Appendix 16.1.1

Protocol and Protocol Amendments

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Clinical trial protocol

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

Sponsor:	Zealand Pharma A/S Smedeland 36 2600 Glostrup, Denmark
Sponsor protocol number:	ZP4207-17145
IND number:	127866
Development phase:	3
Trial drug name:	Dasiglucagon

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.

The information in this document is confidential and is proprietary to Zealand Pharma. It is understood that information in this document shall not be disclosed to any third party, in any form, without prior written consent of an authorized officer of Zealand Pharma

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List of abbreviations and definition 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
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
EudraCT	European Medicines Agency's Clinical Trials Database
FAS	Full analysis set
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HbA1c	Glycated hemoglobin
ICH	International Council for Harmonization
IEC	Independent Ethics Committee
IMP	Investigational medicinal product
IRB	Institutional Review Board
IV	Intravenous(ly)
IWRS	Interactive Web Response System
KM	Kaplan-Meier
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
SC	Subcutaneous(ly)
SUSAR	Suspected unexpected serious 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

1 Protocol synopsis

Title of trial

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

Trial site(s)

The trial will be conducted in up to 4 sites in North America.

Clinical phase

3

Objectives

Primary objective

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

Secondary objectives

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

Trial design

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

Subjects will be randomized to receive a single SC dose of dasiglucagon or matching placebo in a ratio of 3:1. Each subject will undergo a screening visit up to 30 days prior to dosing, an overnight in-house visit, where dosing will take place, and a follow-up visit. Depending on the development of anti-drug antibodies (ADAs) during the trial, additional follow-up visits may be required.

Number of subjects

A total of 40 subjects is planned to complete the dosing visit (Visit 2), with 30 subjects randomized to the dasiglucagon group and 10 subjects randomized to the placebo group. It is expected that up to 46 subjects will be randomized to have 40 subjects completing Visit 2.

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Criteria for inclusion/exclusion

Key inclusion criteria

- Female or male subjects with T1DM for at least 1 year; diagnostic criteria as defined by the American Diabetes Association (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
- Hemoglobin A1c <10%.
- Aged between 18 and 75 years, both inclusive.

Key exclusion criteria

- Previous participation in a clinical trial within the dasiglucagon in the rescue treatment of hypoglycemia program.

- Known or suspected allergy to trial drug(s) or related products.
- History of anaphylaxis or symptoms of severe systemic allergy (such as angioedema).
- Previous participation in this trial. Participation being defined by signing the informed consent document.

Investigational medicinal product(s)/trial drugs

Trial drug: dasiglucagon, liquid formulation, 1.0 mg/mL, 0.6 mL delivered in auto-injector.
Comparator drug: Placebo, liquid formulation, 0.6 mL delivered in auto-injector.

Duration of treatment

Subjects will receive one single SC dose of dasiglucagon 0.6 mg or placebo

Duration of participation in the trial

The duration for subjects in this trial, including screening, treatment and follow-up will approximately be 8 weeks.

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

Trial procedures/assessments

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

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

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

Statistical methods

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

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.

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 before 45 minutes will be censored (and 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) 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) using Fisher's exact test.

The key secondary endpoints of plasma glucose changes from baseline at 30 minutes, at 20 minutes, at 15 minutes, and at 10 minutes after trial drug injection or at the time of rescue, will be analyzed with the plasma glucose changes from baseline at rescue carried forward in those subjects who require rescue IV glucose before plasma glucose ≥20 mg/dL (1.1 mmol/L) recovery. Each of these changes from baseline 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 first for the 30 minute changes from baseline, and then sequentially for the 20 minute, 15 minute, and 10 minute variables, until the first failure to reject.

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

The trial will be conducted in accordance with the Declaration of Helsinki and Good Clinical Practice.

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 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). Hypoglycemia 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 1.0 mg, given by intramuscular, 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 (hereafter referred to as dasiglucagon) is in development for the treatment of severe hypoglycemia in 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).

A total of five clinical trials have been conducted with dasiglucagon to date. Four trials were conducted using a 1.0 mg/mL dasiglucagon formulation: two clinical pharmacology trials and two phase 2 trials. The fifth trial (ZP4207-16098) was a phase 2 trial using a 4.0 mg/mL dasiglucagon formulation.

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.

No major differences in PD responses were observed between dasiglucagon doses under euglycemic or hypoglycemic conditions in the phase 2 trial ZP4207-16098. Under euglycemic conditions, all subjects achieved an increase in PG of at least 20 mg/dL (1.1 mmol/L) within 30 minutes after dosing with 0.08 mg dasiglucagon and above, while only the 0.2 mg dose of marketed glucagon (Lilly Glucagon™) reached this target. Under hypoglycemic conditions, dasiglucagon met the target at doses of 0.2 mg and above. The PD response over the entire observation time of 240 minutes was significantly higher with dasiglucagon than with Lilly Glucagon at the same doses.

2.1.3.2 Safety of dasiglucagon

No safety concerns were raised for dasiglucagon at the doses investigated in the clinical trials. The most frequently reported adverse event (AE) in each of the trials was nausea, which is a known side-effect following administration of glucagon and appeared at a similar frequency to marketed glucagon, which was used as active comparator in some of the trials. No local tolerability issues occurred. Injection site reactions were observed only sporadically after administration with dasiglucagon, placebo or marketed glucagon in trials with SC or IM administration, and all events 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 and bradycardia were observed in a few healthy volunteers receiving investigational medicinal product (IMP) doses of at least 1.0 mg (4 with dasiglucagon and 1 with GlucaGen). This is not considered a safety concern; however, hemodynamic changes after dosing will be considered an adverse event of special interest (AESI) and information will be collected for this type of adverse events, to support the evaluation of the data at the end of the trial.

For further information on the safety of dasiglucagon, please refer to the Investigator's Brochure (14).

2.2 Rationale

The aim of this phase 3 trial is to confirm the superiority of dasiglucagon for the treatment of hypoglycemia in subjects with T1DM as compared to placebo.

2.3 Risk-benefit assessment

2.3.1 Nonclinical 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.

2.3.2 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 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. Based on the 4 clinical studies completed with dasiglucagon to date (see Section 2.1.3), no anti-dasiglucagon or anti-glucagon antibodies have been detected, except for trial ZP4207-16098, in which there was one transient low titer anti-drug antibody (ADA) incidence showing reactivity towards both dasiglucagon and glucagon. The subject was tested positive for both anti-dasiglucagon and anti-glucagon antibodies at the follow-up visit, 24 days after the last drug exposure (titer: 35.4 and 33.8, respectively). The subject was additionally found to be positive for dasiglucagon and glucagon in vitro neutralizing activities. The titer of anti-dasiglucagon activity was equal to the assay minimum required dilution. Due to the crossover nature of the trial, it was impossible to establish the presence of ADA in association with dosing of dasiglucagon or glucagon. Testing performed 3.5 months after last dosing, confirmed a positive finding for anti-dasiglucagon antibodies (titer of 38.8), while the test was negative for anti-glucagon antibodies. At a final follow-up visit performed 7 months after last dosing, the subject was negative for anti-dasiglucagon antibodies. There was no evidence for altered PK, PD, or safety profile for this subject.

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 drugs 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 clinical trials conducted with dasiglucagon. Direct access to resuscitation equipment is ensured at the clinical trial sites.

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.

Two phase 1 and three 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. These trials have demonstrated that SC and intramuscular administration of dasiglucagon is efficacious and well tolerated, with a safety profile that does not give rise to specific safety concerns (14). 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 benefit-risk balance for patients participating in the trial is considered acceptable.

3 Trial objectives and endpoints

The objectives and endpoints of this trial are shown in Table 1.

Table 1 Objectives and endpoints

Objectives	Endpoints
Primary objective	
To demonstrate superiority of dasiglucagon compared to placebo following a single SC 0.6 mg dose administered to subjects with T1DM with insulin-induced hypoglycemia.	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. Key secondary endpoints: • Plasma glucose recovery within 30 minutes, within 20 minutes, within 15 minutes, and within 10 minutes after trial drug injection without administration of rescue IV glucose. • Plasma glucose changes from baseline at 30 minutes, at 20 minutes, at 15 minutes, and at 10 minutes after trial drug injection or at the time of rescue.
Secondary objectives	**Secondary endpoints (other)**
To evaluate the safety, immunogenicity and PK of dasiglucagon following a single SC dose administered to subjects with T1DM with insulin-induced hypoglycemia as compared to placebo.	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 effect curve (AUE) above baseline from time zero to 30 minutes, $AUE_{0\text{-}30min}$. 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). • Local tolerability. • Number of rescue infusions of IV glucose after trial drug administration. • Time to first rescue infusion of IV glucose after trial drug administration. Immunogenicity endpoint: • Occurrence of anti-drug-antibodies.
Tertiary/Exploratory objective(s)	**Tertiary/Exploratory endpoints**
Tertiary/exploratory objective(s) serve as a basis for explaining or supporting findings of primary analyses and for suggesting further hypotheses for later research.	• Plasma glucose concentration ≥70 mg/dL (3.9 mmol/L) or increase of ≥20 mg/dL (1.1 mmol/L) within 30 minutes after trial drug injection without administration of rescue IV glucose. • Plasma insulin response as area under the curve above baseline from time zero to 60 minutes, $AUC_{0\text{-}60\ min}$.

4 Overall design and plan of the trial

4.1 Overview

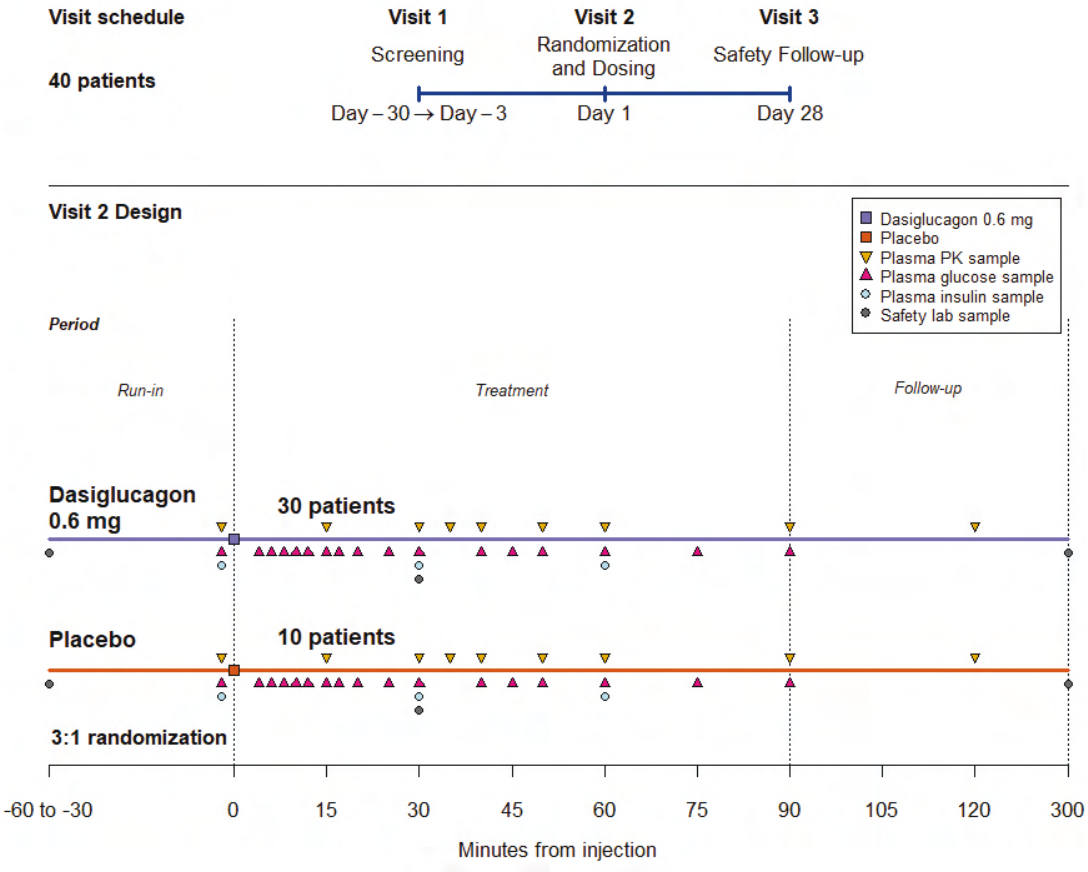
The trial is a multi-center, randomized, parallel-group, double-blind, clinical efficacy and safety trial evaluating rescue treatment of insulin-induced hypoglycemia in subjects with T1DM. The design of the trial is shown in Figure 1.

The subjects will be randomized 3:1 to receive a single fixed SC dose of dasiglucagon or placebo and will be followed for at least 28 days after dosing.

Prior to the dosing visit (Visit 2), the subjects will discontinue their current insulin therapy in due time to ensure wash-out. At the dosing visit, hypoglycemia will be gradually induced by a controlled IV infusion of a fast-acting insulin, i.e. insulin glulisine (Apidra®), targeting a plasma glucose level of 55 mg/dL (3.1 mmol/L). Trial drug will be administered when the subject's plasma glucose level is 45-60 mg/dL (2.5-3.3 mmol/L).

During the insulin-induced hypoglycemia, plasma glucose levels will be monitored closely at site for safety reasons.

Figure 1 Trial design



4.2 Justification of the trial design

4.2.1 Design and parameters

In order to avoid bias in subject selection and in the evaluation of clinical assessments, subjects will be randomly assigned 3:1 to either dasiglucagon or placebo and the trial will be conducted in a double-blinded manner.

A superiority trial design is used because the aim is to show that treatment with dasiglucagon is an effective treatment compared to placebo.

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 following a single SC dose, the primary endpoint and secondary efficacy endpoints involve the measurement of plasma glucose concentrations at different time points.

Subjects will be followed for at least 28 days after dosing in order to perform an adequate immunogenicity evaluation of treatment. Depending on the development of ADAs during the trial, additional follow-up visits may be performed.

4.2.2 Drug, route, dosage and treatment duration

Dasiglucagon will be administered as a single fixed dose of 0.6 mg independent of body weight because this is the intended therapeutic dosing regimen in the emergency treatment of hypoglycemia. Data from the trials 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.

Dasiglucagon or placebo will be administered in the buttock or deltoid by SC injection, as this is one of the intended routes of administration for dasiglucagon, besides intramuscular and IV.

4.3 End of trial definition

A subject is considered to have completed the trial if he or she has completed the 28 days follow-up visit or the last scheduled procedure shown in Table 4.

The end of the trial is defined as completion of the last visit or procedure shown in the Table 4.

5 Trial population

5.1 Rationale for trial population

Dasiglucagon is in development for the treatment of severe hypoglycemia in patients with diabetes mellitus. In the present trial, subjects with T1DM are selected to avoid the endogenous glucagon counter-regulatory response to insulin induced hypoglycemia that may be 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 sites

A total of 40 subjects is planned to complete the dosing visit (Visit 2). It is expected that up to 46 subjects will be randomized to have 40 subjects completing Visit 2.

The trial will be conducted at up to 4 sites in North America.

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 A1c <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)
      iv) Condom with spermicide
   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 are not eligible:

1. Previous participation in a clinical trial within the dasiglucagon in the rescue treatment of hypoglycemia program.
2. Known or suspected allergy to trial drug(s) or related products.
3. History of anaphylaxis or symptoms of severe systemic allergy (such as angioedema).
4. Previous participation in this trial. Participation being defined by signing the informed consent document.
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 (an episode requiring assistance from another person) in the last month prior to screening.
8. Receipt of any investigational medicinal product 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 at screening.
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. Significant history of alcoholism or non-prescribed opioid misuse as judged by the investigator.
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 within 7 (+2) 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 since screening, 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 discontinuation of subjects or trial

5.6.1 Subject discontinuation

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).

A subject will be discontinued from dosing and/or trial 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 drug, 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 the trial drug, every effort should be made to have the subject return and participate in the complete follow-up visit on Day 28 (see Table 4) to avoid missing data.

If trial participation is terminated due to an AE possibly related to the trial drug 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.

5.6.2 Site discontinuation

The site can be closed for the following reasons:

- The site is unlikely to be able to recruit sufficient subjects within the agreed time frame
- The site does not respond to trial management requests
- Repeat protocol violations
- The site cannot comply with the requirements of the protocol
- It is not possible for the site to comply with GCP standards
- The site is unable to comply with the timely entry of data in the eCRF

5.6.3 Trial termination

If the investigator, the sponsor 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
- New scientific knowledge that makes the objectives of the trial no longer feasible/valid
- Failure to enroll subjects at an acceptable rate
- A decision on the part of the sponsor to suspend or discontinue development of the drug

5.7 Subject identification and randomization

Subjects who have given written informed consent will be provided with a subject number. Once assigned, the subject number will be used throughout the trial and must not be reused by any other subject.

Subjects who meet all inclusion and none of the exclusion criteria or dosing day exclusion criteria will be assigned a subject randomization number and randomized in a 3:1 ratio to 0.6 mg dasiglucagon (n=30) or placebo (n=10) via an Interactive Web Response System (IWRS). This randomization will also determine whether the subject is to be injected in the buttock or deltoid. Randomization will continue until a total of 40 subjects have completed Visit 2.

If the subject fails to be randomized or to start treatment for any reason, the reason will be entered into the screening disposition page. The IWRS must be notified within 2 days that the subject was not randomized.

In the event of an emergency, e.g. when it becomes necessary for the investigator to know which trial drug 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, see Section 6.4.

5.8 Screening failures

Screening failures are defined as subjects who consent to participate in the clinical trial but do not meet the inclusion or exclusion criteria at the screening visit (Visit 1). A minimal set of screen failure information is required to ensure transparent reporting of screen failure subjects, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria and any serious adverse event (SAE).

Re-screening is not allowed.

6 Trial drug

6.1 Identity

The following trial drugs will be administered:

- Dasiglucagon, liquid formulation, 1 mg/mL, 0.6 mL delivered in an auto-injector.
- Placebo, liquid formulation, 0.6 mL delivered in an auto-injector.

The description of the trial drugs is provided in Table 2. Dasiglucagon is a stable peptide analog of human glucagon in a ready-to-use liquid formulation for treatment of severe hypoglycemia in patients with diabetes mellitus.

The quantities of ingredients for dasiglucagon and placebo are provided in Table 3.

Table 2 Description of trial drug

Name of active ingredient	Dasiglucagon	Placebo
Active substance	Dasiglucagon	N/A
Formulation	Liquid formulation	Liquid formulation
Strength	1.0 mg/mL	N/A
Device	Single use auto-injector	Single use auto-injector
Manufacturer	Zealand Pharma A/S, Glostrup (Copenhagen), Denmark	
Storage	Store between 2 and 8°C	

N/A not applicable

Table 3 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 1.0 mL	To make 1.0 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 Dosing and administration

The trial drug (dasiglucagon or placebo) will be administered by SC injection in the buttock or deltoid (as determined by randomization).

All doses will be administered by staff at the trial site.

6.3 Packaging, labelling and storage

6.3.1 Packaging and labelling

The trial drug 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 trial drug label will describe the storage conditions for the trial drug. The labels will supply no information about the subjects. Each auto-injector will have a unique Dispensing Unit Number for drug allocation, drug accountability and traceability purposes, and is labelled in a blinded fashion.

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

6.3.2 Trial drug storage and stability

The investigator must ensure the availability of proper storage conditions. All trial drug supplies provided for this trial will be stored in the refrigerator in a secure area with restricted access at the trial site. The trial drugs ready for use should be separated from any other clinical supplies, to avoid medication errors.

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 trial termination.

The trial drug (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.

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

Please see the Trial Materials Manual/Pharmacy Manual for additional information on handling the trial drug.

6.4 Blinding and breaking the blinding code

In this double-blind trial, the trial drug (dasiglucagon and placebo) are identical liquid formulations in auto-injectors.

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 site 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]) will be able to break the code in case of a suspected unexpected serious adverse reaction (SUSAR).

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

6.5 Drug accountability

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

All unused supplies and all empty packaging will be stored until the trial closure visit has been performed and then sent for destruction. Destruction must not take place until approved by the sponsor.

6.6 Drug exposure and compliance

All trial drugs will be administered by trial personnel. Time and date of the administered doses will be recorded in the eCRF.

PK assessments will support the surveillance of compliance with trial drug administration.

6.7 Concomitant therapy

6.7.1 Previous and concomitant medication

Concomitant medication is any medication apart from the trial drug that is used during the subject's participation in the trial. All concomitant medication used must be recorded from the time of signature of the informed consent form (ICF) and to the end of the trial. The investigator or delegate must seek information on concomitant medication use at each trial visit. The information collected for concomitant medication must include:

- Medication name (preferably generic name)
- Indication
- Dosage
- Frequency
- Route of administration
- Start and stop date or continuation

6.7.2 Prohibited medication

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)
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.

6.8 Rescue medication

During the hypoglycemic clamp procedure, it may be necessary to administer a rescue glucose infusion. Provisions for hypoglycemia rescue are described in Section 8.2.3.2.

Use of rescue glucose is to be recorded in the eCRF.

7 Parameters and methods of assessment

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 ($AUE_{0\text{-}30min}$) 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, 45, 50, 60, 75 and 90 minutes after dosing (see the schedule of procedures in Table 4). The actual time of blood sampling for evaluation of plasma glucose should not deviate from the nominal time by more than ±1 minute. Pre-dose is defined as within 2-5 minutes prior to dosing.

7.1.2 Pharmacokinetic measurements

The exposure to dasiglucagon 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 4. The actual time of blood sampling for exposure to trial drug should not deviate from the nominal time by more than ±1 minute. Pre-dose is defined as within 2-5 minutes prior to dosing.

7.2 Safety parameters

7.2.1 Adverse events and pregnancies

Timely, accurate and complete reporting and analysis of safety information from clinical trials are crucial for the protection of subjects, investigators and the sponsor, and are mandated by regulatory agencies worldwide.

7.2.1.1 Definitions

7.2.1.1.1 Adverse events

An adverse event (AE) is any untoward medical occurrence in a clinical trial subject administered a medicinal (investigational or non-investigational) product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporarily associated with the use of a product, whether or not related to the product.

AEs include:

- A clinically significant worsening of a concomitant illness.
- A clinical laboratory adverse event: a clinical 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, e.g. change of dose or more frequent follow-up due to the abnormality.

The following should not be considered as AEs:

- Pre-existing conditions, including those found as a result of screening procedures (pre-existing conditions should be reported as medical history or concomitant illness).
- Pre-planned procedures, 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.

When assessing an adverse event, the following definitions are used:

Severity:

- **Mild:** No or transient symptoms, no interference with the subject's daily activities.
- **Moderate:** Marked symptoms, moderate interference with the subject's daily activities.
- **Severe:** Considerable interference with the subject's daily activities, which the subject finds unacceptable. A severe reaction does not necessarily deem the AE as serious and an SAE are not always severe in nature.

Causality:

- **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 product.
- **Not Related:** An AE that does not follow a reasonable temporal sequence from administration of a drug and/or that can reasonably be explained by other factors, such as underlying diseases, complications, concomitant drugs and concurrent treatments.

Outcome:

- **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 ICF.
- **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 or has died from another AE.
- **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, or the outcome is not known.
- **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.

7.2.1.1.2 Treatment-emergent adverse events (TEAEs)

A treatment emergent adverse event (TEAE) is defined as an AE with an onset at the time of or following the start of treatment with the trial drug.

7.2.1.1.3 Serious adverse event

A serious adverse event (SAE) is any untoward medical experience that at any dose result in any of the following:

- 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 defects.
- Is otherwise medically important, that may not result in death, be life threatening or require hospitalization may be considered an SAE when (based on appropriate medical judgement) it may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in the definition of SAE. Examples could be emergency room or home treatment of allergic bronchospasm or convulsion.

7.2.1.1.4 Other important adverse event

The following events must always be reported to the sponsor according to SAE timelines, regardless of whether the event is non-serious or serious:

- Risk of liver injury defined as ALT or AST > 3x upper limit of normal (ULN) and total bilirubin > 2x ULN, where no alternative etiology exists.
- Suspicion of transmission of infectious agents via the trial drug.
- Overdose of the trial drug.
- Medication error involving the trial drug.
- Inadvertent or accidental exposure to the trial drug.

7.2.1.1.5 Non-serious adverse events

A non-serious AE is any AE which does not fulfil the definition of an SAE.

7.2.1.1.6 Adverse event of special interest

In this trial the following adverse events are considered adverse events of special interest (AESI), with data collected under a specific eCRF form:

- 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.

7.2.1.1.7 Suspected unexpected serious adverse reactions

An AE is considered a SUSAR if the nature or severity is not consistent with the applicable product Reference Safety Information (RSI). For dasiglucagon, the expectedness of an AE will be determined by whether or not it is listed in the RSI section of the Investigator's Brochure.

7.2.1.1.8 Adverse events associated with the use of the drug

An AE is considered associated with the use of the drug if the causality assessment is possible or probable by the definition listed in Section 7.2.1.1.1.

7.2.1.2 Collection, recording and reporting of adverse events

All AEs, whether serious or non-serious, will be reported from the time a signed and dated ICF is obtained until the end of the post-treatment follow-up period (which may include contacts for follow-up of safety). In addition, subjects will be observed for any signs or symptoms and asked about their condition by open questioning, such as "How have you been feeling since you were last asked?", at each contact with the trial site (visit or telephone). Subjects will also be encouraged to spontaneously report AEs occurring at any other time during the trial.

All AEs, regardless of seriousness, severity, or presumed relationship to trial drug, must be recorded and evaluated by the investigator. Whenever possible, diagnoses should be given when signs and symptoms are due to a common etiology. If no diagnosis can be made, the investigator should record each sign and symptom as individual AEs. Investigators must record their opinion concerning the relationship of the AE to the trial drug. All assessments completed during AE evaluation must be recorded in the source document and reported according to sponsor instructions.

Each AE must be reported on an AE eCRF within 5 calendar days of the investigator becoming aware of the event.

AESI will be reported by adding a tick-mark in the appropriate box on the AE and/or SAE form and by completing 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 immediately (within 24 hours) after obtaining knowledge about the event.

AE information should as a minimum include the following:

- Date and time of onset
- Date and time of investigator's first information about the AE
- Seriousness
- Severity
- Causal relationship with trial drug
- Measures taken due to AE
- Interruption or withdrawal of treatment during IMP administration
- Date and time of resolution and final outcome

SAEs, including those spontaneously reported to the investigator within 30 days after the last dose of trial drug, must be reported within 24 hours after obtaining knowledge about the event by completing the SAE form in the Electronic Data Capture (EDC) system. Once additional information becomes available, a follow-up SAE form must be reported. For each SAE, a separate SAE form should be completed.

All SAEs will be reported in EDC, and for each reported event a system-generated email will be sent to the safety CRO (PharmaLex), the medical monitor, sponsor medical director, and trial manager

Pharmacovigilance for this trial is outsourced to PharmaLex Denmark A/S.

It is the responsibility of the Safety CRO to report all SUSARs that occur in this trial to competent authorities. It is the responsibility of clinical CRO to report all SUSARs that occur in this trial to IRBs in accordance with the local requirements in force and ICH guideline for GCP.

7.2.1.3 Follow-up of adverse events

The investigator must record follow-up information on the eCRF for non-serious AEs, on the AESI form for AESIs, and on the SAE form for SAEs during the follow-up periods as described below. Follow-up questions to investigators regarding SAEs are queried directly by PharmaLex to the investigator.

Follow-up information must be reported according to the following:

- **SAEs:** All SAEs must be followed until the outcome of the events is "recovered/resolved", "recovered/resolved with sequelae" or "fatal" and until all queries have been resolved. Cases of chronic conditions, cancer or AEs ongoing at time of death (where death is due to another AE) may be closed with the outcome "recovering/resolving" or "not recovered/not resolved". Cases can be closed with the outcome of "recovering/resolving" when the subject has completed the follow-up period and is expected by the investigator to recover.

The SAE follow-up information should only include new (e.g. corrections or additional) information and must be reported within 24 hours of the investigator's first knowledge of the information. This is also the case for previously non-serious AEs which subsequently become SAEs.

- **Non-serious AEs:** Non-serious AEs must be followed until the outcome of the event is "recovering/resolving", "recovered/resolved" or "recovered/resolved with sequelae" or until the end of the follow-up period stated in the protocol, whichever comes first, and until all queries related to these AEs have been resolved. Cases of chronic conditions, cancer or AEs ongoing at time of death (where death is due to another AE) may be closed with the outcome of "recovering/resolving" or "not recovered/not resolved". Cases can be closed with the outcome of "recovering/resolving" when subject has completed the follow-up period and is expected by the investigator to recover.

If a potential hypersensitivity reaction is observed, additional blood samples may be required to further characterize the potential hypersensitivity reaction. If an anaphylactic shock is suspected, samples may be taken for the measurement of tryptase. In this case, a blood sample should be taken 3 to 4 hours after the event and again approximately 1 to 2 weeks later to determine tryptase baseline levels. In addition, assessments for elevated histamine levels may be considered.

The investigator must ensure that the maximum severity of an event is kept throughout the trial, starting at the time of exposure to trial drug. A worsening of an unresolved AE must be reported as follow up with re-assessment of severity of the event.

Queries or follow-up requests must be responded within 14 calendar days from the date of receipt of the request, unless otherwise specified in the follow-up request.

7.2.1.4 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 trial drug, a plasma glucose value of <45 mg/dL (2.5 mmol/L) will be treated by an IV glucose solution. After administration of the trial drug 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 trial drug 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.

7.2.1.5 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. All initial reports of pregnancy in female subjects must be reported to the sponsor by the trial site personnel within 24 hours of their knowledge of the event using the appropriate pregnancy form. Abnormal pregnancy outcomes (e.g. spontaneous abortion, fetal death, stillbirth, congenital anomalies, ectopic pregnancy) are considered SAEs and must be reported using the SAE form. If a subject becomes pregnant during the trial, a determination regarding the trial drug discontinuation must be made by the investigator.

Because the effect of the trial drug on sperm is unknown, pregnancies in partners of male subjects must be reported by the trial site personnel within 24 hours of their knowledge of the event using the appropriate pregnancy form. Male subjects must be instructed to notify the investigator immediately if their partner becomes pregnant or suspects to be pregnant.

The investigator must follow the pregnancy until the pregnancy outcome is known and the newborn infant is one month of age. The investigator must report information about the pregnancy, pregnancy outcome and health of the newborn infant(s). Any AEs in connection with the pregnancy or AEs in the fetus and newborn infant must be reported.

7.2.1.6 Precautions

Normal precautions taken for a human trial, including the provision of emergency equipment, will be taken during this trial. Qualified and well-trained physicians and medical staff will instruct the subjects. During a subject's participation in the trial, the investigator should ensure that adequate medical care is provided to the subjects 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, refer to the current version of the Investigator's Brochure (14).

7.2.1.7 Safety Committee

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

If safety signals or concerns are observed, whether based on reported SAEs, review of all AEs and laboratory parameters reported or any other notification of significant findings, the Safety Committee will respond appropriately to protect the safety of the subjects. The Safety Committee meets quarterly and additionally on ad-hoc basis as needed.

7.2.2 Safety laboratory parameters

7.2.2.1 Clinical chemistry, hematology and urinalysis

Routine clinical laboratory tests will be performed centrally.

Samples for clinical laboratory parameters (biochemistry, hematology) and urinalysis will be collected at screening (Visit 1), the dosing visit (Visit 2) and the follow-up visit (Visit 3). Samples

for glycated hemoglobin (HbA_{1c}), C-peptide and coagulation parameters will be collected at screening only (Visit 1). The following parameters will be assessed:

- Clinical biochemistry: sodium, potassium, calcium, glucose, urea, creatinine, total bilirubin, AST, ALT, gamma-glutamyl transferase, alkaline phosphatase, total protein, C-reactive protein, C-peptide, HbA_{1c}.
- Hematology: hemoglobin, red blood cell count (erythrocytes), hematocrit, platelet count (thrombocytes), total white blood cell count (leukocytes).
- Coagulation: international normalized ratio, fibrinogen (only Visit 1).
- Urinalysis: pH, blood (leukocytes and erythrocytes), protein, glucose, ketones, nitrite.

At the dosing visit, samples for clinical laboratory parameters (biochemistry, hematology) will be collected 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. The sample for urinalysis will be collected prior to the start of the insulin-induced hypoglycemic procedure (within 120 minutes).

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.

7.2.2.2 Pregnancy test

Pregnancy tests will be performed at each visit for women of childbearing potential. A serum pregnancy test will be performed at screening (Visit 1) and urine stick tests will be performed at the trial site at the dosing visit (Visit 2) and the follow-up visit (Visit 3). Test sticks will be provided to the trial site.

7.2.2.3 Plasma glucose measurements for safety

During insulin-induced hypoglycemia, plasma glucose levels will be monitored closely at site. After the start of the IV insulin infusion, plasma glucose should be checked approximately every 10 minutes while plasma glucose is above 110 mg/dL (6.1 mmol/L), and approximately every 5 minutes once plasma glucose is at or below 110 mg/dL (6.1 mmol/L) and until after dosing when the subject's plasma glucose is ≥ 70 mg/dL (3.9 mmol/L). Plasma glucose will continue to be checked every 5 minutes after dosing until the 45 min time point. 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.

7.2.2.4 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 insulin should not deviate from the nominal time by more than ±1 minute. Pre-dose is defined as within 2-5 minutes prior to dosing.

7.2.2.5 Other safety laboratory tests

Alcohol breath tests and a urine drug screen (12 panel drug test; including benzodiazepines, barbiturates, cocaine, marijuana, metamphetamine, morphine, methadone, buprenorphine, oxycodone, methylendiaxymetamphetamine, phencyclidine, amphetamine) 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 urine drug screen will be provided to the trial site.

7.2.3 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 assessment of Exclusion Criteria #11, and this arm should be used for all subsequent measurements of the subject's blood pressure in this trial.
  If blood pressure measurements are within 10 mmHg in both arms, one arm will be chosen and 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 aural (°C).

At the dosing visit vital signs will be measured pre-dose (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.

7.2.4 Physical examination

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.

7.2.5 Electrocardiogram

A standard 12-lead ECG (safety ECG) will be performed after 5 minutes in a supine position at the screening visit (Visit 1), the dosing visit (Visit 2) and the follow-up visit (Visit 3).

At the dosing visit single recordings will be made pre-dose (within 30 minutes) and at 20, 35, 45, 60 and 300 minutes. The actual time of the assessment should not deviate from the nominal time by more than ±5 minutes.

Safety ECGs will be printed and assessed locally by the investigator or a qualified designee.

ECG parameters (heart rate, PQ, QRS, QTcF [corrected using the Fridericia correction]) 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').

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), at the follow-up visit (Visit 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.

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 in the eCRF. The assessments will be performed by a trial physician or nurse. In case of an observation, the Local Tolerability form will be completed, as well as the (S)AE form.

7.2.7 Immunogenicity

Antibodies against dasiglucagon 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 injection. The ADA samples will be analyzed at a special laboratory, York Bioanalytical Solutions.

The clinical ADA assay specific for dasiglucagon has been validated in accordance with existing guidelines and recommendations (17-20).

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. Any anti-dasiglucagon antibody-positive subject must be followed up every 3 months at extra visits, where a blood sample for the assessment of ADAs and plasma glucose must be collected until the ADA levels return to baseline levels.

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, 21). The calculated sensitivity in previous studies was about 51.8 ng/mL. 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.

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

The results from the neutralizing antibody assay may be included in the clinical trial report (CTR) or reported separately pending availability of the results.

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.

7.3 Demographics and baseline characteristics

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

7.3.1 Demographics and body measurements

Subject demographics and body measurements consist of:

- Age
- Race and ethnicity
- Sex
- Height (meters or inches), without shoes
- Body weight (kg), wearing light clothing and measured using standard scales
- Body mass index (kg/m^2) calculated by the system based on height and body weight

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 as an AE.

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

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 drugs, 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 7.2.1.2. If the change in medication influences the subject's eligibility to continue in the trial, the sponsor and trial monitor must be informed.

8 Trial conduct

8.1 Schedule of procedures

The schedule of procedures is provided in **Table 4** below.

Table 4 Schedule of procedures

Visit number	**V1**	**V2**	**V3**
Trial day	**-3**	**-1 and 1**	**28**
Visit type	**Screening**	**In-house stay/ Dosing on Day 1**	**Follow-up**
Window **Procedure**	**Day -30 to -3**		**+5 days**
Subject related info/assessments			
Informed consent	X		
Inclusion and exclusion criteria	X	X[1,2]	
Demographics	X		
Body measurements	X		
Medical history and concomitant illness	X		
Diabetes diagnosis and current diabetes treatment	X		
Previous and concomitant medication	X	X[1]	X
History of alcohol and drug abuse	X		
Randomization		X[1]	
Withdrawal criteria		X[1]	
Dosing day exclusion criteria		X[1]	
Insulin-induced hypoglycemia		X	
Safety assessments			
Physical examination	X		X
Vital signs	X	X[3]	X
12-lead safety ECG	X	X[4]	X
Local tolerability		X[5]	X
Adverse events	X	X	X
Laboratory			
Biochemistry, hematology, coagulation, HbA_{1c} (coagulation and HbA_{1c} at Visit 1 only)	X	X[6]	X
Urinalysis	X	X[1]	X
Pregnancy test (only women of childbearing potential)	X[7]	X[1,8]	X[8]
Urine drug screen	X	X[1]	
Alcohol breath test	X	X[1]	
PK/Clinical efficacy			
Dasiglucagon PK sampling		X[9]	
PD sampling (plasma glucose)		X[10]	
Other assessments			

Visit number	V1	V2	V3
Trial day	**-3**	**-1 and 1**	**28**
Visit type	**Screening**	**In-house stay/ Dosing on Day 1**	**Follow-up**
Window **Procedure**	**Day -30 to -3**		**+5 days**
Antibodies against dasiglucagon		X[1]	X[11]
Plasma insulin		X[12]	
Trial material			
Administration of trial drug (during hypoglycemic clamp procedure)		X	

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

8.2 Procedures by visit

8.2.1 Visit 1 (screening)

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
- Previous and concomitant medications
- History of alcohol/drug abuse

- Physical examination
- Vital signs
- 12-lead ECG
- AEs
- Biochemistry, hematology, coagulation, HbA1c
- Serum pregnancy test (women of childbearing potential only)
- Urinalysis
- Urine drug screen
- Alcohol breath test

Eligible or potentially eligible subjects (laboratory results pending) will be provided with an Identification 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 Identification 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 up to Visit 2: 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 site 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 by IV 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 or placebo.

The following assessments will 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 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 2-5 minutes prior to dosing (baseline).
- Plasma glucose concentrations:
  - Pre-dose, 4, 6, 8,10, 12, 15, 17, 20, 25, 30, 40, 45, 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 ±1 minute. Pre-dose is defined as within 2-5 minutes prior to dosing (baseline).
- Antibodies against dasiglucagon (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 2-5 minutes prior to dosing.

8.2.3.1 Hypoglycemic clamp procedure and administration of trial drug

The following procedure is based on precedent procedures for hypoglycemia induction in patients with T1DM (22, 23).

The dosing 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 within 7 (+2) 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 metacarpal dorsal hand 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 (but distal to the infusion vein) may be used at the discretion of the investigator. A third catheter for blood sampling will be placed into a metacarpal dorsal hand 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 (but distal to the infusion vein) 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. 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 PK, and insulin PK will be collected. The samples are the baseline samples and should be collected within 2 minutes before trial drug administration.

  - If plasma glucose is ≥45 mg/dL and <60 mg/dL (2.5-3.3 mmol/L), trial drug will be administered, defining time, t=0. The trial drug will be delivered in the buttock or deltoid (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 (2.5-3.3 mmol/L) target range. The run-in period will be adequately extended (at least 30 minutes) until the above target is achieved and new baseline samples for plasma glucose, dasiglucagon, and insulin PK will be collected. Glucose should not be infused within 10 minutes before trial drug administration. If plasma glucose is not within target range after the second attempt, the subject should be rescheduled for a new dosing visit within 7 days (+ 2 days).

Administration of trial drug should not occur later than 12:00 hours.

As shown in Table 5, serial blood samples for plasma glucose will be collected at t=0, 4, 6, 8, 10, 12, 15, 17, 20, 25, 30, 40, 45, 50, 60, 75 and 90 minutes post-dosing. Samples for assessing plasma dasiglucagon 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 5 Post-treatment blood sampling times

Times	0	4	6	8	10	12	15	17	20	25	30	35	40	45	50	60	75	90	120
Plasma glucose	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y		Y	Y	Y	Y	Y	Y	
PK dasiglucagon	Y						Y				Y	Y	Y		Y	Y		Y	Y
PK insulin	Y										Y					Y			

PK pharmacokinetics; Y yes

Refer to Section 7.2.2 for details of laboratory safety sampling and to Section 7.2.2.3 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 a light snack. Drinking of water is allowed *ad libitum* during the entire procedure.

8.2.3.2 Hypoglycemia rescue provisions

During insulin-induced hypoglycemia, plasma glucose levels will be monitored closely at site for safety reasons as described in Section 7.2.2.3. 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 (3.9 mmol/L).

2. If plasma glucose is <45 mg/dL (2.5 mmol/L) between t=8 and t=44 minutes (both inclusive), 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 (3.1 mmol/L).
3. If plasma glucose is <70 mg/dL (3.9 mmol/L) 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 trial drug will be administered SC according to Section 6.2. The time of trial drug administration will be recorded. At the time point when the insulin infusion is stopped, the total insulin dose which was required to induce hypoglycemia will be recorded.

During the procedure, the Investigator will assess whether any subjective AEs have occurred. Subjects may report AEs occurring at any time during the procedure.

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

The subject may be released from the clinical site 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 site for a longer period.

8.2.4 Visit 3 (follow-up visit)

Visit 3 will take place on Day 28. 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

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 treatment unblinding, a separate statistical analysis plan (SAP) will be finalized, providing detailed methods for the analyses outlined below.

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

9.1 Determination of sample size

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

9.1.1 Disposition of subjects

All randomized subjects will be accounted for. All post-randomization discontinuations will be summarized by reason for discontinuation. The number and characteristics of subjects screened but not found eligible will be stated in the CTR, together with a summary of reasons and types of failures. A detailed listing of these subjects will be provided.

The number of subjects in each analysis set will be summarized by frequency and percentage in a subject disposition table.

9.1.2 Analysis populations

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

- Safety analysis set: All randomized subjects who received at least one dose of trial drug.
- Full analysis set (FAS): All randomized subjects who received at least one dose of trial drug.
- Per protocol (PP) analysis set: All subjects of the FAS for whom no relevant protocol deviations were documented.

The decision to exclude a subject or exclude specific data from a subject should be taken at the blinded data review meeting before unblinding, and the exclusion from efficacy or other analyses justified in the signed notes of the meeting.

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

9.2 General considerations

All statistical analysis of the trial will be performed using the statistical software SAS for Windows Version 9.4. In all calculations, zero will be substituted for concentrations below the quantification limit of the assay. All data collected from all subjects will be presented in data listings. Both absolute values and change from baseline values for each subject will be given where applicable. All continuous data will be listed with the same precision as will be presented in the database. Data listings will be sorted by treatment, subject ID and time point. A missing value will be represented by an empty cell and no imputation will be made.

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

9.3 Demographics, baseline characteristics and concomitant medications

Baseline and demographic data will be summarized using descriptive statistics.

9.4 Treatment compliance

Treatment compliance will be summarized and listed for the safety analysis set.

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

Analogous supportive analyses will be conducted in the PP set.

9.5.2 Analysis of primary endpoint

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

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 before 45 minutes will be censored (and set to 'not recovered') at 45 minutes after dosing. In sensitivity analyses, the time to plasma glucose recovery will be analyzed 1) without censoring those subjects who require rescue IV glucose before 45 minutes and 2) with censoring at the time of administration of rescue IV glucose before 45 minutes.

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 ratio (dasiglucagon versus placebo) will be estimated together with the 95% confidence intervals, and the treatment group inference (dasiglucagon vs placebo) will be evaluated using the two-sided likelihood ratio test.

9.5.3 Analysis of key secondary endpoints

The key secondary endpoints are in this order:

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

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) using Fisher's exact test.

The key secondary endpoints of plasma glucose changes from baseline at 30 minutes, at 20 minutes, at 15 minutes, and at 10 minutes after trial drug injection or at the time of rescue, will be analyzed with the plasma glucose changes from baseline at rescue carried forward in those subjects who require rescue IV glucose before plasma glucose ≥20 mg/dL (1.1 mmol/L) recovery. Each of these changes from baseline 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 first for the 30 minute changes from baseline, and then sequentially for the 20 minute, 15 minute, and 10 minute variables, until the first failure to reject.

9.5.4 Analysis of secondary efficacy endpoints

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.

Time to first plasma glucose concentration ≥70 mg/dL (3.9 mmol/L) from baseline will be evaluated using a KM approach, with treatment group as a stratification factor, analogous to that used for the primary endpoint analysis. Differences between the KM curves (dasiglucagon versus placebo) will be evaluated inferentially using pairwise two-sided log rank tests. If the ≥70 mg/dL (3.9 mmol/L) endpoint is not met within 45 minutes post-dosing, the time of the last valid plasma glucose measurement up to 45 minutes will be the censoring time.

The $AUE_{0\text{-}30min}$ will be calculated as the baseline-adjusted area under the plasma glucose profile from time zero to 30 minutes. The log-transformed AUE endpoint will be analyzed using an 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 Analysis of exposure endpoints

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

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

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

These exposure endpoints will be summarized descriptively for each treatment group.

9.6 Safety analyses

9.6.1 Drug exposure

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

9.6.2 Adverse events

AEs will be coded using the latest available version of the MedDRA, the version of which will be provided in the CTR.

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

- Treatment-emergent adverse event (TEAE)
- Severe TEAE
- Treatment-emergent SAE
- Drug-related TEAE
- Drug-related severe TEAE
- Treatment-emergent drug-related SAE
- TEAE leading to withdrawal
- TEAE with outcome death
- AESI

9.6.3 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.

9.6.4 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.

9.6.5 Other safety parameters

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).

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) 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.

9.7 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, trial drug 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 $AUE_{0\text{-}30min}$.

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

9.8 Interim analyses

No interim analyses are currently planned.

9.9 Other analyses

9.9.1 Subgroup analyses

Summary tables describing the primary endpoint will be created based on age, sex, race/ethnicity or other demographic characteristic(s).

9.9.2 Exploratory analyses

Exploratory analyses cannot be used as confirmatory proof for registration trials.

10 Ethical, legal and administrative aspects

10.1 Statement of compliance

The information contained within this protocol is consistent with the current risk/benefit evaluation of the IMP as well as with the ethical and scientific principles governing clinical research as set out in the current version of the Declaration of Helsinki (1) and the guidelines on GCP (2).

10.2 Quality management

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

Each clinical site will perform internal quality management of trial conduct, data and biological specimen collection, documentation and completion, according to GCP guidelines.

Quality control procedures will be implemented beginning with the data entry system and data quality checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

Following written standard operating procedures (SOPs), the monitors will verify that the clinical trial is conducted, and data are generated, and biological specimens are collected, documented (recorded) and reported in compliance with the protocol, GCP and applicable regulatory requirements, Good Manufacturing Practices (GMP)).

The investigational site will provide direct access to all trial related sites, source data/documents and reports for the purpose of monitoring and auditing by the sponsor and inspection by local and regulatory authorities.

10.2.1 Clinical monitoring

Clinical site monitoring is conducted to ensure that the rights and well-being of trial subjects are protected, that the reported trial data are accurate, complete and verifiable, and that the conduct of the trial is in compliance with the currently approved protocol/amendment(s), with GCP and with applicable regulatory requirement(s).

During the course of the trial, the monitor will visit the trial site to ensure that the protocol and GCP are adhered to, that all issues have been recorded, to perform source data verification and to monitor drug accountability. The monitoring visit intervals will depend on the trial site’s recruitment rate and the compliance of the trial site with the protocol and GCP.

In order to perform their role effectively, monitors and persons involved in quality assurance and inspections will need direct access to source data, e.g. medical records, laboratory reports, appointment books, etc. If the electronic medical record does not have a visible audit trail, the investigator must provide the monitor with signed and dated printouts. In addition, relevant site staff should be available for discussions at monitoring visits and between monitoring visits (e.g. by telephone).

Details of clinical site monitoring are documented in a monitoring manual. The manual describes in detail who will conduct the monitoring, at what frequency monitoring will be done, at what level of detail monitoring will be performed and the distribution of monitoring reports.

10.3 Data handling and record keeping

10.3.1 Data collection and management

Data will be collected by means of EDC. 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.

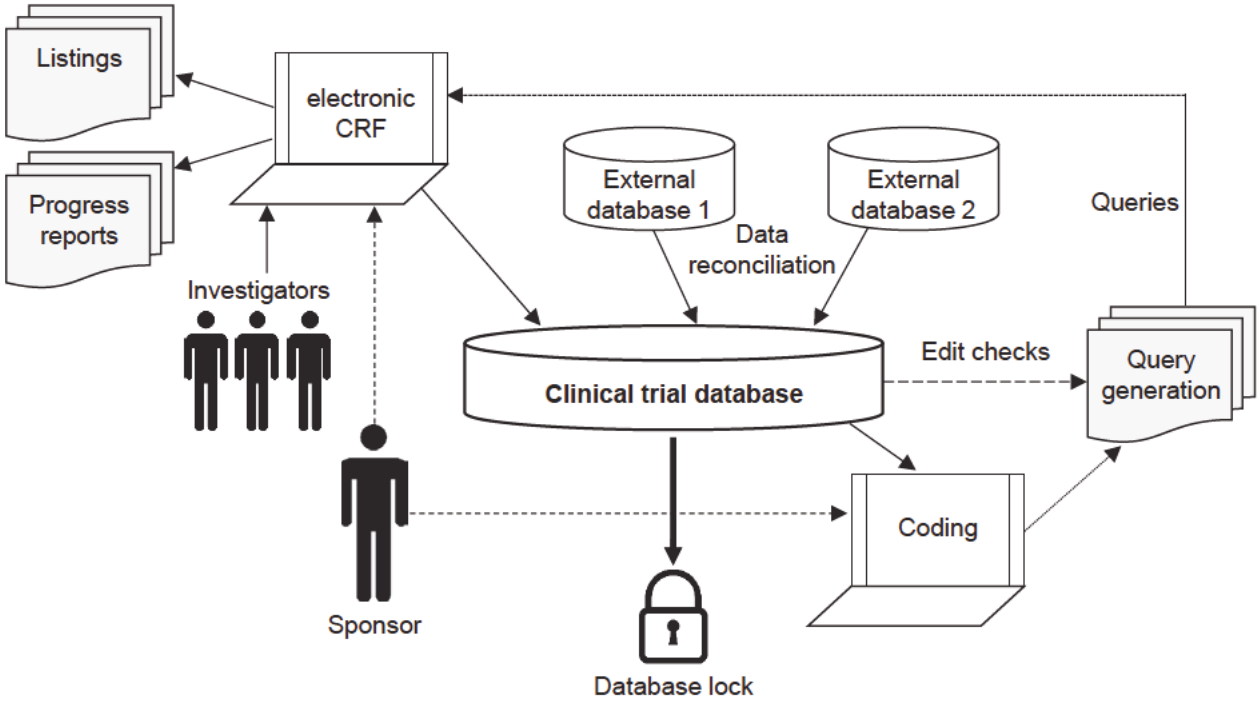
The investigator or staff authorized by the investigator will enter subject data into eCRFs. Data recorded in the eCRFs will be accessible to the trial site and sponsor personnel immediately after entry. The eCRFs must be maintained in an up-to-date state at the trial site at all times.

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site investigator. The investigator is responsible for ensuring the accuracy, completeness, legibility and timeliness of the data reported. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. The investigator must sign all eCRFs used. This signature information will be kept in the audit trail and cannot be altered. Any correction(s) made by the (sub)investigator or authorized site staff to the eCRF after original entry will be documented in the audit trail. Changes to data already approved will require the re-signature by the investigator. The person making the change to the data, and the date, time and reason for the change will be identified in the audit trail.

Data integrity must be secured through the complete data life cycle, from data creation to data deletion. Data integrity means that data must be attributable, legible, contemporaneous, original or a true copy, accurate, complete, consistent, enduring and available (ALCOA+).

Figure 2 illustrates the flow of data collected for this trial.

Figure 2 Flow of electronic data



10.3.2 Source documentation

For all data recorded, the source document must be defined in a source document agreement at each trial site. There must only be one source defined at any time for any data element.

The investigator is required to prepare and maintain adequate and accurate source documents designed to record all observations and other data pertinent to the trial for each trial participant. All information recorded in the eCRFs for this trial must be consistent with the subject's source documentation.

The trial monitor will check the eCRFs for accuracy and completeness by verifying data recorded in the eCRF against source data to ensure such records are consistent. If data are to be entered directly into the eCRF, this must be specified in the source document agreement prior to the start of the trial.

10.3.3 Access to source data

Subject data should be entered into the eCRF as soon as possible after the visit (e.g. as close to the date and time of the assessment as possible and no more than 5 days between assessment and data entry). Queries for discrepant data may be generated automatically by the system upon entry or generated manually by the monitor or the trial data manager. All queries, whether generated by the system or by a user, will be in an electronic format. This systematic validation will ensure that a clean and consistent database is provided prior to the statistical analysis being performed.

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, competent authorities , 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 subject confidentiality.

10.3.4 Archiving of trial records

The investigator is responsible for maintaining and archiving all trial records during and after the trial in compliance with GCP guidelines.

According to the GCP 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 IMP. However, these documents should be retained for a longer period if required by the applicable legal requirements.

No trial site document may be destroyed without prior written agreement between the investigator and sponsor. Should the investigator elect to assign the trial documents to another party, or move them to another location, the sponsor must be notified.

10.4 Subject consent and participation

10.4.1 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.

If a protocol amendment is required, the ICF 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.

10.4.2 Subject participation card

The subjects will be provided with a subject participation card bearing the following information:

- That he/she is participating in a blinded clinical trial, including the trial ID
- That he/she is treated with dasiglucagon or placebo
- The address, name and phone number of the investigator

The subjects will be asked to keep the subject participation card in their possession at all times during the trial and to destroy or return it at the end-of-trial visit.

10.5 Protocol requirements

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 l 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.

10.5.1 Protocol deviations

Deviations from the protocol should be avoided. A protocol deviation is any noncompliance with the clinical trial protocol or ICH GCP requirements. The noncompliance may be either on the part of the participant, the investigator or the trial site staff. As a result of deviations, corrective and preventive actions are to be developed by the site and implemented promptly.

It is the responsibility of the investigator to use continuous vigilance to identify and report deviations. The investigator must inform the site monitor. All deviations must be addressed in trial source documents and reported to sponsor. The implications of the deviation must be reviewed and discussed.

10.6 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) must be maintained in confidence by the investigator.

10.7 Other ethical and regulatory issues

If a significant safety issue is identified, either from an individual case report or from 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.

10.8 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 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 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 insofar as the individual subject is concerned.

Any injury to health, which might have occurred as 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.

10.9 Publication and public disclosure

The information obtained during the conduct of this trial is considered confidential and may be used by or on behalf of Zealand Pharma for regulatory purposes as well as for the general development of the trial drug. All information supplied by Zealand Pharma in connection with this trial shall remain the sole property of Zealand Pharma and is to be considered confidential information.

No confidential information shall be disclosed to others without prior written consent from Zealand Pharma. Such information shall not be used except in the performance of this trial. The information obtained during this trial may be made available to other physicians who are conducting other clinical trials with the trial drug, if deemed necessary by Zealand Pharma. Provided that certain conditions are fulfilled, Zealand Pharma may grant access to information obtained during this trial to researchers who require access for research projects studying the same disease and/or trial drug studied in this trial.

One investigator will be appointed to review and sign the clinical trial report (signatory investigator) on behalf of all participating investigators.

10.9.1 Communication of results

No permission to publish shall be granted to any CRO involved in the trial.

The results of this trial will be subject to public disclosure on external web sites according to international and national regulations.

Zealand Pharma reserves the right to defer the release of data until specified milestones are reached, for example when the clinical trial report is available. This includes the right not to release the results of interim analyses, because the release of such information may influence the results of the entire trial.

At the end of the trial, one or more scientific publications (including abstracts, posters and presentations) may be prepared collaboratively by the investigator(s) and Zealand Pharma. Zealand Pharma reserves the right to postpone publication and/or communication for up to 60 days to protect intellectual property. In all cases the trial results will be reported in an objective, accurate and balanced manner. In the event of any disagreement on the content of any publication, the opinions of both the investigators and Zealand Pharma will be fairly and sufficiently represented in the publication.

Authorship of publications should be in accordance with the Uniform Requirements of the International Committee of Medical Journal Editors (sometimes referred to as the Vancouver Criteria).

10.9.2 Site-specific publication(s) by investigator(s)

For a multi-centre clinical trial, analyses based on single-site data usually have significant statistical limitations and frequently do not provide meaningful information for healthcare professionals or trial patients, and therefore may not be supported by Zealand Pharma. It is a Zealand Pharma policy that such individual reports do not precede the primary manuscript and should always reference the primary manuscript of the trial. Zealand Pharma reserves the right to prior review of such publications. Further to allow for the primary manuscript to be published as the first, Zealand Pharma asks for deferment of publication of individual site results until the primary manuscript is accepted for publication. As Zealand Pharma wants to live up to the industry publication policy, submission of a primary publication will take place no later than 18 months after trial completion.

The results of this trial may be published or presented at scientific meetings. If this is foreseen, the investigator agrees to submit all manuscripts or abstracts to the sponsor before submission. This allows the sponsor to protect proprietary information and to provide comments.

10.9.3 Investigator access to data and review of results

As owner of the trial database, Zealand Pharma has the discretion to determine who will have access to the database. Individual investigators will have their own research subjects' data and will be provided with the randomization code after results are available.

11 Reference list

1. WMA Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Subjects. Available from URL: https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/
2. 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.
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;40:155-57.
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 4, 25 Oct 2017.
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;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. 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.
22. 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.
23. 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.

Sponsor protocol approval page

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

Protocol version: **2.0**

Protocol date: **04-Jul-2018**

The sponsor agrees to conduct the trial according to the protocol and in compliance with Declaration of Helsinki, the FDA regulations for clinical trials, the current Good Clinical Practice (GCP) guidelines, and with other applicable regulatory requirements.

[redacted]
Date (dd-Mmm-yyyy)

Signature

Name: [redacted] MSc.
Title: Clinical Trial Manager
Institution: Zealand Pharma A/S
Phone: [redacted]
Email: [redacted]

[redacted]
Date (dd-Mmm-yyyy)

Signature

Name: [redacted] MD, PhD, EMBA
Title: Medical Project Director
Institution: Zealand Pharma A/S
Phone: [redacted]
Email: [redacted]

[redacted]
Date (dd-Mmm-yyyy)

Signature

Name: [redacted] MSc Stat
Title: Statistical Project Director
Institution: Zealand Pharma A/S
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