to plasma glucose ≥20 mg/dL recovery, defined as first increase in plasma glucose of ≥20 mg/dL (1.1 mmol/L) from baseline during the hypoglycemic clamp procedure. In the primary analysis, those subjects who require rescue intravenous (IV) glucose will be censored at the time to plasma glucose recovery. In a sensitivity analysis, the time to plasma glucose recovery will be analyzed without censoring those subjects who require rescue IV glucose before 45 minutes.

3.4 Key secondary endpoints

- Plasma glucose changes from baseline within 30 minutes, within 20 minutes, within 15 minutes, and within 10 minutes after study drug injection or at the time of rescue.
- Incidences of plasma glucose recovery (achieving a plasma glucose concentration increase of ≥20 mg/dL [1.1 mmol/L]) within 30 minutes, within 20 minutes, within 15 minutes, and within 10 minutes after study drug injection.

3.5 Other secondary endpoints

- Clinical efficacy (PD) endpoints:
  - Time to first plasma glucose concentration ≥70 mg/dL (3.9 mmol/L).
  - Plasma glucose response as area under the curve (AUC) above baseline from time zero to 30 minutes, $AUC_{0\text{-}30min}$.
- Exposure (PK) endpoints:
  - Area under the drug concentration curve from time zero to 90 minutes, $AUC_{0\text{-}90min}$.
  - Maximum plasma drug concentration (C_{max}).

- Time to maximum plasma drug concentration (t_{max}).

- Safety endpoints:
  - Adverse events, clinical laboratory assessments (biochemistry, hematology, coagulation, urinalysis), vital signs, physical examination, electrocardiogram (ECG), and local tolerability.
  - Incidence of rescue infusion of IV glucose during the hypoglycemic clamp procedure.
  - Time to first rescue infusion of IV glucose during the hypoglycemic clamp procedure.
- Immunogenicity endpoint:
  - Incidence of anti-drug antibodies

3.6 Exploratory endpoint

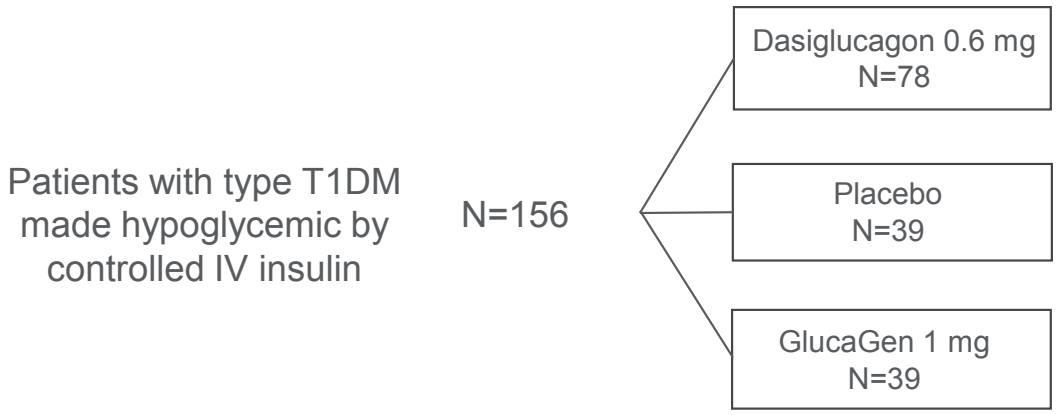
- Incidences of plasma glucose recovery (achieving a plasma glucose concentration ≥70 mg/dL [3.9 mmol/L] or an increase of ≥20 mg/dL [1.1 mmol/L]) within 30 minutes after study drug injection.
- Plasma insulin response as area under the curve above baseline from time zero to 60 minutes, $AUC_{0\text{-}60\ min}$.

4 OVERALL DESIGN AND PLAN OF THE TRIAL

4.1 Overview

This trial is a global, multicenter, randomized, parallel, double-blind, clinical trial confirming the efficacy and safety of dasiglucagon for insulin-induced hypoglycemia in subjects with T1DM. The subjects will be randomized 2:1:1 to receive a single fixed SC 0.6 mg dose of dasiglucagon, placebo, or a 1 mg dose of GlucaGen and followed for at least 28 days after receiving treatment. A total of 156 subjects with T1DM are expected to complete the treatment visit. The trial will be conducted in the European Union (EU) and North America.

Figure 1 Trial design



4.2 Justification of the trial design

4.2.1 Justification for design and parameters

In order to avoid bias in subject selection and in the evaluation of clinical assessments, subjects will be randomly assigned 2:1:1 to either dasiglucagon, placebo, or GlucaGen and the trial will be conducted in a double-blinded manner. The randomized parallel treatment design with administration of fixed SC doses of dasiglucagon, placebo, or GlucaGen to subjects with T1DM and insulin-induced hypoglycemia allows for direct comparison of the clinical efficacy of the treatments.

The trial is double-blind to increase trial validity and to reduce bias during evaluation of the treatments. However, since the trial medications are not identical in appearance, the

handling, preparation and administration of trial medication will be done by unblinded trial personnel who will not be involved in any other trial procedures or assessments. See Section 6.6 for more information about which assessments are blinded and which are not, with reasons.

A superiority trial design is used because the aim is to show that treatment with dasiglucagon is an effective treatment compared to placebo. The secondary objective was chosen to allow a comparison between treatment with dasiglucagon and an established comparator treatment for severe hypoglycemia, GlucaGen.

Administration of glucagon is intended to quickly raise blood glucose levels in subjects with T1DM with insulin-induced hypoglycemia. Therefore, in order to assess the clinical efficacy of dasiglucagon as compared to placebo and GlucaGen following a single SC dose, the primary endpoint and secondary efficacy endpoints involve the measurement of plasma glucose concentrations at different timepoints.

4.2.2 Justification for drug, route, dosage and treatment duration

Dasiglucagon and GlucaGen will be administered as 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 approved dose for treatment of severe hypoglycemia. Data from the studies conducted to date with dasiglucagon, including the phase 2 trial in subjects with T1DM, have been used to establish that 0.6 mg of dasiglucagon is an effective dose and also represents a therapeutically equivalent dose to 1 mg of GlucaGen (see also Section 6.1).

Dasiglucagon, placebo, and GlucaGen will be administered in the abdomen, buttocks, or thigh by SC injection, as this is one of the intended routes of administration for dasiglucagon, besides IM and IV.

Subjects will be followed for at least 28 days after dosing in order to perform an adequate immunogenicity evaluation of treatment.

5 TRIAL POPULATION

5.1 Rationale for trial population

Dasiglucagon is in development for the treatment of severe hypoglycemia in insulin-dependent patients with diabetes mellitus. In the present trial, subjects with T1DM are included in the evaluation of efficacy and safety of dasiglucagon under hypoglycemic conditions as this is part of the intended target population. Subjects with T1DM are selected to avoid the endogenous glucagon counter-regulatory response to insulin-induced hypoglycemia that is present in patients with T2DM. The inclusion and exclusion criteria are set to include a trial population that represents the general population of subjects with T1DM.

5.2 Planned sample size and number of trial centers

A total number of 156 subjects with T1DM are expected to complete the treatment visit (Visit 2). To qualify as completed, the subject must be dosed and have blood drawn for the PK and PD assessments described in the protocol. It is expected that up to 176 subjects will be randomized to have 156 subjects completing Visit 2. Completion of 156 subjects (78 subjects in the dasiglucagon group and 39 subjects in each of the placebo and GlucaGen groups) would be expected to provide adequate power for the primary efficacy evaluation, as described in Section 9.1.

The planned date for first subject first visit is expected to take place in Q4, 2017 and the planned date for last subject last visit is expected to take place in Q3, 2018.

This trial is planned to be conducted at 4 to 6 trial sites in the United States of America [US], Canada, and Europe.

5.3 Inclusion criteria

Subjects will be entered into this trial only if they meet 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 subject).
2. Female or male subjects with T1DM for at least 1 year, diagnostic criteria as defined by the American Diabetes Association (3).
3. Treated with insulin for T1DM for at least 1 year and with stable insulin treatment (defined as no more than a 10-unit daily variation in total daily insulin dose) 30 days prior to screening

4. Hemoglobin A_{1c} <10%.
5. Aged between 18 and 75 years, both inclusive.
6. A female subject 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 and until last follow-up visit. Additionally, if the participant is using systemic contraceptives, she must use an additional form of acceptable contraception. An acceptable method of contraception includes one of the following:
        i. Abstinence from heterosexual intercourse;
        ii. Systemic contraceptives (birth control pills, injectable/implant/ insertable hormonal birth control products, transdermal patch);
        iii. Intrauterine device (with and without hormones); or
        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).
7. A male subject must meet the following criteria: Surgically sterilized or willing to refrain from sexual intercourse from screening and until last follow-up visit or, if sexually active, uses condom and partner practices contraception during the trial from screening and until last follow-up visit.

5.4 Exclusion criteria

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

1. Previously treated with dasiglucagon (previously referred to as ZP4207).
2. Known or suspected allergy to trial product(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, are actively attempting to get pregnant, or are lactating.
6. History of hypoglycemic events associated with seizures in the last year prior to screening.
7. History of severe hypoglycemia (defined as plasma glucose <54 mg/dL [3.0 mmol/L]) in the last month prior to screening.
8. Receipt of any investigational drug within 3 months prior to screening.
9. Active malignancy within the last 5 years.
10. Congestive heart failure, New York Heart Association class II-IV.

11. Inadequately treated blood pressure, defined as systolic ≥160 mmHg or diastolic ≥90 mmHg) at screening (15).
12. Current bleeding disorder, including anti-coagulant treatment.
13. Known presence or history of pheochromocytoma (i.e. adrenal gland tumor) or insulinoma (i.e. insulin secreting pancreas tumor).
14. Use of a daily systemic beta-blocker drug, indomethacin, warfarin or anticholinergic drugs in the previous 28 days before Day 1 of this trial.
15. Aspartate aminotransferase (AST) or alanine aminotransferase (ALT) >2.5 × the upper limit of the normal range (ULN), bilirubin >1.5 × ULN, estimated glomerular filtration rate <30 mL/min/1.73 m^2 according to the Modification of Diet in Renal Disease study definition (16), or altered electrolyte values of clinical relevance for cardiac conduction, as judged by the investigator.
16. Clinically significant abnormal ECG at screening as judged by the investigator.
17. Clinically significant illness within 4 weeks before screening, as judged by the investigator.
18. Donation of blood or plasma in the past month, or in excess of 500 mL within 12 weeks prior to screening.
19. Surgery or trauma with significant blood loss within the last 2 months prior to screening.
20. 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.
21. Subjects with mental incapacity or language barriers which 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.
22. Any condition interfering with trial participation or evaluation or that could be hazardous to the subject.
23. The use of prescription or non-prescription medications known to cause QT prolongation.

5.5 Dosing day exclusion criteria

Subjects who meet one or more of the following dosing day exclusion criteria at the dosing visit will be excluded from the dosing visit, but the visit can be rescheduled 1-7 days later. The dosing visit can only be rescheduled once.

1. Atypically strenuous exercise within 4 days prior to dosing, as judged by the Investigator. Exercise during the trial should follow subject's typical routine, and should not exceed a near maximum intensity for more than 20 minutes per day, or moderate intensity for more than 90 minutes per day.

2. Clinically significant illness within 4 weeks before dosing, as judged by the investigator.
3. Consumption of alcohol within 24 hours prior to dosing visit, or positive results from an alcohol breath test.
4. Not fasting from 22:00 hours the evening prior to dosing, apart from water. Small amounts of carbohydrates (up to 20 g) to prevent hypoglycemia are allowed.
5. The use of any non-prescribed systemic or topical medication, except routine vitamins and occasional use (as judged by the investigator) of acetylsalicylic acid and paracetamol within 2 weeks prior to dosing. Treatment with insulin, including analogs, is allowed.
6. Use of any basal insulin within 24 hours prior to dosing.
7. Use of any short acting (bolus) insulin within 12 hours prior to dosing.
8. Changes in medical history or concomitant medication resulting in fulfillment of clinical exclusion criteria, as judged by the investigator.
9. Plasma glucose value <50 mg/dL (2.8 mmol/L) within the last 24 hours or plasma glucose value <60 mg/dL (3.3 mmol/L) within the last 5 hours prior to dosing.

5.6 Premature treatment discontinuation and withdrawal

Participation in the trial is strictly voluntary. A subject 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 subject at any time if the investigator deems it in the subject's best interest. The reason and circumstances for withdrawal will be documented in the electronic case report form (eCRF).

5.6.1 Possible reasons for treatment visit discontinuation

A subject will be discontinued from treatment if the following applies:

- Withdrawal of consent by subject.
- If a protocol deviation occurs which, in the clinical judgement of the investigator, can invalidate the trial or endpoints or can interfere pharmacokinetically or pharmacodynamically with the trial product, the subject will be discontinued by the investigator.
- Adverse events occur which are considered unacceptable by the subject or the investigator.

If discontinuation occurs following administration of trial medication, every effort should be made to have the subject return and participate in the complete follow-up visit on Day 28 (see Table 3) to avoid missing data.

If trial participation is terminated due to an AE possibly related to any of the trial medications or trial examinations, the subject 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.

A total of 156 subjects must complete the treatment visit (Visit 2). To qualify as completed, the subject must be dosed and have blood drawn for the PK and PD assessments described in the protocol.

5.6.2 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 subjects within the agreed time frame.
- The center does not respond to trial management requests.
- Repeat protocol violations.

5.6.3 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 subjects.
- New scientific knowledge becomes known that makes the objectives of the trial no longer feasible/valid.
- Unsatisfactory enrolment of subjects.

5.7 Subject identification and randomization

Subjects who have given written informed consent and meet all inclusion and none of the exclusion criteria will be randomized in a 2:1:1 ratio to either 0.6 mg dasiglucagon (n=78), placebo (n=39), or 1 mg GlucaGen (n=39) via an Interactive Web Response System (IWRS). Randomization will continue until 156 subjects have completed Visit 2.

Subjects 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 subject is taking, the subject code can be broken by the

investigator, preferably after consultation with the medical monitor. Emergency code breaks can be performed using the IWRS.

6 TRIAL DRUG

6.1 Identity

The following trial drugs will be administered:

- Dasiglucagon, liquid formulation, 1 mg/mL, 0.6 mL delivered in a prefilled syringe.
- Placebo, liquid formulation, 0.6 mL delivered in a prefilled syringe.
- Recombinant glucagon hydrochloride, 1 mg for reconstitution (GlucaGen®, Novo Nordisk) in 1 mL sterile water.

The description of the three trial drugs is provided in Table 1. Dasiglucagon is a stable peptide analog of human glucagon in a ready-to-use liquid formulation for treatment of severe hypoglycemia in insulin-dependent patients with diabetes mellitus. 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.

Table 1 Description of trial drugs

	Test product	Placebo Product	Comparator product
Name	Dasiglucagon	Placebo	GlucaGen®
Active substance	Dasiglucagon	N/A	Recombinant glucagon hydrochloride
Formulation	Liquid formulation, 0.6 mL	Liquid formulation, 0.6 mL	Powder and solvent for reconstitution as 1 mL solution for injection
Strength	1 mg/mL	N/A	1 mg
Device	Single use pre-filled syringe	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	Zealand Pharma A/S, Glostrup (Copenhagen), Denmark	Novo Nordisk A/S, Bagsværd, Denmark

	Test product	Placebo Product	Comparator product
Storage requirements	Store between 2 and 8°C	Store between 2 and 8°C	Store between 2 and 8°C

The quantities of ingredients for dasiglucagon and placebo are provided in Table 2.

Table 2 Quantities of ingredients in dasiglucagon and placebo injection

Component	Amount per mL (dasiglucagon)	Amount per mL (placebo)	Function
Dasiglucagon*	1.0 mg	N/A	[redacted]
Sodium chloride	10.23 mg	10.23 mg	
Trometamol/Tromethamine	6.06 mg	6.06 mg	
Water for injection	To make 1 mL	To make 1 mL	
Sodium hydroxide	q.s.	q.s.	
Hydrochloric acid	q.s.	q.s.	

*The quantity of drug substance to be used is calculated according to net peptide content and purity.
q.s. = quantum sufficit (quantity required).

6.2 Treatment assignment and randomization

Subjects successfully completing screening and who fulfill entry eligibility and randomization criteria will be randomized to one of three treatment groups in a ratio of 2:1:1:

- Test treatment: Dasiglucagon, liquid formulation, 1 mg/mL, 0.6 mL delivered in a prefilled syringe.
- Placebo treatment: Placebo, liquid formulation, 0.6 mL delivered in a prefilled syringe.
- Reference treatment: Recombinant glucagon hydrochloride, 1 mg for reconstitution.

Randomization will be performed using a fixed-block randomization scheme. The randomization scheme will be generated prior to the initiation of the study by an independent statistician/programmer who will not be a member of the study team; all investigators will not be aware of the block size of the randomization scheme. Randomization will be stratified by treatment group and by injection site (abdomen, buttocks, or thigh) and controlled via the IWRS.

Subjects will be randomized to study treatment using an interactive, automated system which has been validated for the intended use under the International Society of Pharmaceutical Engineers Good Automated Manufacturing Process guidelines, 21CFR Part 11 (FDA regulation for Electronic Records and Electronic Signatures) and the International Conference on Harmonization (ICH) Guidance E6 for Industry on Good Clinical Practice (GCP).

6.3 Administration

Dasiglucagon, placebo, and GlucaGen will be administered by SC injection in the abdomen, buttocks, or thigh.

An unblinded person (appropriately trained) authorized to prepare the dose and administer the treatment in accordance with the randomization will prepare the treatment required for each subject on each dosing day. The dose will be administered by the unblinded, trained and qualified person. The content of the syringe has to be checked for clarity and absence of bubbles. To ensure proper administration, the unblinded person should administer the SC injection at a 90 degree angle by grasping skin between the thumb and first finger. The injection should be given within 5 seconds.

Syringes will be discarded after dose administration. Used GlucaGen vials will be stored in a lockable box (separated from unused vials) at ambient temperature.

6.4 Packaging and labelling

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

The study drug label will describe the storage conditions for study drug. The labels will supply no information about the subjects. Each treatment kit (pre-filled syringe/vial for reconstitution) will have a unique Dispensing Unit Number for drug allocation, drug accountability, and traceability purposes.

Labelling will be performed according to Annex 13 of the Good Manufacturing Practice guidelines of the European Commission, ICH GCP guidelines, and local law.

6.5 Storage of study drugs

The Investigator must ensure the availability of proper storage conditions. All study drug supplies provided for this study will be stored in a secure area with restricted access at the study site.

The temperature should be monitored by recording the actual, minimum, and maximum temperatures using a calibrated thermometer or thermocouple, or by continuous recording using a qualified temperature monitoring system. The temperature should be evaluated and documented at least on working days on a temperature log. This log must be included in the Investigator Site File upon study termination.

Dasiglucagon and placebo must be stored in a refrigerator (at a temperature of 2–8°C), and should be handled in accordance with guidelines from the sponsor. GlucaGen must also be stored in a refrigerator (at a temperature of 2–8°C), and should be handled in accordance with the Summary of Product Characteristics (13).

The unblinded person responsible for study drug handling must contact the unblinded monitor in case of temperature deviations outside the acceptable range.

Please see the Pharmacy Manual for additional information on handling study drug.

6.6 Blinding and breaking the blind

This is a double-blind trial. As the trial products are not identical in appearance, dasiglucagon and placebo being available as a liquid formulation and GlucaGen as a powder for reconstitution, unblinded trial personnel will be responsible for handling, preparing (according to the prescription from the IWRS), and administering the trial medication, as well as for keeping the records strictly confidential and accessible only to unblinded staff until after the database has been locked. To maintain double-blind conditions, all trial assessments at the trial center will be done by blinded trial personnel not involved in the administration of trial medications. However, exposure assessments and anti-drug antibody (ADA) assessments will be performed by unblinded personnel at the special laboratories, to ensure that dasiglucagon, placebo, or GlucaGen administration is matched with the applicable bioanalytical assay.

Treatment assignment will be kept strictly confidential and accessible only to authorized persons until after the time of unblinding. Codes with treatment assignment will, however, be readily available in the IWRS to the blinded personnel in case of an emergency.

The code for a particular subject may be broken in a medical emergency if knowing the identity of the treatment allocation would influence the treatment of the subject. The emergency code break can be performed using the IWRS. Whenever a code is broken, the person breaking the code must record the time, date and reason as well as his/her initials in the source documents. The breaking of blinded codes in case of medical emergency for one subject should not unblind the trial personnel to the treatment information of other subjects. The person performing the unblinding should inform as few people as possible about the result of the unblinding. All persons unblinded for a specific subject should be documented.

If the trial center needs to break the code, the medical monitor should, if at all possible, be contacted prior to breaking the code and the monitor must be notified within 24 hours after the code has been broken.

The pharmacovigilance unit (safety contract research organization [CRO]; see the list of trial personnel in Section 12.1) will be able to break the code in case of a serious unexpected suspected adverse reaction (SUSAR).

The central and specialty laboratories will be provided with a copy of the randomization list.

6.7 Drug accountability

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 for destruction. This does not apply to the used syringes as they will be discarded after dose administration. Destruction must not take place until approved by the Sponsor.

6.8 Treatment compliance

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

PK assessments will support the surveillance of compliance with IMP administration.

6.9 Prior and concomitant medications

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

Subjects using any new concomitant medication resulting in fulfillment of a dosing day exclusion criterion (see Section 5.5) will be excluded from the dosing visit, but can be rescheduled to one of the following days (1–7 days later). The dosing visit can only be rescheduled once.

6.9.1 Prohibited medications

The use of anti-coagulant treatments and medications (prescription and non-prescription) that are known to cause QT prolongation are prohibited during the course of the trial.

Within 28 days prior to dosing, the use of daily systemic beta-blockers, indomethacin, warfarin, and anticholinergic drugs is prohibited.

Within 2 weeks prior to dosing, the use of any non-prescribed systemic or topical medication (with the exception of vitamins and the occasional use of acetylsalicylic acid and paracetamol) is prohibited.

Within 24 hours prior to dosing, the use of any basal insulin is prohibited.

Within 12 hours prior to dosing, the use of any short acting (bolus) insulin, except insulin glulisine (Apidra®) is prohibited.

During the insulin-induced hypoglycemic procedure, continuous SC insulin infusion must be stopped.

7 PARAMETERS AND METHODS OF ASSESSMENT

Overall, approximately 180 mL of blood will be drawn from each subject for PK, PD, ADA, and safety laboratory assessments.

7.1 Efficacy parameters

A description of the sample handling and sample processing at the site will be included in the laboratory manuals. Validation documentation for the assays must be available prior to sample analyses. A bioanalytical report for each analysis of trial PD and PK samples will be prepared.

7.1.1 Pharmacodynamic measurements

The plasma glucose profile for evaluation of the primary and secondary clinical efficacy endpoints will be assessed based on plasma concentration data ($AUC_{0\text{-}30min}$) from samples collected at the dosing visit (Visit 2). The samples will be sent to the clinical laboratory and analyzed using a sensitive and validated assay for glucose measurements.

Samples will be collected pre-dose, and at 4, 6, 8, 10, 12, 15, 17, 20, 25, 30, 40, 50, 60, 75, and 90 minutes after dosing (see the schedule of procedures in Table 3). The actual time of blood sampling for evaluation of plasma glucose should not deviate from the nominal time by more than ±30 seconds until the 20 minute collection time point and by more than ±1 minute for the subsequent collection time points. Pre-dose is defined as within 2 minutes prior to dosing.

7.1.2 Pharmacokinetic measurements

The exposure to trial medication (dasiglucagon or GlucaGen) for evaluation of PK will be assessed based on plasma concentration data ($AUC_{0\text{-}90\ min}$, C_{max}, t_{max}) from samples collected at the dosing visit (Visit 2).

Samples (including back-up samples) will be collected pre-dose, and at 15, 30, 35, 40, 50, 60, 90, and 120 minutes after dosing (see the schedule of procedures in Table 3). The actual time of blood sampling for exposure to trial medication should not deviate from the nominal time by more than ±1 minute. Pre-dose is defined as within 2 minutes prior to dosing.

7.2 Safety parameters

7.2.1 Adverse events

The investigator is responsible for the detection and documentation of events meeting the criteria and definition of an AE or serious adverse event (SAE), as provided in this protocol. During the trial, the investigator or center staff will be responsible for reporting AEs and SAEs, as detailed in this section of the protocol.

Based on the investigator's clinical judgment it will be determined whether an AE is related to treatment and of sufficient severity to require the subject's removal from treatment or from the trial. A subject may also voluntarily withdraw from treatment due to what he or she perceives as an intolerable AE. If either of these situations arises, the subject should be under medical supervision until symptoms cease or the condition becomes stable.

7.2.1.1 Definitions

Adverse event

An AE is any untoward medical occurrence in a trial subject given an IMP which does not necessarily have a causal relationship with the treatment. An AE can therefore be any unfavorable and/or 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.

In this trial, only treatment-emergent adverse events (TEAEs) will be collected and reported. TEAEs are events that occur from the first trial-related activity after the subject has signed the informed consent form until the end of the post-treatment follow-up period.

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 7.2.2).
- Injection site reactions (see Section 7.2.6).

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

- Pre-planned procedure, unless the condition for which the procedure was planned has worsened from the first trial related activity after the subject 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

An 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 events may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above.

Adverse drug reaction

All noxious and unintended responses to an investigational product related to any dose should be considered adverse drug reactions. The phrase responses to an investigational product means that a causal relationship between an investigational product and an adverse event is at least a reasonable possibility, i.e. the relationship cannot be ruled out.

Suspected unexpected serious adverse reactions (SUSARs)

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. dasiglucagon Investigator's Brochure or package leaflet/Summary of Product Characteristics for GlucaGen).

Adverse event of special interest

An AESI 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 AESIs:

- Post-dose clinical signs, or measured vital signs, indicating a clinically significant drop in blood pressure including signs of orthostatic hypotension, vasovagal responses or bradycardia.
- Post-dose 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 AE 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 AE 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 subject.

Severe: A type of AE 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 an SAE may not necessarily 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 subject 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 subject signed the informed consent.
Recovering/resolving:	The condition is improving and the subject is expected to recover from the event. This term is only applicable if the subject has completed the trial.
Recovered/resolved with sequelae:	The subject 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 subject has not improved and the symptoms are unchanged.
Fatal:	This term is only applicable if the subject died from a condition related to the reported AE. Outcomes of other reported AEs in a subject 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 subject is lost to follow-up.

7.2.1.2 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 subject has signed the informed consent until the end of the post-treatment follow-up period. At each contact with the center (visit or telephone, excluding visits where the subject is not seeing the investigator or his/her staff [e.g. visits to the laboratory]) the subject must be asked about AEs. All AEs, either observed by the investigator or reported by the subject, 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.

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 AESIs, the AESI 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 during IMP administration 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 AESI, the investigator must tick the AESI box on the AE form and complete the AESI form. The AESI 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 all AESIs to the sponsor's responsible pharmacovigilance unit (Safety CRO; see the list of trial personnel in Section 12.1) immediately (within 24 hours) after obtaining knowledge about the event.

The investigator must report initial information electronically (e.g. in PDF format) on all SAEs to the sponsor's responsible pharmacovigilance unit (Safety CRO; see the list of trial personnel in Section 12.1) immediately (within 24 hours) after obtaining knowledge about the event. 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 (see Section 7.2.1.1) that occur in this trial to the Competent Authorities and Institutional Review Boards (IRBs)/Independent Ethics Committees (IECs) in accordance with the local requirements in force and ICH guideline for GCP. The trial monitor must be informed accordingly.

7.2.1.3 Follow-up of adverse events

All AEs that are ongoing at the end of the subject's participation in the trial will be followed-up until the event is resolved or reaches a satisfactory outcome as deemed by the investigator or until the last visit of the last subject enrolled in the trial, whichever occurs first.

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 an SAE.

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

The sponsor and/or CROs acting on behalf of the sponsor can upgrade a non-serious AE to an SAE. In these situations the investigator will be informed and asked to fill out an SAE form and forward to the Safety CRO immediately (within 24 hours).

7.2.1.4 Clinical laboratory abnormalities and other abnormal assessments as adverse events or serious adverse events

Abnormal laboratory findings (e.g. biochemistry, hematology, urinalysis) or other abnormal assessments (e.g. vital signs) that are judged by the investigator as **clinically significant** will be recorded as AEs or SAEs if they meet the definition of an AE or SAE. Clinically significant abnormal laboratory findings or other abnormal assessments that are detected during the trial or are present at baseline and significantly worsen following the start of the trial will be reported as AEs or SAEs. However, clinically significant abnormal laboratory findings or other abnormal assessments that are associated with the disease being studied, unless judged by the investigator as more severe than expected for the subject's condition, or that are present or detected at the start of the trial and do not worsen, will not be reported as AEs or SAEs.

The investigator will exercise his or her medical and scientific judgment in deciding whether an abnormal laboratory finding or other abnormal assessment is clinically significant.

7.2.2 Hypoglycemia

Hypoglycemia will be regarded as an AE and recorded and documented on an AE form (and SAE form, if applicable).

Hypoglycemia is defined as a decline in plasma glucose to below 70 mg/dL (3.9 mmol/L). However, in the time period from initiation of the hypoglycemic clamp procedure (see Section 8.2.3.1) until 45 minutes after dosing, hypoglycemia is defined as a decline in plasma glucose to below 45 mg/dL (2.2 mmol/L).

During the dosing visit, prior to administration of the IMP, a plasma glucose value of <45 mg/dL (2.5 mmol/L) will be treated by an IV glucose solution. After administration of the IMP in the period from 8 minutes until 44 minutes after dosing, a plasma glucose value of <45 mg/dL (2.5 mmol/L) will be treated by an IV glucose solution, and if a plasma glucose value of ≥70 mg/dL (3.9 mmol/L) is not achieved within the 45 minutes after IMP administration, IV glucose infusion will also be initiated.

If the subject experiences symptoms of hypoglycemia, a plasma glucose measurement should be taken in order to classify the event (please refer to Section 7.4.2 for additional details).

7.2.3 Physical examination

The physical examination will be carried out at screening (Visit 1) and at the follow-up visit (Visit 3; see Table 3).

The physical examination includes examination of the following body systems: head, ears, eyes, nose, throat, including the thyroid gland; heart, lung, chest; abdomen; skin and mucosae; musculoskeletal system; nervous system; lymph node; other findings.

At the screening visit, any abnormality will be recorded and described in the eCRF, including the investigator's assessment of clinical significance ('abnormal, not clinically significant' or 'abnormal, clinically significant'). Clinically significant findings at the screening visit should be recorded as concomitant illness.

At subsequent visits, any clinically significant deterioration of a pre-existing condition as well as any new clinically significant findings will be recorded as AEs (see Section 7.2.1).

7.2.4 Vital signs

An examination of the following vital signs will be performed at screening (Visit 1), the dosing visit (Visit 2) and at the follow-up visit (Visit 3):

- Diastolic and systolic blood pressure (mmHg) will be measured after at least 5 minutes rest in a supine position. At Visit 1, blood pressure will be measured in both arms. The blood pressure from the arm with the higher systolic value is

transcribed into the eCRF and this arm should be used for all subsequent measurements of the subject's blood pressure in this trial.
- Pulse (beats per min) measured after at least 5 minutes rest in a supine position.
- Body temperature (°C).

At the dosing visit, measurements will be taken prior to the start of the insulin-induced hypoglycemic procedure (within 30 minutes) and at 30, 90, and 300 minutes after dosing (see Table 3). The actual time of the assessment should not deviate from the nominal time by more than ±10 minutes. In addition to the pre-specified assessments as listed in Table 3, blood pressure and pulse may be assessed at any time during the trial at the discretion of the investigator.

7.2.5 Electrocardiogram

A standard 12-lead ECG will be performed at the screening visit (Visit 1), the dosing visit (Visit 2; prior to the start of the insulin-induced hypoglycemic procedure [within 30 minutes]) and at 20, 35, 45, 60, and 300 minutes after dosing) and at the follow-up visit (Visit 3; see Table 3). The actual time of the assessment should not deviate from the nominal time by more than ±5 minutes.

ECG parameters (heart rate, PQ, QRS, QT, QTcB) and any abnormality will be recorded and described in the eCRF including the investigator's assessment of clinical significance ('abnormal, not clinically significant' or 'abnormal, clinically significant').

At subsequent visits, any clinically significant deterioration of a pre-existing condition as well as any new clinically significant findings will be recorded as AEs (see Section 7.2.1).

7.2.6 Local tolerability

Immediately prior to treatment administration, it should be verified that the injection site is normal. To ensure all injection site assessments are performed at the injection site, the site will be marked with a pen prior to injection. Assessment of local tolerability at the injection site will be performed at the dosing visit (Visit 2; at 30, 120 and 300 minutes after dosing), and at the follow-up visit (Visit 3) (see Table 3) and more frequently, if deemed necessary by the investigator. The actual time of the assessment should not deviate from the nominal time by more than ±10 minutes. In the eCRF, the time of assessment and any injection site reaction observed will be recorded. In case of an observation, the Local Tolerability form will be completed, as well as the (S)AE form.

The local tolerability at the injection site will be evaluated by means of the following assessments: spontaneous pain, pain on palpation, itching, redness, edema,

induration/infiltration, and other. Each of these assessments will be reported on a scale of 0 (none), 1 (mild), 2 (moderate), and 3 (severe). The evaluation and the actual time of the assessment will be recorded. The assessments will be performed by a trial physician or nurse.

Digital pictures will be taken of the injection site at the time of identification, and thereafter as often as judged necessary by the investigator. The pictures should include a subject identifier, visit number, time after dosing, and a ruler for scaling.

7.2.7 Clinical laboratory assessments

The safety parameters that will be assessed at the clinical laboratory are listed in Table 3. Routine clinical laboratory tests will be performed centrally. Samples for clinical laboratory parameters (biochemistry, hematology, coagulation) will be collected at screening (Visit 1), the dosing visit (Visit 2, prior to the start of the insulin-induced hypoglycemic procedure [within 30 minutes]) and at 30 and 300 minutes after dosing), and at the follow-up visit (Visit 3). The actual time of the assessment should not deviate from the nominal time by more than ±10 minutes. Samples for glycated hemoglobin (HbA_{1c}) will be collected at screening only (Visit 1). Samples for urinalysis will be collected at screening (Visit 1), at the dosing visit (Visit 2, prior to the start of the insulin-induced hypoglycemic procedure [within 120 minutes]) and at the follow-up visit (Visit 3). The following parameters will be assessed:

- Clinical biochemistry: sodium, potassium, calcium, glucose, urea, creatinine, total bilirubin, AST, ALT, gamma-glutamyltransferase, 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 (leukocytes).
- Coagulation: international normalized ratio, fibrinogen (at screening visit only).
- Urinalysis: pH, blood (leukocytes and erythrocytes), protein, glucose, ketones, nitrite.

Pregnancy tests will be performed at each visit for women of childbearing potential only. A serum pregnancy test will be performed at screening (Visit 1) and urine stick tests will be performed at the dosing visit (Visit 2, prior to the start of the insulin-induced hypoglycemic procedure) and the follow-up visit (Visit 3). Test sticks will be provided to the trial centers.

Alcohol breath tests and a urine drug screen will be performed at screening (Visit 1) and at the dosing visit (Visit 2, prior to the start of the insulin-induced hypoglycemic procedure). Equipment for the alcohol breath test and uring drug screen will be provided to the trial centers.

Re-assessment of laboratory parameters will be allowed only if handling issues, damaged samples, or hemolyzed samples have confounded the measurement results.

For further details of the clinical laboratory assessments, please refer to the laboratory manual.

7.2.8 Pregnancy

Female subjects 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 subjects must be instructed to notify the investigator immediately if their partner becomes pregnant or suspects pregnancy. The sponsor has a responsibility to monitor the outcome of all pregnancies reported during the clinical trial. During an information meeting at the trial center, the subject'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 immediately (within 24 hours), according to the procedure stated in Section 7.2.1.2. 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 follow 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 7.2.1.2. Any (S)AEs in the newborn must be reported on the (S)AE form.

The SAEs that must be reported include 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 until the age of 1 month.

7.2.9 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 subjects. During a subject's participation in the trial, the investigator should ensure that adequate medical care is provided to the subject for any AEs, including clinically significant laboratory values related to the trial. The investigator should inform the subject when medical care is needed for intercurrent illnesses of which the investigator becomes aware.

For further information on safety precautions for dasiglucagon and GlucaGen, please refer to the current version of the Investigator's Brochure (14) and the Summary of Product Characteristics for GlucaGen (13), respectively.

7.2.10 Safety committee

The internal Zealand Pharma Safety Committee is constituted to perform ongoing blinded 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 Safety Committee meetings, or on notification of significant findings, the Safety Committee will take appropriate measures to safeguard the subjects.

7.3 Demography, concomitant illness, medical history and concomitant medication

Demographics, body measurements, concomitant illness and medical history will be assessed only at screening (Visit 1). Concomitant medication will be assessed at screening (Visit 1), the dosing visit (Visit 2, prior to the start of the insulin-induced hypoglycemic procedure) and at the follow-up visit (Visit 3).

7.3.1 Demography and body measurements

Subject demographics and body measurements will include:

- Age
- Race, ethnicity
- Sex
- Height (meters or inch), without shoes
- Body weight (kg or lb), only wearing underwear and measured using standard scales
- Body mass index (kg/m^2) calculated based on height and body weight (body weight/height^2).

7.3.2 Concomitant illness and medical history

A concomitant illness is any illness that is present at the start of the trial (i.e. at the screening visit). Concomitant illnesses present at the start of the trial will be recorded in the eCRF at screening.

Medical history is an account of medical events that the subject has experienced in the past, including prior medications. Relevant medical conditions/illnesses in the past obtained by asking the subject or by inspecting his/her medical records will be recorded in the eCRF at screening. History of alcohol or drug abuse will also be recorded.

The information collected for concomitant illness and medical history should include diagnosis, date of onset, date of resolution or continuation.

Any change to a concomitant illness should be recorded during the trial, including end date, if applicable. A clinically significant worsening of a concomitant illness must be reported according to Section 7.2.1.

All previous and concomitant diseases will be coded with Medical Dictionary for Regulatory Activities (MedDRA), the version of which will be provided in the clinical trial report.

7.3.3 Diabetes diagnosis and current treatment

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

7.3.4 Concomitant medication

A concomitant medication is any medication, other than the trial products and current diabetes treatment (including insulin glulisine [Apidra®] for diabetes therapy wash-out), which is taken during the trial, including screening and follow-up periods.

Details of any concomitant medication must be recorded at trial entry (i.e. at screening). Any changes in concomitant medication must be recorded at each visit as they occur. The information collected for each concomitant medication includes (at a minimum) trade name or generic name, indication, start date and stop date or continuation. A change in medication due to an AE must be recorded and reported according to Section 7.2.1. If the change in medication influences the subject's eligibility to continue in the trial, the sponsor and trial monitor must be informed.

7.4 Other assessments

7.4.1 Immunogenicity

Antibodies against dasiglucagon/GlucaGen will be measured at the dosing visit (Visit 2) and at follow-up (Visit 3). At the dosing visit (Visit 2), samples will be collected prior to the start of the insulin-induced hypoglycemic procedure.

The clinical ADA assays, specific for dasiglucagon and GlucaGen, respectively, have been validated in accordance with existing guidelines and recommendations (17-21).

Confirmed positive anti-dasiglucagon antibody samples (treatment-induced or treatment-boosted) from anti-dasiglucagon antibody-positive subjects 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 neutralizing antibody analysis.

The in vitro neutralizing effect of the antibodies will be measured using an assay based on glucagon receptor transfected human embryonic kidney cells (20,22). The calculated sensitivity in previous studies was about 51.8 ng/mL. The assay was also validated for recombinant glucagon with similar results (21,23). In case of a positive result in the neutralizing antibody assay, a titer estimation will be performed. The cell-based neutralizing antibody analyses will be performed by a special laboratory, BioAgilytix, Durham, NC, USA.

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.

7.4.2 Plasma glucose measurements for safety

During insulin-induced hypoglycemia, plasma glucose levels will be monitored closely at site. After the start of insulin infusion, plasma glucose should be checked every 10 minutes while plasma glucose is above 110 md/dL, and every 5 minutes once plasma glucose is at or below 110 mg/dL and until after dosing when the subject's plasma glucose is ≥70mg/dL (3.9 mmol/L). Hereafter, plasma glucose should be checked every 30 minutes until 300 minutes (5 hours) after dosing. Plasma glucose concentrations will be measured using a US FDA-approved glucose analyzer (e.g., YSI 2300, Yellow Springs Instruments, Yellow Springs, OH).

At the discretion of the investigator, additional plasma glucose measurements can be taken at any time during the trial, for example when there is a suspicion (e.g. symptoms) of a hypoglycemic episode.

Plasma glucose measurements for safety should only be recorded in the eCRF if they are related to an AE (e.g. a hypoglycemic episode).

In case of persistent post-treatment hypoglycemia, subjects will receive rescue treatment with an IV glucose infusion (see Section 8.2.3.1 for details). Blood samples for PD and PK assessments should still be taken at the specified timepoints.

7.4.3 Plasma insulin measurements

Samples for insulin assessment will be collected at the dosing visit (Visit 2, pre-dose and at 30 and 60 minutes after dosing). The actual time of blood sampling for evaluation of plasma insulin should not deviate from the nominal time by more than ±1 minute. Pre-dose is defined as within 2 minutes prior to dosing.

8 TRIAL CONDUCT

8.1 Schedule of procedures

The schedule of procedures is provided in Table 3. Informed consent will be obtained prior to any trial-related procedures; see Section 10.8.

8.2 Procedures by visit

8.2.1 Visit 1 (screening, Day -30 to Day -3)

Visit 1 will take place between 3 and 30 days before Visit 2, Day -1 to Day 1 (dosing day).

Informed consent can be obtained prior to or at Visit 1, however it must in any case be obtained prior to any trial related procedures. During the screening visit, the following assessments will take place:

- Informed consent (obtain or check)
- Check of subject eligibility (inclusion/exclusion criteria)
- Demography
- Body measurements
- Medical history, diabetes diagnosis, current diabetes treatment
- Concomitant illnesses
- Concomitant medications
- History of alcohol/drug abuse
- Physical examination
- Vital signs
- 12-lead ECG
- AEs
- Biochemistry, hematology, coagulation, HbA_{1c}
- Serum pregnancy test (women of childbearing potential only)
- Urinalysis
- Urine drug screen
- Alcohol breath test

Table 3 Schedule of procedures

Visit number	V1	V2	V3
Trial day	**-3**	**-1 and 1**	**28**
Visit type	**Screening**	**Dosing**	**Follow-up**
Window	**-30 to -3**		**+5 days**
Subject related information/assessments			
Informed consent	x		
Inclusion/exclusion criteria	x	x[1,2]	
Demography	x		
Body measurements	x		
Medical history, diabetes diagnosis, and current diabetes treatment	x		
Concomitant illnesses	x		
Concomitant medications	x	x[1]	x
History of alcohol/drug abuse	x		
Randomization		x[1]	
Withdrawal criteria		x[1]	
Dosing day exclusion criteria		x[1]	
Insulin-induced hypoglycemia		x	
Safety assessments			
Physical examination	x		x
Vital signs	x	x[3]	x
12-lead ECG	x	x[4]	x
Local tolerability		x[5]	x
Adverse events	x	x	x
Laboratory			
Biochemistry, hematology, coagulation, HbA_{1c} (HbA_{1c} at Visit 1 only)	x	x[6]	x
Pregnancy test (women of childbearing potential only)	x[7]	x[1,8]	x[8]
Urinalysis	x	x[1]	x
Urine drug screen	x	x[1]	
Alcohol breath test	x	x[1]	
PK/Clinical efficacy			
Plasma dasiglucagon/GlucaGen		x[9]	
Plasma glucose		x[10]	
Other assessments			
Antibodies against dasiglucagon/GlucaGen		x[1]	x[11]
Plasma insulin		x[12]	
Trial material			
Administration of trial product (during hypoglycemic clamp procedure)		x	

[1]Prior to the start of the insulin-induced hypoglycemic procedure.
[2]Only check of dosing day exclusion criteria and changes between screening visit and Visit 2.
[3]Prior to the start of the insulin-induced hypoglycemic procedure (within 30 minutes), and at 30, 90 and 300 minutes after dosing. The actual time of the assessment should not deviate from the nominal time by more than ±10 minutes.

[4]Prior to the start of the insulin-induced hypoglycemic procedure (within 30 minutes), and at 20, 35, 45, 60 and 300 minutes after dosing. The actual time of the assessment should not deviate from the nominal time by more than ±5 minutes.
[5]Local tolerability assessed at 30, 120, and 300 minutes after dosing. The actual time of the assessment should not deviate from the nominal time by more than ±10 minutes.
[6]Prior to the start of the insulin-induced hypoglycemic procedure (within 30 minutes), and at 30 and 300 minutes after dosing. The actual time of the assessment should not deviate from the nominal time by more than ±10 minutes.
[7]Serum pregnancy test.
[8]Urine stick pregnancy test.
[9]Pre-dose, and at 15, 30, 35, 40, 50, 60, 90, and 120 minutes after dosing. The actual time of blood sampling should not deviate from the nominal time by more than ±1 minute. Pre-dose is defined as within 2 minutes prior to dosing.
[10]Pre-dose, and at 4, 6, 8, 10, 12, 15, 17, 20, 25, 30, 40, 50, 60, 75, 90 minutes after dosing. The actual time of blood sampling should not deviate from the nominal time by more than ±30 seconds until the 20 minute collection time point and by more than ±1 minute for the subsequent collection time points. Pre-dose is defined as within 2 minutes prior to dosing.
[11]Any subject that tests positive for ADA will be monitored until the ADA levels return to baseline levels.
[12]Pre-dose, and at 30 and 60 minutes after dosing. The actual time of blood sampling should not deviate from the nominal time by more than ±1 minute. Pre-dose is defined as within 2 minutes prior to dosing.
ADA = anti-drug antibodies; ECG = electrocardiogram; HbA_{1c} = glycated hemoglobin; NPH = neutral protamine Hagedorn.

Eligible or potentially eligible subjects (laboratory results pending) will be provided with an Identification card (ID card), stating that the subject is participating in the trial and whom to contact (site address, investigator name and telephone number). The subjects should be instructed to return the ID card to the investigator at the last visit or to destroy the card after the last visit.

8.2.2 Instructions to subjects prior to the dosing visit (Visit 2)

At the screening visit, the investigator will inform the subject about the changes to his/her insulin therapy leading up to the start of the insulin-induced hypoglycemic procedure. The subject's current insulin therapy will be washed out as defined in Section 5.5: 24 hours prior to dosing and during the dosing visit, treatment with any basal insulin is not allowed; 12 hours prior to dosing and during the dosing visit, treatment with any short acting (bolus) insulin, except insulin glulisine (Apidra®), is not allowed. The basal rate of insulin pumps (continuous SC insulin infusion) will be discontinued on the morning of the dosing day.

On the day prior to dosing (Day -1), the subjects will need to attend the clinical center and will be required to stay onsite overnight. On the morning of the dosing day (Day 1), patients are required to be in a fasting condition, defined as having consumed only water since 22:00 hours the night before. However, the subjects are allowed to consume small amounts (up to 20 g) of carbohydrates to prevent hypoglycemia. The subjects must also not consume any alcohol within 24 hours prior to dosing (refer to Section 5.5 for all dosing day exclusion criteria).

8.2.3 Visit 2 (dosing visit)

Visit 2 will take place on Day -1 to Day 1.

The subjects will attend the clinical center the day prior to dosing (Day -1) and should stay onsite overnight. Dosing will take place the following morning (Day 1).

On Day 1 and prior to the start of the insulin-induced hypoglycemic procedure, subject eligibility is rechecked (check of changes between the screening visit and Visit 2) and those subjects eligible to participate will be randomized to treatment with dasiglucagon, placebo, or GlucaGen.

The following assessments will also take place:

- Document all changes in concomitant medication (prior to the start of the insulin-induced hypoglycemic procedure)
- Check of withdrawal criteria (prior to the start of the insulin-induced hypoglycemic procedure)
- Check of dosing day exclusion criteria (prior to the start of the insulin-induced hypoglycemic procedure)
- Vital signs (prior to the start [within 30 minutes] of the insulin-induced hypoglycemic procedure, and at 30, 90 and 300 minutes after dosing). Blood pressure should be measured first in sitting and then in standing.
- 12-lead ECG (prior to the start [within 30 minutes] of the insulin-induced hypoglycemic procedure, and at 20, 35, 45, 60, and 300 minutes after dosing)
- Local tolerability (at 30, 120, and 300 minutes after dosing)
- AEs
- Biochemistry, hematology, coagulation (prior to the start of the insulin-induced hypoglycemic procedure [within 30 minutes], and at 30 and 300 minutes after dosing)
- Urine stick pregnancy test (women of childbearing potential only; prior to the start of the insulin-induced hypoglycemic procedure)
- Urinalysis (prior to the start of the insulin-induced hypoglycemic procedure [within 120 minutes])
- Urine drug screen (prior to the start of the insulin-induced hypoglycemic procedure)
- Alcohol breath test (prior to the start of the insulin-induced hypoglycemic procedure)
- Dasiglucagon/GlucaGen plasma concentrations:
  - Pre-dose, 15, 30, 35, 40, 50, 60, 90, and 120 minutes after dosing. The actual time of blood sampling for exposure should not deviate from the nominal time by more than ±1 minute. Pre-dose is defined as within 2 minutes prior to dosing.
- Plasma glucose concentrations:
  - Pre-dose, 4, 6, 8 ,10, 12, 15, 17, 20, 25, 30, 40, 50, 60, 75, and 90 minutes after dosing. The actual time for blood sampling for plasma glucose should not deviate

from the nominal time by more than ±30 seconds until the 20 minute collection time point and by more than ±1 minute for the subsequent collection time points. Pre-dose is defined as within 2 minutes prior to dosing.

- Antibodies against dasiglucagon/GlucaGen (prior to the start of the insulin-induced hypoglycemic procedure).
- Plasma insulin concentrations:
  - Pre-dose, 30 and 60 minutes after dosing. The actual time of blood sampling for plasma insulin should not deviate from the nominal time by more than ±1 minute. Pre-dose is defined as within 2 minutes prior to dosing.

8.2.3.1 Hypoglycemic clamp procedure and administration of trial medication

The following procedure is based on precendent procedures for hypoglycemia induction in patients with T1DM [24, 25].

The treatment day (Visit 2, Day 1) will be conducted after an overnight fast of at least 8 hours, targeting a starting plasma glucose around 90 to 110 mg/dL (5.0-6.1 mmol/L).

Subjects who meet one or more of the dosing day exclusion criteria (Section 5.5) at the dosing visit will be excluded from the dosing visit, but the visit can be rescheduled 1-7 days later. The dosing visit can only be rescheduled once.

For participants using an insulin pump, the continuous subcutaneous insulin infusion will be suspended during the procedure, while participants using multiple daily injections of insulin will take their last long-acting insulin dose at least 24 hours before testing.

At approximately 08:00 hours, an infusion catheter will be inserted into each arm (forearm cephalic vein) for the manual glucose clamp procedure, with the glucose infusion in one arm and the insulin infusion in the opposite arm. A third catheter for blood sampling will be placed into a metacarpel vein for blood sampling. This hand will be warmed (55-65ºC) to arterialize venous blood.

Hypoglycemia will be gradually induced by a fast-acting IV insulin glulisine (Apidra®) infusion (15 U [100 U/mL] in 49 mL saline and 1 mL of subject's blood or plasma), initially at 150% of the subject's usual basal rate and can be increased or decreased over a range of 75% to 200% as judged necessary by the investigator, to achieve a controlled decline in plasma glucose, targeting a plasma glucose level of 55 mg/dL (3.0 mmol/L).

Plasma glucose concentrations will be measured using a US FDA-approved glucose analyzer (e.g., YSI 2300, Yellow Springs Instruments, Yellow Springs, OH). After the start of the insulin infusion, plasma glucose will be measured every 10 minutes while

plasma glucose is above 110 mg/dL, and every 5 minutes once plasma glucose is at or below 110 mg/dL.

Once the glucose concentration declines to <60 mg/dL (3.3 mmol/L), the insulin infusion will be stopped, and 5 min later plasma glucose concentration will be measured at the glucose analyzer and blood samples for baseline assessment of plasma glucose, dasiglucagon/GlucaGen PK, and insulin PK will be collected. The samples are the baseline samples and should be collected within 2 minutes before IMP administration.

- If plasma glucose is >45 mg/dL and <60 mg/dL (3.0-3.3 mmol/L), study treatment (IMP) will be administered, defining time, t=0. The study treatment will be delivered in the abdomen, buttock, or thigh (according to stratification) via SC injection, with the subject lying in a lateral recumbent position.
- If plasma glucose is <45 mg/dL (2.8 mmol/L), IV glucose solution will be administered sufficient to raise plasma glucose to within the 45-60 mg/dL target range. The run-in period will be adequately extended (at least 30 min) until the above target is achieved and new baseline samples for plasma glucose, dasiglucagon/GlucaGen PK, and insulin PK will be collected. Glucose should not be infused within 10 minutes before IMP administration. If plasma glucose is not within target range after the second attempt, the subject should be rescheduled for a new treatment visit within 7 days (+ 2 days).

Administration of IMP should not occur earlier than 9:00 hours in the morning or later than 12:00 hours.

As shown in Table 4, serial blood samples for glucose will be collected at t=0, 4, 6, 8, 10, 12, 15, 17, 20, 25, 30, 40, 50, 60, 75 and 90 minutes post-dosing. Samples for assessing plasma dasiglucagon/GlucaGen concentration will be collected at t=0, 15, 30, 35, 40, 50, 60, 90, and 120 minutes. Samples for assessing plasma insulin concentration will be collected at t=0, 30, and 60 minutes.

Table 4 Post-treatment blood sampling times

Times	0	4	6	8	10	12	15	17	20	25	30	35	40	50	60	75	90	120
Plasma glucose	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y		Y	Y	Y	Y	Y	
PK Dasiglucagon/ GlucaGen	Y						Y				Y	Y	Y	Y	Y		Y	Y
PK Insulin	Y										Y				Y			

Refer to Section 7.2.7 for details of laboratory safety sampling and to Section 7.4.2 for details of blood glucose safety sampling.

When the t=90-minute blood sampling for plasma glucose has been collected the subjects are allowed to eat moderately. Drinking of water is allowed *ad libitum* during the entire procedure.

Hypoglycemia Rescue Provisions

During insulin-induced hypoglycemia, plasma glucose levels will be monitored closely at site for safety reasons as described in Section 7.4.2. Subjects should receive post-treatment rescue glucose infusion to ameliorate persistent hypoglycemia, as follows.

1. Glucose infusion should be initiated if a subject experiences severe alarming escalation of symptoms of hypoglycemia (e.g. symptoms suggesting a change in consciousness) at any time during the trial; glucose infusion should be initiated targeting a plasma glucose levels >70 mg/dL.
2. If plasma glucose is <45 mg/dL (2.5 mmol/L) between t=8 and t=44 minutes, rescue glucose infusion (1-2 mg/kg administered IV over about 5 seconds) should be initiated to maintain plasma glucose between 45 mg/dL and 55 mg/dL (2.5-3.0 mmol/L). Pause glucose infusion if plasma glucose is >55 mg/dL.
3. If plasma glucose is <70 mg/dL at t=45 minutes, rescue glucose infusion (2-3 mg/kg administered IV over about 5 seconds) should be initiated to maintain plasma glucose between 70 mg/dL and 80 mg/dL (3.9-4.4 mmol/L). Pause glucose infusion if plasma glucose is >75 mg/dL (4.2 mmol/L).

Subjects should remain in bed until completion of the test procedure 300 minutes after dosing (bathroom visits are allowed).

The IMP will be administered SC according to Section 6.3. The time of IMP administration will be recorded. At the timepoint when the insulin infusion is stopped, the total insulin dose which was required to induce hypoglycemia will be recorded.

AEs will be specifically recorded during the procedure at several timepoints.

The investigator must provide information to the subjects on how to resume their usual diabetes treatment.

The subject may be released from the clinical center if the investigator does not have any safety concerns based on the last safety plasma glucose value and the general condition of the subject. However, at the discretion of the investigator or on request of the subject, the subject may stay at the trial center for a longer period.

8.2.4 Visit 3 (follow-up visit)

Visit 3 will take place on Day 28 + 5 days. The subject does not need to be fasting.

At Visit 3, the following assessments will take place:

- Document all changes in concomitant medication
- Physical examination
- Vital signs
- 12-lead ECG
- Local tolerability
- AEs
- Biochemistry, hematology, coagulation
- Urine stick pregnancy test (women of childbearing potential only)
- Urinalysis
- Antibodies against dasiglucagon/GlucaGen.

After the follow-up visit the End of Trial form must be completed. Even if a subject is not able to attend the follow-up visit, the End of Trial form, the eCRF Accountability/Affirmation Statement form and the Drug Accountability form must be completed.

9 STATISTICAL METHODS

Before database lock and treatment unmasking, a separate statistical analysis plan (SAP) will be finalized, providing detailed methods for the analyses outlined below. Further analysis details may be added or refined in the SAP.

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

9.1 Determination of sample size

Due to requirements in the size of the safety database, the sample size is set to 78 subjects treated with dasiglucagon 0.6 mg. With a 2:1:1 randomization ratio for the three treatments, dasiglucagon 0.6 mg, placebo, and GlucaGen 1 mg, 78 subjects injected with dasiglucagon and 39 subjects with placebo will yield a power of 90% at a 5% two-sided significance level to detect a treatment group difference in recovery incidence within 20 minutes, assuming as low as 80% and as high as 50% recovery incidence for the dasiglucagon and placebo groups, respectively.

9.2 Trial subjects

9.2.1 Analysis samples

For presentation of data and reporting of the statistical analyses, the following analysis samples will be used, depending on the context:

- Safety analysis set (SAS): All randomized subjects who received at least one dose of trial medication.
- Full analysis set (FAS): All randomized subjects who received at least one dose of trial medication and contributed valid information for at least one post-dose endpoint.
- Per protocol (PP) set: All subjects of the FAS for whom no relevant protocol deviations were documented. This sample will primarily be used for sensitivity analysis.

The analysis of the primary endpoint and secondary endpoints will be based on the FAS. A secondary analysis of the primary endpoint will be based on the PP set. All safety analyses will be based upon the SAS.

The decision regarding whether a protocol deviation is relevant or not for the exclusion of subjects from the PP set will be made case-by-case in a data review meeting prior to treatment unmasking and database lock (see Section 9.2.3).

9.2.2 Disposition of subjects

Subject disposition will be tabulated including the number of screened subjects, screening failures, subjects exposed to trial product, subjects completing the trial and subjects in each analysis set.

Subjects withdrawn from the trial will be listed including the primary reason for withdrawal.

9.2.3 Protocol deviations

Before data are released for statistical analysis, a treatment-masked review of all data will take place to identify protocol deviations that may potentially affect the results. This review will be performed without revealing to which trial product the subjects were assigned. The masking of the trial products will be maintained for everyone involved in allocating subjects to the analysis sets until data are released for statistical analysis. Furthermore, spurious outliers will be evaluated. In addition, protocol deviations that may potentially affect the results will be identified and it will be evaluated if subjects and/or data should be excluded from the analysis. Protocol deviations will be classified as minor or major in a consistent way. Major deviations from the protocol may lead to the exclusion of a subject from the PP set.

Major protocol deviations may include deviations related to trial inclusion or exclusion criteria, conduct of the trial, subject management or subject assessment. Unless explicitly decided otherwise during the masked data review, the following will be considered major protocol deviations:

- Violation of one or more major inclusion/exclusion criteria
- Interruption of administration of trial product
- Significant deviation from time windows
- Incorrect treatment allocation
- Missing primary endpoint.

The violation of several major inclusion/exclusion criteria or the complete absence of efficacy data might lead to exclusion of the subject from FAS. In that case, the decision should be taken at the masked data review meeting, and the exclusion from efficacy analysis justified in the signed notes of the meeting.

Obviously erroneous data points may be excluded from the analyses or re-analyzed (in case of e.g. serum concentrations). The decision to re-analyze or exclude data points from the statistical analysis is the joint responsibility of the sponsor and the trial statistician.

The subjects or observations to be excluded and the reason for their exclusion will be documented and signed by those responsible prior to database release. The documentation will be stored together with the remaining trial documentation. The subjects and observations excluded from analysis sets, and the corresponding reasons, will be described in the clinical trial report.

9.3 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 timepoint will be included in the analysis.

Raw data listings and summary tables will be generated using the software SAS© version 9.4 or higher.

Continuous variables will be summarized using means, standard deviations, medians, coefficients of variation, and minimum and maximum values.

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

9.4 Demographics and baseline characteristics

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

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

9.5 Efficacy Analyses

For the confirmatory analyses, the primary and key secondary endpoints for the dasiglucagon 0.6 mg and placebo treated subjects will be compared. A hierarchical procedure will be applied for the control of multiplicity. The primary and secondary endpoints will be evaluated on the full analysis set. In the primary analysis, those subjects who require rescue IV glucose will be censored at the time to plasma glucose recovery. In a sensitivity analysis, the time to plasma glucose recovery will be analyzed without censoring the subjects who received rescue IV glucose.

9.5.1 Hierarchical testing procedure

For the confirmatory analyses, the following a priori defined hierarchical inferential test order will be applied for the control of the type 1 error rate across the planned multiple comparisons. The primary endpoint and key secondary endpoints will be inferentially evaluated within the FAS in the following order, where inference will proceed at the two-sided 0.05 criterion significance level until the first failure to reject the null hypothesis for a dasiglucagon versus placebo comparison:

- Primary: Time to plasma glucose ≥20 mg/dL recovery
- Secondaries 1-4: Plasma glucose changes from baseline within 30 minutes, within 20 minutes, within 15 minutes, and within 10 minutes after study drug injection or at the time of rescue.
- Secondaries 5-8: Incidences of plasma glucose recovery within 30 minutes, within 20 minutes, within 15 minutes, and within 10 minutes after study drug injection.

The GlucaGen versus placebo comparisons will not be included in the inferential testing hierarchy, since the efficacy of GlucaGen is previously established, and these comparisons are intended to support the validity of the study for the dasiglucagon versus placebo comparisons.

Analogous supportive sensitivity analyses will be conducted in the PP set, but without inference intent.

9.5.2 Primary confirmatory endpoint

- Time to plasma glucose ≥20 mg/dL recovery, defined as first increase in plasma glucose of ≥20 mg/dL (1.1 mmol/L) from baseline during the hypoglycemic clamp procedure.

9.5.2.1 Primary analysis

The primary endpoint of time to plasma glucose ≥20 mg/dL recovery will be analyzed using a Cox proportional hazards (CPH) time to event statistical model, if the hazards by treatment group can be assumed proportional using a graphical comparison of the Nelson-Aalen estimates. The CPH model will be used for inferences, with treatment group and injection site modeled as categorical effects, and baseline plasma glucose modeled as a continuous covariate. The treatment group rate ratios, active versus placebo, will be estimated together with the 95% confidence intervals, and pairwise treatment group inferences (dasiglucagon vs placebo, GlucaGen vs placebo) will be evaluated using two-sided likelihood ratio tests.

If the PH assumption for treatment groups is not met, the primary endpoint will be analyzed using a Kaplan-Meier (KM) time to event statistical model, with treatment group and injection site as stratification factors. Treatment group differences between the KM curves (dasiglucagon vs placebo, GlucaGen vs placebo) will be evaluated inferentially using pairwise two-sided log-rank tests.

In the primary analysis, those subjects who require rescue IV glucose will be censored at the time to plasma glucose recovery. In a sensitivity analysis, the time to plasma glucose recovery will be analyzed without censoring those subjects who require rescue IV glucose before 45 minutes.

9.5.3 Secondary endpoints

- Plasma glucose changes from baseline within 30 minutes, within 20 minutes, within 15 minutes, and within 10 minutes after study drug injection or at the time of rescue.
- Incidences of plasma glucose recovery (achieving a plasma glucose concentration increase of ≥20 mg/dL [1.1 mmol/L]) within 30 minutes, within 20 minutes, within 15 minutes, and within 10 minutes after study drug injection.

9.5.3.1 Confirmatory analysis

The key secondary endpoints of plasma glucose changes from baseline (CFB) within 30 minutes, within 20 minutes, within 15 minutes, and within 10 minutes after study drug injection or at the time of rescue, will be analyzed with the plasma glucose CFB at rescue carried forward in those subjects who require rescue IV glucose before plasma glucose ≥20 mg/dL recovery. Each of these CFB variables will be analyzed using an Analysis of Covariance model, with treatment group modeled as a fixed effect and with the baseline plasma glucose modeled as a covariate. The dasiglucagon versus placebo treatment group difference will be evaluated inferentially as a least squares means contrast, using a two-sided t-test at the 0.05 signficance level, first for the 30 minute CFB, and then sequentially for the 20 minute, 15 minute, and 10 minute variables, until the first failure to reject.

The key secondary incidence variables (plasma glucose recovery within 30 minutes, within 20 minutes, within 15 minutes, within 10 minutes, in hierarchical order for inference) will be analyzed using descriptive statistics (frequency and percentage) by treatment group. Inferential treatment group comparisons will be assessed via pairwise tests of independent binomial proportions (dasiglucagon versus placebo, GlucaGen versus placebo).

9.5.4 Secondary clinical efficacy (PD) endpoints

- Time to first plasma glucose concentration of ≥70 mg/dL (3.9 mmol/L).

- Plasma glucose response as area under the curve above baseline from time zero to 30 minutes, $AUC_{0\text{-}30min}$.

Secondary clinical efficacy endpoints will be assessed based on plasma concentration data from samples collected at the dosing visit (Visit 2).

Unless otherwise stated, the population base of analysis will be the FAS.

9.5.4.1 Analysis of secondary clinical efficacy (PD) endpoints

1. Time to first plasma glucose concentration ≥70 mg/dL from baseline. This time-to-event endpoint will be evaluated using a KM time to event statistical model, with treatment group as a stratification factor, analogous to that used for the primary endpoint analysis. Differences between the KM curves (dasiglucagon versus placebo, GlucaGen versus placebo) will be evaluated inferentially using pairwise two-sided stratified log rank tests. If the ≥70 mg/dL endpoint is not met within 45 minutes post-dosing, the time of the last valid plasma glucose measurement up to 45 minutes will be the censoring time.
2. The AUC will be calculated as the baseline-adjusted area under the plasma glucose profile over time:
    a. $AUC_{0\text{-}30min}$: restricting the time window to the 0 to 30 minutes interval.
3. The log-transformed AUC endpoint will be analyzed using an analysis of covariance (ANCOVA) model with treatment as fixed effect and baseline plasma glucose modeled as a covariate. The least squares means treatment group differences will be back-transformed (anti-logged) for presentation as a ratio of the treatment group geometric means, with their corresponding 95% CI.

9.5.5 Exposure (PK) endpoints

- Plasma dasiglucagon and GlucaGen concentrations from time zero to 90 minutes: $AUC_{0\text{-}90min}$, C_{max}, and t_{max}.

9.5.5.1 Analysis of exposure (PK) endpoints

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

C_{max} will be determined as the maximum of all valid plasma dasiglucagon/GlucaGen concentrations.

T_{max} will be determined as the timepoint where the maximum of all valid plasma dasiglucagon/GlucaGen concentration measurements for each measurement series is observed.

The log-transformed PK endpoints AUC and C_{max} will be analyzed in the same way as the AUC endpoints.

As t_{max} is a highly discrete endpoint, Wilcoxon's rank sum test for unpaired observations will be used to assess differences between the two treatment groups.

9.6 Exploratory analyses

Exploratory analyses will include descriptive statistics and modeling analogous to that done for key secondary endpoints. However, treatment group comparisons will be summarized without inference intent.

- Incidence of plasma glucose recovery (achieving a plasma glucose concentration ≥70 mg/dL [3.9 mmol/L] or an increase of ≥20 mg/dL [1.1 mmol/L]) within 30 minutes after study drug injection.
- Plasma insulin response as area under the curve above baseline from time zero to 60 minutes, $AUC_{0\text{-}60\ min}$.

Plasma insulin concentrations measured pre-dose and at 30 and 60 minutes after dosing (see Table 3) will be presented individually. A summary table per timepoint will be provided. The $AUC_{0\text{-}60min}$ will be determined and a summary presented.

9.7 Safety analyses

9.7.1 Adverse events

AEs will be coded using the latest available version of the MedDRA, the version of which will be provided in the clinical trial report. AE summary tables will include the number and percentage of subjects who experienced AEs summarized by system organ class and preferred term.

An overall summary table will be provided showing the number and percentage of subjects with any:

- TEAE
- Severe TEAE
- Serious TEAE
- Drug-related TEAE
- Drug-related severe TEAE
- Drug-related serious TEAE
- TEAE leading to withdrawal
- TEAE with outcome death
- AESI

Refer to Section 7.2.1 for the definition of TEAEs.

9.7.2 Immunogenicity data

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

9.7.3 Clinical laboratory assessments

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

9.7.4 Other safety data

Incidence of rescue infusion of IV glucose during the hypoglycemic clamp procedure will be analyzed using descriptive statistics (frequency and percentage) by treatment group. Inferential treatment group comparisons will be assessed via pairwise tests of independent binomial proportions (dasiglucagon versus placebo, GlucaGen versus placebo).

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

Vital signs, physical examination, 12-lead ECG and local tolerability data will be summarized using descriptive statistics.

9.8 Treatment compliance

Trained unblinded members of staff will perform all administrations of the IMP at the trial center. The administered doses will be recorded in the blinded Drug Accountability form in the eCRF.

PK assessments will support the surveillance of compliance with IMP administration.

9.9 Subject withdrawals and missing data

In the case of subject withdrawal, no imputation of values for PK or PD measurements will be done. Analyses will be done on valid cases only, i.e., no imputation techniques such as last observation carried forward will be applied. For the primary analysis in the FAS, missing values for the primary endpoint will be imputed by a conservative rule considering any missing value as a failure.

9.10 Interim analyses

No interim analyses are currently planned.

10 ETHICAL, LEGAL, AND ADMINISTRATIVE ASPECTS

10.1 Quality assurance

The sponsor or designee will conduct a site visit to verify the qualifications of each investigator, inspect the facilities, and inform the investigator of responsibilities and the procedures for ensuring adequate and correct documentation.

The investigator is required to prepare and maintain adequate and accurate case histories designed to record all observations and other data pertinent to the trial for each trial participant. All information recorded on the eCRFs for this trial must be consistent with the subjects' source documentation (i.e. medical records).

The investigator will permit trial-related monitoring, IRB/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 monitor or a representative of the sponsor will cross-check the data entered in the eCRFs with the source data at the trial center and observe the trial procedures in order to verify adherence to the trial protocol. Any queries will be resolved by the investigator or his/her delegate.

All of the clinical data will be captured via electronic data capture (EDC) using a web-based tool.

The investigator center 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 subjects. The investigator's data will be accessible from the investigator's center throughout the trial. The eCRFs must be kept current to reflect subject status at each phase during the course of the trial. The eCRF will not capture personalized data. The investigator must make a separate confidential record of personalized details (name and initials) on the subject identification and enrollment log. All changes to data are made by the investigator or his/her delegate through the EDC system.

It is the responsibility of the principal investigator of the respective center to ensure that all subject discontinuations or changes in trial or other medications entered on the subject's eCRF are also made on the subject's medical records.

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

10.2 Electronic case report forms

Remote data capture software will be used for data collection. Following training, trial staff will be given access to the software. Access to the software is restricted to staff participating in the trial and the extent of access will depend on the participants' user role in the trial.

The subjects enrolled into the trial will be identified in the database by subject number and trial identification code. The investigator or delegate will enter subject data into the eCRF promptly. All data is to be entered in English. Data recorded in the eCRFs will be accessible to the trial staff throughout the trial.

After data entry, systematic data validation will be performed and data entry discrepancies will be presented electronically directly to the center staff. Queries for discrepant data may be generated automatically by the software upon entry and/or generated manually by the trial monitor or the trial data manager. All queries, whether generated by the system or by trial staff, will be in electronic format.

All sections of the eCRF are to be electronically approved by the investigator or a medically qualified delegate after the data has been entered and all queries have been resolved. Changes to any eCRF page subsequent to the approval require a new approval signature.

All queries and changes/corrections to the data are documented in the eCRF.

10.3 Access to source data

During the course of the trial, a trial monitor will make site visits to review protocol compliance, compare eCRFs with individual subject’s medical records, assess drug accountability, and ensure that the trial is being conducted according to pertinent regulatory requirements.

Checking of the eCRFs for completeness and clarity, and cross-checking with source documents, will be required to monitor the progress of the trial. Moreover, regulatory authorities of certain countries, IRBs/IECs, and/or the sponsor may wish to carry out such source data checks and/or on-site audit inspections. Direct access to source data will be required for these inspections and audits; they will be carried out giving due consideration to data protection and medical confidentiality.

10.4 Source documentation

All source documents from which eCRF entries are derived should be placed in the subject's medical records. If data are to be entered directly into the eCRF this must be specified in a source data agreement prior to the start of the trial.

eCRF entries will be verified with source documentation. The review of medical records will be performed in a manner to ensure that subject confidentiality is maintained.

The trial monitor will check the eCRF for accuracy and completion and perform source data verification. The trial monitor will document source data verification of all reviewed sections of the eCRF.

10.5 Data processing

The trial is run as an EDC trial, i.e. all relevant data is entered by the centers directly into the clinical database. The eCRF is designed to capture all required information in compliance with GCP standards.

10.6 Archiving trial records

According to ICH guidelines, essential documents should be retained for a minimum of 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. However, these documents should be retained for a longer period if required by the applicable legal requirements.

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

10.7 Good clinical practice

The procedures set out in this trial protocol are designed to ensure that the sponsor and investigator abide by the principles of the GCP guidelines of the ICH (1), and of the Declaration of Helsinki (2008) (2). The trial also will be carried out in keeping with local legal requirements.

10.8 Informed consent

Before each subject is admitted to the trial, written informed consent will be obtained from the subject according to the regulatory and legal requirements of the participating country. This consent form must be dated and retained by the investigator as part of the trial records. The investigator will not undertake any investigation specifically required only for the clinical trial until valid consent has been obtained. The terms of the consent and when it was obtained must also be documented in the eCRF.

The investigator will explain to each subject orally and in writing (subject information sheet) the nature, significance, risks and implications of the trial before inclusion. In particular, the subjects 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.
- That his/her medical records may be examined by authorized monitors or clinical quality assurance auditors appointed by the sponsor, by appropriate IRB/IEC members, and by inspectors from regulatory authorities.

All subjects will receive a copy of the subject information sheet and a copy of their signed and dated informed consent form, both of which will be in the subject's local language.

If a protocol amendment is required, the informed consent form may need to be revised to reflect the changes to the protocol. If the consent form is revised, it must be reviewed and approved by the appropriate IEC/IRB, and signed by all subjects subsequently enrolled in the trial as well as those currently enrolled in the trial.

10.9 Protocol approval and amendment

Before the start of the trial, the trial protocol and/or other relevant documents will be approved by the IEC/IRB/competent authorities, in accordance with local legal requirements. The sponsor must ensure that all ethical and legal requirements have been met before the first subject is enrolled in the trial.

This protocol is to be followed exactly. To alter the protocol, amendments must be written, receive approval from the appropriate personnel, and receive IRB/IEC/competent authority approval prior to implementation (if appropriate).

Administrative changes (not affecting the subject benefit/risk ratio) may be made without the need for a formal amendment. All amendments will be distributed to all protocol recipients, with appropriate instructions.

10.10 Duration of the trial

The maximum duration of the trial for each subject will be up to 63 days (including up to 30 days for screening and up to 33 days until the follow-up visit).

The trial will be closed when all subjects have completed Visit 3.

10.11 Premature termination of the trial

If the investigator, the sponsor (e.g. safety committee), or the safety medical monitor becomes aware of conditions or events that suggest a possible hazard to subjects if the trial continues, the trial may be terminated after appropriate consultation between the relevant parties. The trial may also be terminated early at the sponsor's discretion in the absence of such a finding.

Conditions that may warrant termination include, but are not limited to:

- The discovery of an unexpected, significant, or unacceptable risk to the subjects enrolled in the trial
- Failure to enroll subjects at an acceptable rate.
- A decision on the part of the sponsor to suspend or discontinue development of the drug.

The trial can be terminated prematurely by the sponsor at an individual center if:

- The center cannot comply with the requirements of the protocol.
- It is not possible for the center to comply with GCP standards.

10.12 Confidentiality

All trial findings and documents will be regarded as confidential. The investigator and members of his/her research team must not disclose such information without prior written approval from the sponsor.

The anonymity of participating subjects must be maintained. Subjects will be identified on eCRFs submitted to the sponsor by their subject number. On the SAE reports and all other source documents, the subject will be identified via subject number. Documents not

to be submitted to the sponsor that identify the subject (e.g. the signed informed consent form) must be maintained in confidence by the investigator.

10.13 Other ethical and regulatory issues

If a significant safety issue is identified, either from an individual case report or review of aggregate data, then the sponsor will issue prompt notification to all parties: regulatory authorities, investigators and IRB/IECs.

A significant safety issue is one that has a significant impact on the course of the clinical trial or program (including the potential for suspension of the trial program or amendments to protocols) or warrants immediate update of informed consent.

10.14 Liability and insurance

The sponsor will take out reasonable third-party liability insurance cover in accordance with all local legal requirements. The civil liability of the investigator, the persons instructed by him and the hospital, practice or institute in which they are employed and the liability of the sponsor with respect to financial loss due to personal injury and other damage that may arise as a result of the carrying out of this trial are governed by the applicable law.

The sponsor will arrange for liability insurance if subjects should be injured due to the participation in the trial and provided that the sponsor is legally liable for that.

Excluded from the insurance cover are injuries to health and deteriorations of illnesses already in existence which would have occurred or continued to exist even if the subject had not taken part in the clinical trial.

The insurance cover is jeopardized if the subject fails to report immediately to the investigator or responsible physician any injury to health, which might have resulted from participation in the clinical trial, or if he/she undergoes any other medical treatment without their consent before the clinical trial has been completely finished in so far as the individual subject is concerned.

Any injury to health, which might have occurred as a result of participation in the clinical trial must be reported by the subject to the investigator without delay. The investigator is obliged to make such a report in any case.

10.15 Publication policy

By signing the trial protocol, the investigator agrees with the use of results of the trial for the purposes of national and international registration, publication and information for medical and pharmaceutical professionals. If necessary, the authorities will be notified of the investigator's name, address, qualifications and extent of involvement.

A clinical trial report will be prepared and reviewed by the sponsor in co-operation with the coordinating investigator. The coordinatoing investigator will be appointed by Zealand Pharma to review and sign the clinical trial report on behalf of all participating investigators. A summary of the clinical trial report (according to CPMP/ICH137/95) should be sent to the regulatory authorities and IRB/IEC according to the relevant guidelines.

According to the Declaration of Helsinki (2) investigators and sponsors 'have ethical obligations with regard to the publication and dissemination of the results of research'.

The trial design and results may be published as one or more original research manuscripts/abstracts and presented at a scientific meeting. 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 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. Authorship on any publication(s) resulting from this clinical trial will be assigned according to the recommendations of the International Committee of Medicinal Journal Editors (26).

Participating subjects will not be identified by name in any published reports about the clinical trial.

The sponsor is responsible for trial registration at ClinicalTrials.gov (www.clinicaltrials.gov) according to FDA requirements, as well as the European Medicines Agency's Clinical Trials Database (EudraCT).

11 REFERENCE LIST

1. ICH Harmonised Tripartite Guideline. International Conference on Harmonisation. ICH Harmonised Tripartite Guideline. Integrated addendum to ICH E6(R1): Guideline for Good Clinical Practice E6 (R2), Step 4. 09-Nov-2016. Available from: URL: http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E6/E6_R2__Step_4.pdf
2. World Medical Association. Declaration of Helsinki. Ethical Principles for Medical Research Involving Human Subjects. 52nd WMA General Assembly, Edinburgh, Scotland, October 2000. Last amended with Note of Clarification on Paragraph 29 by the WMA General Assembly, Washington 2002, and Note of Clarification on Paragraph 30 by the WMA General assembly, Tokyo 2004
3. 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 May;36:1384-95
4. Cryer PE. The barrier of hypoglycemia in diabetes. Diabetes 2008;57:3169-76
5. American Diabetes Association Position Statement. Standards of medical care in diabetes - 2017. Diabetes Care 2017;40(Supplement 1):S1-S135
6. Davies MJ, Gagliardino JJ, Gray LJ, Khunti K, Mohan V, Hughes R. Real-world factors affecting adherence to insulin therapy in patients with Type 1 or Type 2 diabetes mellitus: a systematic review. Diabetic Medicine 2013;30:512-24
7. International Hypoglycaemia Study Group. Glucose concentrations of less than 3.0 mmol/L (54 mg/dL) should be reported in clinical trials: a joint position statement of the American Diabetes Association and the European Association for the Study of Diabetes. Diabetes Care 2017;40(1):155-57
8. Christensen M, Bagger JI, Vilsbøll T, Knop FK. The alpha-cell as target for type 2 diabetes therapy. Rev Diabet Stud 2011;8:369-81
9. Lund A, Bagger JI, Christensen M, Knop FK, Vilsbøll T. Glucagon and type 2 diabetes: the return of the alpha cell. Curr Diab Rep 2014;14:555
10. Habegger KM, Heppner KM, Geary N, Bartness TJ, DiMarchi R, Tschöp MH. The metabolic actions of glucagon revisited. Nat Rev Endocrinol 2010;6:689-97
11. Onoue S, Ohshima K, Debari K, Koh K, Shioda S, Iwasa S, et al. Mishandling of the therapeutic peptide glucagon generates cytotoxic amyloidogenic fibrils. Pharm Res 2004;21:1274-83

12. Fosgerau K, Hoffmann T. Peptide therapeutics: current status and future directions. Drug Discovery Today 2015;20:122-8
13. Novo Nordisk Limited. SPC for GlucaGen Hypokit 1 mg. 2015. http://www.medicines.org.uk/emc/medicine/4258/SPC/GlucaGen+Hypokit+1+mg - Accessed 13 Mar 2017 .
14. Zealand Pharma A/S. Dasiglucagon Investigator's Brochure, Edition 3, 12 Dec 2016.
15. The Task Force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). 2013 ESH/ESC guidelines for the management of arterial hypertension. European Heart Journal 2013;34:2159-219
16. Levey AS, Coresh J, Greene T, Stevens LA, Zhang Y, Hendriksen BA, et al. Using standardized serum creatinine values in the modification of diet in renal disease study equation for estimating glomerular filtration rate. Annals of Internal Medicine 2006;145:247-54
17. Food and Drug Administration (FDA). Guidance for Industry on Bioanalytical Method Validation. Food and Drug Administration (FDA) 2001
18. Viswanathan CT, Bansal S, Booth B, DeStefano AJ, Rose MJ, Sailstad J, et al. Quantitative bioanalytical methods validation and implementation: best practices for chromatographic and ligand binding assays. Pharm Res 2007 Oct;24:1962-73
19. Shankar G, Devanarayan V, Amaravadi L, Barrett YC, Bowsher 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;48:1267-81
20. Zealand Pharma report SS. Commissioning and validation of a method for the validation of anti-ZP4207 antibodies in human serum. 2014
21. Zealand Pharma report SS. Commissioning and validation of a method for the validation of anti-glucagon antibodies in human serum. 2014
22. Bioagilytix Labs. Validation of an assay to detect neutralizing antibodies to ZP4207 by measuring cyclic adenosine monophosphate (cAMP) in a glucagon receptor transfected cell line, BAL-14-206-004-REP. 2016. Report No.: Zealand Pharma, study 15-039
23. 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. 2016. Report No.: Zealand Pharma, Study 15-040

24. Amiel SA, Tamborlane WV, Simonson DC, et al. Defective glucose counterregulation after strict glycemic control of insulin-dependent diabetes mellitus. N Eng J Med 1987; 316: 1376-83.

25. Rickels MR, Ruedy KJ, Foster NC, et al. Intranasal glucagon for treatment of insulin-induced hypoglycemia in adults with type 1 diabetes: A randomized crossover noninferiority study. Diabetes Care 2016; 39: 264-70.

26. ICMJE. Recommendations for the conduct, reporting, editing, and publication of scholarly work in medical journals. Updated December 2016

12 APPENDICES

12.1 List of trial personnel

Sponsor **Clinical Trial Manager**	[redacted] Zealand Pharma Smedeland 36 2600 Glostrup, Denmark Phone: [redacted]
Medical Officer	[redacted] Zealand Pharma Smedeland 36 2600 Glostrup, Denmark Phone: [redacted]
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Project Manager	[redacted] Chiltern International (Ltd) 171 Bath Road Slough Berkshire SL1 4AA United Kingdom Phone: [redacted]
Senior Medical Officer and Safety medical monitor	[redacted] Chiltern International kft Canada Square Office House Ganz u

	12-14, 4 emelet 1027 Budapest Hungary Phone: [redacted]
Pharmacovigilance unit Responsible for Serious Adverse Event (SAE) Management and 24-hour SAE reporting	[redacted] PharmaLex Agern Allé 24 2970 Hørsholm, Denmark Phone: [redacted] (8 a.m. to 4 p.m.) Phone: (outside 8 a.m. to 4 p.m.) Fax: email: PV-nordic@pharmalex.com
Central laboratory	MLM Laboratory Dohrweg 63 41066 Mönchengladbach Germany
Special laboratory (GlucaGen PK, insulin PK)	MLM Laboratory Dohrweg 63 41066 Mönchengladbach Germany
Special laboratory (dasiglucagon PK, dasiglucagon ADA, GlucaGen ADA)	York Bioanalytical Solutions (YBS) Cedar House Northminster Business Park Northfield Lane York, YO26 6QR, United Kingdom
Special laboratory (neutralizing antibodies)	BioAgilytix 2300 Englert Drive Durham, NC, 27713, USA

A list of all investigators, IECs and IRBs will be provided in a separate document and in the clinical trial report.