16.1 TRIAL INFORMATION

16.1.1 PROTOCOL AND PROTOCOL AMENDMENTS

This appendix includes

Document	**Date, Version**
Clinical trial protocol	• Version 1.0, 08 June 2018 (valid in Slovenia and the USA) • Version 3.0, 08 January 2019 (valid in Germany)

Clinical Trial Protocol

A phase 3, randomized, double-blind, placebo- and active-controlled, parallel-arm trial to assess the efficacy, safety, and pharmacokinetics of dasiglucagon relative to placebo and GlucaGen® when administered as a rescue therapy for severe hypoglycemia in children with T1DM treated with insulin

Sponsor code: ZP4207-17086
Synteract: ZEA-DNK-02170
EudraCT number: 2018-000892-33

Coordinating investigator: Prof. Dr. med. Thomas Danne

Allgemeine Kinderheilkunde

Diabetologie, Endokrinologie, Klinische Forschung
Diabeteszentrum für Kinder und Jugendliche AUF DER BULT

Kinder- und Jugendkrankenhaus

Janusz-Korczak-Allee 12

30173 Hannover

Germany

Sponsor: Zealand Pharma A/S

Smedeland 36

2600 Glostrup, Copenhagen

Denmark

Version: final version 1.0

Date: 08 June 2018

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

1. Signatures and agreement with protocol

Title: A phase 3, randomized, double-blind, placebo- and active-controlled, parallel-arm trial to assess the efficacy, safety, and pharmacokinetics of dasiglucagon relative to placebo and GlucaGen® when administered as a rescue therapy for severe hypoglycemia in children with T1DM treated with insulin

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

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

Coordinating investigator: Prof. Dr. med. Thomas Danne

[redacted]

Date Signature

Statistician

[redacted]

Senior Director Biometrics
Synteract Deutschland GmbH
Albrechtstr. 14
80636 Munich, Germany

[redacted]

Sponsor's representative

[redacted]

Clinical Project Director
Zealand Pharma A/S
Smedeland 36
2600 Glostrup, Denmark

[redacted]

Dr. [redacted]
Medical Director
Zealand Pharma A/S
Smedeland 36
2600 Glostrup, Denmark

[redacted]

Title: A phase 3, randomized, double-blind, placebo- and active-controlled, parallel-arm trial to assess the efficacy, safety, and pharmacokinetics of dasiglucagon relative to placebo and GlucaGen® when administered as a rescue therapy for severe hypoglycemia in children with T1DM treated with insulin

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

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

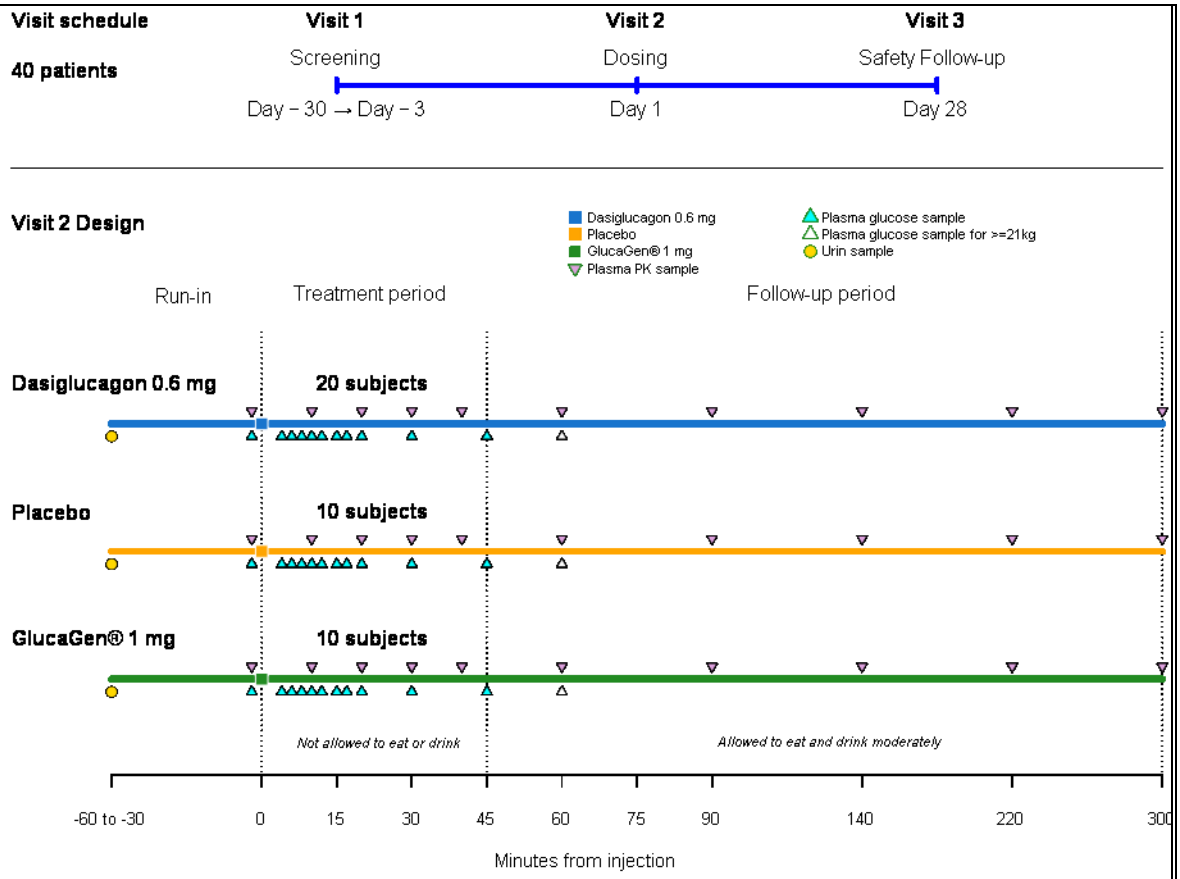
Investigator

Date	Signature

Name and address

2. Trial synopsis

Title of the trial: A phase 3, randomized, double-blind, placebo- and active-controlled, parallel-arm trial to assess the efficacy, safety, and pharmacokinetics of dasiglucagon relative to placebo and GlucaGen® when administered as a rescue therapy for severe hypoglycemia in children with T1DM treated with insulin	
EudraCT number: 2018-000892-33	**Protocol codes:** Sponsor: ZP4207-17086 Synteract: ZEA-DNK-02170
Sponsor or sponsor's representative in the European Union: Zealand Pharma A/S, Smedeland 36, 2600 Glostrup (Copenhagen), Denmark	
Coordinating investigator: Prof. Dr. med. Thomas Danne, Kinder- und Jugendkrankenhaus AUF DER BULT, Janusz-Korczak-Allee 12, 30173 Hannover, Germany	
Trial center(s): 2-3 centers in the EU (Germany, Slovenia) and 1-2 centers in the USA	
Planned trial period: First Patient First Visit: September 2018 Last Patient Last Visit: Second quarter 2019	**Phase of development:** Phase 3
Objectives: The primary objective is to demonstrate that dasiglucagon is superior to placebo following a single injection of 0.6 mg of dasiglucagon in treating hypoglycemia in children with type 1 diabetes mellitus (T1DM). Secondary objectives are: • To confirm that a single dose of dasiglucagon [0.6 mg] is comparable to a single dose of GlucaGen® [1 mg/mL] in treating hypoglycemia in children with T1DM, (in accordance with the label, children below 25 kg body weight will be administered 0.5 mg GlucaGen®), • To assess safety profile of dasiglucagon in children with T1DM, • To assess pharmacokinetic (PK) profile of dasiglucagon in children with T1DM.	
Trial design: This is a phase 3, randomized, double-blind, placebo- and active-controlled, parallel-arm trial designed to assess efficacy, safety and PK of dasiglucagon vs. placebo and vs. GlucaGen® in children with diabetes mellitus type 1 (T1DM). Patients will receive a single subcutaneous injection of the investigational product during a hypoglycemic clamp procedure. Handling, preparation and administration of trial product will be done by unblinded trial personnel. All trial assessments will be done by blinded trial personnel. The trial design is illustrated in the figure below.	



Abbreviation: PK=pharmacokinetic

Planned number of patients:

At least 40 children ≥6 years and <18 years of age with T1DM will be randomized into the trial (2:1:1 dasiglucagon/placebo/GlucaGen®) and stratified by age intervals: 6 years to <12 years, and 12 years to <18 years and by injection site (abdomen/thigh). A minimum of 16 patients will be enrolled into each age group, including a minimum of 8 patients in either of age groups on dasiglucagon treatment arm.

Patients who are dosed will not be replaced.

Medical condition or disease under investigation:

This is the first dedicated efficacy and safety trial in children; thus, the target population for this trial is children with T1DM ≥6 to <18 years of age. Dasiglucagon is in development for treatment of severe hypoglycemia.

Inclusion criteria:

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

1. Following receipt of verbal and written information about the trial, patient, parent(s) or guardian(s) of the patient must provide signed informed consent before any trial-related activity is carried out*
2. Female or male patients with T1DM for at least 1 year, diagnostic criteria as defined by the American Diabetes Association; and receiving daily insulin
3. At least 6.0 years of age (inclusive) and less than 18.0 years
4. Body weight ≥20 kg
5. Female patients must meet one of the following criteria:
   a. Participant is of childbearing potential and agrees to use one of the accepted contraceptive regimens throughout the entire duration of the trial from screening until last follow-up visit. An acceptable method of contraception includes at least one of the following:
      i. Abstinence from heterosexual intercourse

ii. Systemic contraceptives (birth control pills, injectable/implant/ insertable hormonal birth control products, transdermal patch); if the participant is using systemic contraceptives, she must use an additional form of acceptable contraception (iii or iv, below)
iii. Intrauterine device (with and without hormones)
iv. Condom with spermicide

or

b. Participant is of non-childbearing potential due to pre-puberty status or a medical condition confirmed by the investigator

6. Male patients must meet the following criteria: If sexually active, uses condom and partner practices contraception during the trial from screening and until last follow-up visit
7. Willingness to adhere to the protocol requirements

* Informed consent signatures must be obtained according to local regulations.

Exclusion criteria:

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

1. Females who are pregnant according to a positive urine pregnancy test, actively attempting to get pregnant, or are lactating
2. Known or suspected allergy to trial product(s) or related products
3. History of anaphylaxis or symptoms of severe systemic allergy (such as angioedema)
4. Previous randomization in this trial
5. History of an episode of severe hypoglycemia that required a third party assistance within a month prior to screening visit
6. History of hypoglycemic events associated with seizures or hypoglycemia unawareness in the last year prior to screening
7. History of epilepsy or seizure disorder
8. Receipt of any investigational drug within 3 months prior to screening
9. Active malignancy within the last 5 years
10. Congestive heart failure, New York Heart Association class II-IV
11. Current bleeding disorder, including anti-coagulant treatment
12. Known presence or history of pheochromocytoma (i.e. adrenal gland tumor) or insulinoma (i.e. insulin secreting pancreas tumor)
13. Use of a daily systemic beta-blocker drug, indomethacin, warfarin or anticholinergic drugs in the previous 28 days before Day 1 of this trial
14. 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, or altered electrolyte values of clinical relevance for cardiac conduction, as judged by the investigator
15. Clinically significant abnormal electrocardiogram (ECG) at screening as judged by the investigator
16. Clinically significant illness within 4 weeks before screening as judged by the investigator
17. Surgery or trauma with significant blood loss within the last 2 months prior to screening
18. Patients 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
19. Any condition interfering with trial participation or evaluation or that could be hazardous to the patient
20. The use of prescription or non-prescription medications known to cause QT prolongation

In addition, the following exclusion criteria at clinic admission on Visit 2, day 0 apply at the time of admission to the clinic, which is the day before clamp procedure:

Patients who meet one or more of the following exclusion criteria at the time of admission to the clinic will be excluded from the dosing visit, however, the visit can be rescheduled 1-7 days later. The dosing visit can only be rescheduled once.

1. Atypically strenuous exercise within 4 days prior to dosing, as judged by the investigator. Exercise during the trial should follow patient's typical routine, and should not exceed a near maximum intensity for more than 20 minutes per day, or moderate intensity for more than 90 minutes per day
2. Clinically significant illness within 4 weeks before dosing, as judged by the investigator
3. Consumption of alcohol within 24 hours prior to dosing visit, determined by positive results from an alcohol breath test
4. Not fasting from 22:00 hours the evening prior to dosing, apart from water.
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 72 hours prior to dosing; or use of other long-acting insulins (e.g. insulin glargine U100 or insulin detemir) within 48 hours prior to dosing; or use of neutral protamine Hagedorn insulin NPH within 22 hours prior to dosing
7. Use of any short acting (bolus) insulin within 12 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 admission to the clinic.

Test product, dose and mode of administration:

Dasiglucagon, liquid formulation, 1 mg/mL, 0.6 mL

Reference product, dose and mode of administration:

Placebo for dasiglucagon (hereafter referred to as placebo): Placebo, liquid formulation, 0.6 mL
Active control: Recombinant glucagon hydrochloride, 1 mg lyophilized powder for reconstitution (GlucaGen®, Novo Nordisk) in 1 mL sterile water (hereafter referred to as GlucaGen®). In accordance with the label, children below 25 kg body weight will be administered 0.5 mg GlucaGen®

Duration of treatment:

Patients will receive a single subcutaneous dose of investigational product. Patients will be subjected to a hypoglycemic clamp procedure and have to be within a plasma glucose target range of 54-80 mg/dL at dosing. The total individual trial duration will be a maximum of 63 days (up to 30 days screening, 1 dosing, 28 [+5] days of follow-up).

Criteria for evaluation:

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.

Secondary endpoints

Secondary efficacy endpoints:

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

Safety endpoints:

- Adverse events (AEs)
- Clinical laboratory assessments (biochemistry, hematology, coagulation, urinalysis)
- Vital signs
- Physical examination
- Clinically significant changes in the electrocardiogram
- Local tolerability
- Administration of IV glucose infusion during the hypoglycemic clamp procedure

- Time to first IV glucose infusion, after trial product administration. (N.B. IV glucose infusion prior to trial product administration should not be included, as it is part of hypoglycemic clamp procedure)
- Immunogenicity endpoint: occurrence of anti-drug antibodies

Pharmacokinetic endpoints

PK endpoints will be derived from plasma dasiglucagon and GlucaGen® profiles from 0 to 300 minutes:

- Area under the plasma dasiglucagon or GlucaGen® concentration versus time curve from 0 to 30 minutes post-dose ($AUC_{0\text{-}30min}$)
- Area under the plasma dasiglucagon or GlucaGen® concentration versus time curve from 0 to 300 minutes post-dose ($AUC_{0\text{-}300min}$)
- Area under the plasma dasiglucagon or GlucaGen® concentration versus time curve from 0 to infinitely post-dose ($AUC_{0\text{-}inf}$)
- Maximum of all valid plasma dasiglucagon or GlucaGen® concentration measurements from 0 to 300 minutes post-dose (C_{max})
- Time to maximum of plasma dasiglucagon or GlucaGen® concentration measurements (t_{max})
- Terminal elimination rate constant of plasma dasiglucagon or GlucaGen® (λ_z)
- Terminal plasma elimination half-life of dasiglucagon or GlucaGen® ($t_{½}$)
- Total body clearance of plasma dasiglucagon or GlucaGen® (CL/f)
- Volume of distribution of plasma dasiglucagon or GlucaGen® (V_z/f)
- Mean residence time of plasma dasiglucagon or GlucaGen® (MRT)

Pharmacodynamic endpoint

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

Statistical methods:

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

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

Other summaries (e.g. quartiles, 95% confidence intervals) may be used as appropriate. Categorical efficacy and safety variables will be summarized by counts and by percentage of patients in corresponding categories. The primary endpoint, time to recovery, will be summarized using Kaplan-Meier estimates for each treatment group in total and stratified by age intervals and injection site. The median time to recovery with 95% confidence interval will be estimated by treatment group.

Log-rank tests will be applied to compare the 2 treatment groups to the placebo group.

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

All safety data will be analyzed with descriptive methods.

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

Plasma dasiglucagon and glucagon concentrations will be described and PK metrics determined.

$AUE_{0\text{-}30min}$ will be summarized with descriptive statistics.

Sample size calculation:
The primary comparison is between the dasiglucagon and placebo treatment arms. From phase 2 results, the median time to an increase of 20 mg/dL of the 0.6 mg dose was approximately 10

minutes. For placebo-treated patients, the median time to recovery is assumed to be more than 50 minutes. Assuming an exponential time-to-recovery distribution with median times of 10 and 50 minutes, a 2-sided log-rank test will be able to detect a difference between dasiglucagon 0.6 mg and placebo with 90% power with a follow-up time of 45 minutes at a 5% significance level with 20 patients randomized to the dasiglucagon arm and 10 patients to placebo. GlucaGen® is included as a reference to compare the responses and AE profile to dasiglucagon with those to a marketed product. It is expected that 10 patients in the GlucaGen® group will suffice for the comparison.

Table 2-1: Schedule of Assessments

Visit number	V1	V2	V3	Vx
Trial day	-3	0 and 1	28	Unscheduled[1]
Visit type	Screening	Dosing	Follow-up	Unscheduled[1]
Window	-30 to -3	–	+5 days	Unscheduled[1]
Patient related information/assessments				
Informed consent	x	–	–	–
Inclusion/exclusion criteria	x	x[2,3]	–	–
Demography	x	–	–	–
Body measurements	x	–	–	–
Diabetes diagnosis and current diabetes treatment	x	x	x	x
Medical history including concomitant illnesses	x	–	–	–
Concomitant medications	x	x[2]	x	–
Randomization	–	x[2]	–	–
Exclusion criteria at clinic admission on Visit 2, day 0	–	x[2]	–	–
Insulin-induced hypoglycemia	–	x	–	–
Safety assessments				
Physical examination	x	x	x	x
Vital signs	x	x[4]	x	x
12-lead ECG	x	x[5]	x	–
Local tolerability	–	x[6]	x	–
Adverse events	x	x	x	x
Laboratory				
Biochemistry, hematology, coagulation, HbA1c (HbA1c at Visit 1 only)	x	–	x	–
Pregnancy test (women of childbearing potential only)	x[7]	x[2,8]	x[8]	–
Urinalysis	x	x[2]	x	–
Alcohol breath test	–	x[2]	–	–
PK/Clinical efficacy				
Plasma dasiglucagon/GlucaGen®	–	x[9]	–	–
Plasma glucose	–	x[10]	–	–
Other assessments				

Visit number	V1	V2	V3	Vx
Trial day	-3	0 and 1	28	Unscheduled[1]
Visit type	Screening	Dosing	Follow-up	Unscheduled[1]
Window	-30 to -3	–	+5 days	Unscheduled[1]
Antibodies against dasiglucagon/GlucaGen®	x	–	x[11]	x
Trial material				
Administration of trial product (during hypoglycemic clamp procedure)	–	x	–	–
End of trial status	–	–	x	–

Abbreviations: ADA=Anti-drug antibody; ECG=Electrocardiogram, HbA1c=Hemoglobin A1c, PK=Pharmacokinetics

[1]For ADA-positive patients only.
[2]Prior to the start of the insulin-induced hypoglycemic procedure.
[3]Only check exclusion criteria at clinic admission on Visit 2, day 0 and changes between screening visit and Visit 2.
[4]Prior to the start of the insulin-induced hypoglycemic procedure (within 30 minutes), and at 30, 60 and 300 minutes after dosing. The actual time of the assessment should not deviate from the nominal time by more than ±10 minutes.
[5]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.
[6]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.
[7]Serum pregnancy test.
[8]Urine stick pregnancy test.
[9]Pre-dose, and at 10, 20, 30, 40, 60, 90, 140, 220, and 300 minutes after dosing. The actual time of blood sampling should not deviate from the nominal time by more than ±1 minute between t=5 minutes and t=90 minutes, ±5 minutes between t=150 minutes and t=300 minutes.
Pre-dose is defined as within 2 minutes prior to dosing.
[10]Pre-dose, and at 4, 6, 8, 10, 12, 15, 17, 20, 30 and 45 minutes (as well as 60 minutes if the patient´s body weight is ≥21 kg) after dosing. The actual time of blood sampling should not deviate from the nominal time by more than ±30 seconds until the 20-minute collection time point, and by more than ±1 minute for the subsequent collection time points. Pre-dose is defined as within 2 minutes prior to dosing.
[11]Antibodies against dasiglucagon/GlucaGen® (any treatment-induced or treatment-boosted [titer increase above 5 fold] ADA-positive patients will be monitored until the ADA levels return to baseline levels).

3. Table of contents

Appendices:

(1) List of names and addresses

Index of in-text tables

Index of in-text figures

4. List of abbreviations and definition of terms

4.1 Abbreviations

ADA	anti-drug antibody
AE	adverse event
AESI	adverse event of special interest
ALT	alanine aminotransferase
AST	aspartate aminotransferase
CA	competent authority
CDISC	Clinical Data Interchange Standards Consortium
CFR	Code of Federal Regulations
CRO	contract research organization
ECG	electrocardiogram
eCRF	electronic case report form
EDC	electronic data capture
FAS	full analysis set
GCP	Good Clinical Practice
ICH	International Council for Harmonisation
IEC	independent ethics committee
IMP	investigational medicinal product
IRB	institutional review board
IV	intravenous(ly)
IWRS	interactive web response system
MRT	mean residence time
NPH	neutral protamine Hagedorn
ODM	OpenDocument Master
PD	pharmacodynamic(s)
PK	pharmacokinetic(s)
PPS	per protocol set
PR	PR interval; i.e. the measure of the time between the start of the p wave and the end of the r wave in the heart's electrical cycle
PT	preferred term
QRS	QRS interval; i.e. the measure of the time between the start of the q wave and the end of the s wave in the heart's electrical cycle
QT	QT interval; i.e. the measure of the time between the start of the q wave and the end of the t wave in the heart's electrical cycle
SAE	serious adverse event
SAS	safety analysis set

SC	subcutaneous(ly)
SOC	system organ class
SUSAR	suspected unexpected serious adverse reaction
T1DM	type 1 diabetes mellitus
ULN	upper limit of normal

Plasma concentrations of dasiglucagon/GlucaGen

$AUC_{0\text{-}30min}$	Area under the plasma concentration versus time curve from 0 to 30 minutes post-dose
$AUC_{0\text{-}300min}$	Area under the plasma concentration versus time curve from 0 to 300 minutes post-dose
$AUC_{0\text{-}inf}$	Area under the plasma concentration versus time curve from 0 to infinitely post-dose
C_{max}	Maximum of all valid plasma concentration measurements from 0 to 300 minutes post-dose
t_{max}	Time to maximum of plasma concentration measurements
λ_z	Terminal elimination rate constant
$t_{½}$	Terminal plasma elimination half-life
CL/f	Total body clearance
V_z/f	Volume of distribution
MRT	Mean residence time

Plasma glucose concentrations

AUE0-30min	Plasma glucose response as area under the effect curve above baseline from time 0 to 30 minutes

4.2 Definitions of terms

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

5. Introduction

5.1 Background of the trial

Hypoglycemia

Hypoglycemia in patients with diabetes is defined as episodes of an abnormally low plasma glucose concentration.[9] This is a common, unpredictable, and potentially dangerous side effect of treatment of diabetes mellitus with especially insulin or sulfonylureas. It is more frequent in patients with profound endogenous insulin deficiency, such as occurs in type 1 diabetes mellitus (T1DM) and advanced type 2 diabetes mellitus. Treatment of type 2 diabetes mellitus with insulin causes hypoglycemia progressively and more frequently over time, whereas in T1DM, hypoglycemia is experienced throughout the course of established disease.

Symptoms and signs of hypoglycemia are not specific. Patients undergoing a hypoglycemic episode experience unpleasant symptoms such as anxiety, sweating, hunger, tremors, palpitations, paresthesia, nausea and pallor. Depending on its severity, the hypoglycemia may lead to mild confusion, behavioral changes, loss of consciousness, seizures, coma, and death.[1]

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

Glucagon

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

Insulin decreases blood glucose levels and cases of hypoglycemia can be reversed by glucagon. Therefore, glucagon is indicated for the treatment of severe hypoglycemia.

Dasiglucagon

Dasiglucagon (ZP4207) is a stable peptide analog of human glucagon, available in a ready-to-use liquid formulation and is in development for the treatment of severe hypoglycemia in insulin dependent patients with diabetes mellitus. Dasiglucagon is a specific and full glucagon receptor agonist designed to mimic the effects of glucagon, having a fast absorption and elimination (minutes). It is a peptide of 29 amino acids, with 7 amino acid substitutions compared to native glucagon. The main purpose of the substitutions is to increase the physical and chemical stability of the glucagon analog compared to marketed glucagon products such as Lilly´s Glucagon or GlucaGen®. Dasiglucagon exhibits improved physical and chemical stability and is available in an aqueous solution at neutral pH.[2]

Five clinical trials have been completed with dasiglucagon: A first human dose trial (ZP4207-14013), a multiple-dose dose-escalation trial (ZP4207-15007) to evaluate the safety, tolerability, pharmacokinetics (PK) and pharmacodynamics (PD) of dasiglucagon, a phase 2 crossover trial to assess the PK and PD of a single dose of an optimized formulation of dasiglucagon administered subcutaneously (SC) in patients with T1DM (ZP4207-15126), and a feasibility trial testing the bionic pancreas with dasiglucagon was completed (ZP4207-16051).[2] Finally, a cross-over trial (ZP4207-16098) assessing PK and PD responses after micro-doses of dasiglucagon administered SC to patients with type 1 diabetes mellitus under euglycemic and hypoglycemic conditions, compared to marketed glucagon, was completed.

Pharmacokinetics and pharmacodynamics of dasiglucagon

The results of the phase 1 and 2 clinical trials confirm 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 (C_{max}) was later for dasiglucagon than for GlucaGen® (35 versus 20 minutes). For C_{max}, the results indicated that 0.3 mg dasiglucagon was comparable to 0.5 mg GlucaGen® (90% confidence interval: 0.8167; 1.0068) and 0.6 mg dasiglucagon was comparable to 1.0 mg GlucaGen® (90% confidence interval: 0.8850; 1.1991).[2] At these dose levels, the total exposures ($AUC_{0\text{-}inf}$) were higher for dasiglucagon compared to GlucaGen®.

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

Safety of dasiglucagon

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

Immunogenicity of dasiglucagon

In adults, anti-drug antibodies (ADA) have been assessed in the completed clinical trials. In one of the 5 completed clinical trials (ZP4207-16098) 1 transient ADA incidence have been detected in one T1DM patient. The antibodies were able to bind to dasiglucagon and glucagon confirming the cross-reactivity potential found in the non-clinical toxicity studies (IND 135869 Section 2.4.4.6). The antibodies were also capable of neutralizing the in vitro activity of both dasiglucagon and glucagon, but could not be associated with any AEs or effects on PK or PD in the patient. The trial was a multiple dose trial and completers had received a total of 11 investigational drug administrations (8 injections of dasiglucagon and 3 injections of recombinant glucagon) (IND 135869 Section 2.5.6.3) and indicated that increased exposure will likely increase the risk of ADA formation. However, due to the crossover trial design, the induction of ADA cannot be decisively attributed to either dasiglucagon or glucagon treatment. The ADA incidence in that trial was 4.3% and considered low. From the long-term non-clinical toxicology studies, the detection of antibodies was not associated with a change in toxicity profile compared to ADA negative animals. In the 13- and 26-week rat studies, an increase in exposure was observed in dose groups ≥8 mg/kg/day, which correlates with the dose groups with the highest frequency of ADA-positive animals. So far, these findings have not been observed in humans.

The consequences of an immune response towards endogenous glucagon is not fully known. In a recent review summarizing the current physiology of glucagon and learnings from recent glucagon antagonist research, it was concluded that although glucagon is an important hormone for controlling postprandial glucose levels, it was not essential for the maintenance of fasting glucose and glucagon antagonism did not cause hypoglycemia.[5] Rather, the lack of glucagon signaling induced imbalance in amino acid metabolism leading to elevated amino acid plasma

levels. As excess levels of amino acids can be excreted by the kidneys and the detected ADA incidence have been transient, the impact is not considered a major risk for the trial population.

The overall immunogenicity risk of dasiglucagon in a clinical context is therefore considered low and the potential effects of induced ADAs judged to be of limited clinical consequence.

5.2 Trial rationale

The SC bolus dose of 0.6 mg dasiglucagon that is the most appropriate dosing to ensure rapid rescue from hypoglycemia in adults appears to be appropriate also in children 6 years old and above.

To simulate the potential PK/PD response in children, a previously developed population PK/PD model for dasiglucagon was updated with an allometric PK component derived from published glucagon pediatric data from weight groups of 25.4±5.2, 43.2±8.9, and 61.2±13.8 kg (mean±SD) and adults. The simulations suggested that a 0.3 mg SC bolus dose of dasiglucagon would result in a slightly slower increase of blood glucose compared to the 0.6 mg dose in the 3 pediatric body weight groups. Considering the intended clinical indication of treating patients for severe hypoglycemia where time to PD response is considered essential, the 0.3 mg dose appears less attractive than the 0.6 mg dose.

The higher total drug exposure (AUC and C_{max}) predicted in children at lower body weight, relative to that observed in adults at a dose of 0.6 mg, is within the tolerable exposure achieved in adults administered 2.0 mg dasiglucagon, and the duration of exposure is truncated due to a shorter $t_{½}$ in children. In addition, rapid saturation of the PD response results in similar total and maximum glucose response (AUE and CE_{max}) throughout the body weight range from 25 to 77 kg.

Based on all data generated to date across 4 clinical trials performed in adults receiving dasiglucagon at different dose levels, no clear dose-response for nausea and vomiting was observed above a certain threshold. It is therefore not expected that the intensity and frequency of nausea and vomiting at a dose of 0.6 mg will differ between adults and children/adolescents. This expectation is further consistent with results of the recently conducted trial ZP4207-16098 in adults with T1DM, which indicated a similar degree of nausea and vomiting at SC bolus doses of 0.2 and 0.6 mg dasiglucagon.

5.3 Assessment of anticipated benefits and risks

Glucagon emergency kits are often underutilized in the treatment of patients experiencing severe hypoglycemia. Considering the seriousness and potential complications that can arise from severe hypoglycemic episodes, it is essential that parents and caregivers receive adequate, hands on training for use of glucagon rescue kits to ensure safe, timely, and effective administration. Given the challenges with parent/caregiver training and use of current glucagon products, Zealand Pharma A/S is developing dasiglucagon as a ready to use rescue treatment for severe hypoglycemia. The development of dasiglucagon will provide parents and caregivers of children with diabetes mellitus a more efficient alternative for the treatment of severe hypoglycemia. The ready-to-use kit addresses an unmet need and may potentially prove to be life-saving.

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

Treatment with an investigational medicinal product may result in undesired effects or complaints. Undesired effects and complaints such as nausea and vomiting are known AEs occurring with glucagon administration. Similar AEs have also been observed to a limited degree in the 5 clinical studies conducted with dasiglucagon. As with every novel drug substance, new and yet unknown side effects also may occur.

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

immunogenic potential. Based on the 5 clinical studies completed with dasiglucagon to date (see Section 5.2), no anti-dasiglucagon or anti-glucagon antibodies have been detected, except for trial ZP4207-16098, in which there was one transient low titer ADA incidence showing reactivity towards both dasiglucagon and glucagon. The patient 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 patient was also 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 this trial, the induction of ADAs could not be associated with a specific treatment. 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 patient was negative for anti-dasiglucagon antibodies. There was no evidence for altered PK, PD, or safety profile for this patient.

Administration of dasiglucagon may be associated with a risk of allergic reactions similar to those observed for other therapeutic peptides or proteins. Patients with known or suspected allergies to the trial products 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 4 clinical trials conducted with dasiglucagon. Direct access to resuscitation equipment is ensured at the clinical trial centers.

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

Overall, the benefit to risk ratio for patients entering the ZP4207-17086 trial is considered acceptable.

6. Trial objectives

Primary objective

- To demonstrate that dasiglucagon is superior to placebo following a single injection of 0.6 mg of dasiglucagon in treating hypoglycemia in children with T1DM

Secondary objectives

- To confirm that a single dose of dasiglucagon [0.6 mg] is comparable to a single dose of GlucaGen® [1 mg/mL] in treating hypoglycemia in children with T1DM (in accordance with the label, children below 25 kg body weight will be administered 0.5 mg GlucaGen®)
- To assess safety profile of dasiglucagon in children with T1DM
- To assess PK profile of dasiglucagon in children with T1DM

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

Secondary endpoints

Secondary efficacy endpoints

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

Safety endpoints

- AEs
- Clinical laboratory assessments (biochemistry, hematology, coagulation, urinalysis)
- Vital signs
- Physical examination
- Clinically significant changes in the electrocardiogram
- Local tolerability
- Administration of rescue IV glucose infusion after trial product injection
- Time to first IV glucose infusion, after trial product administration (N.B. IV glucose infusion prior to trial product administration should not be included, as it is part of hypoglycemic clamp procedure)
- Immunogenicity endpoint: occurrence of ADAs

Pharmacokinetic endpoints

PK endpoints will be derived from plasma dasiglucagon and GlucaGen® profiles from 0 to 300 minutes:

- Area under the plasma dasiglucagon or GlucaGen® concentration versus time curve from 0 to 30 minutes post-dose ($AUC_{0\text{-}30min}$)

- Area under the plasma dasiglucagon or GlucaGen® concentration versus time curve from 0 to 300 minutes post-dose ($AUC_{0\text{-}300min}$)
- Area under the plasma dasiglucagon or GlucaGen® concentration versus time curve from 0 to infinitely post-dose ($AUC_{0\text{-}inf}$)
- Maximum of all valid plasma dasiglucagon or GlucaGen® concentration measurements from 0 to 300 minutes post-dose (C_{max})
- Time to maximum of plasma dasiglucagon or GlucaGen® concentration measurements (t_{max})
- Terminal elimination rate constant of plasma dasiglucagon or GlucaGen® (λ_z)
- Terminal plasma elimination half-life of dasiglucagon or GlucaGen® ($t_{½}$)
- Total body clearance of plasma dasiglucagon or GlucaGen® (CL/f)
- Volume of distribution of plasma dasiglucagon or GlucaGen® (V_z/f)
- Mean residence time of plasma dasiglucagon or GlucaGen® (MRT)

Pharmacodynamics endpoint

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

7. Investigational plan

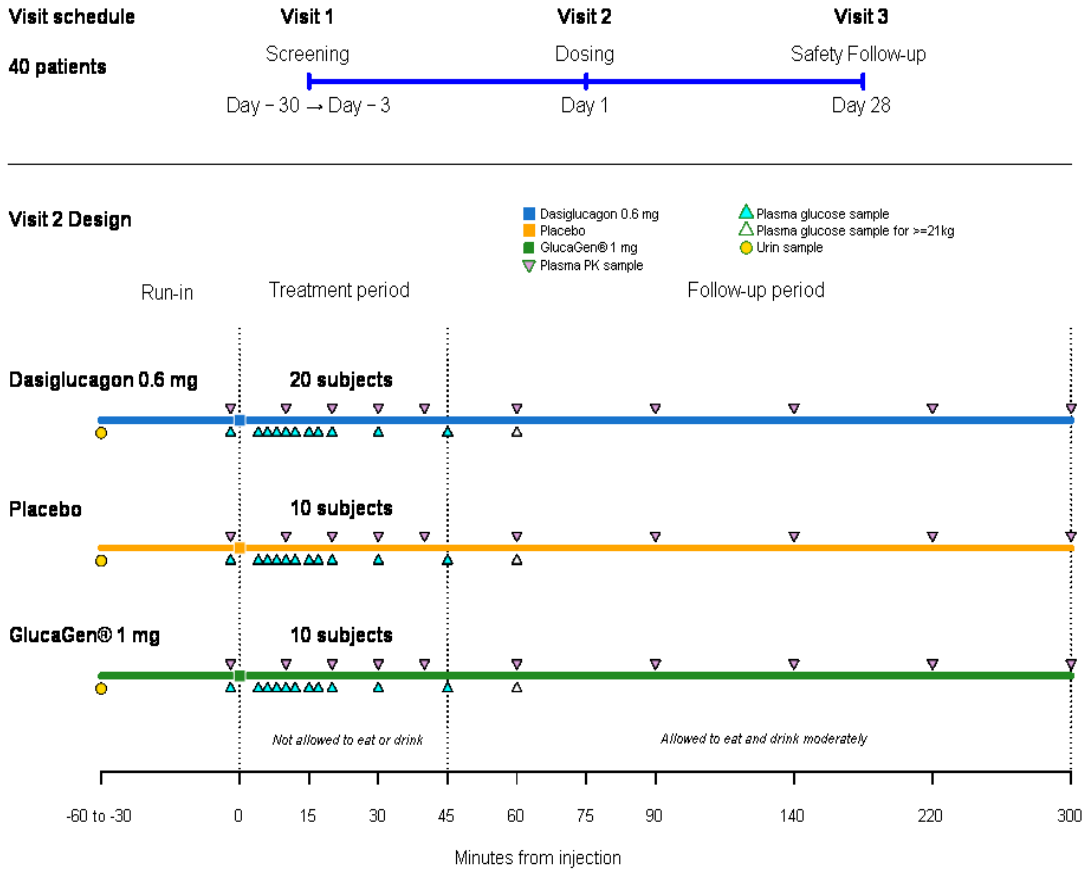
7.1 Overall trial design and plan

This is a phase 3, randomized, double-blind, placebo- and active-controlled, parallel-arm trial designed to assess efficacy, safety and PK of dasiglucagon vs. placebo and vs. GlucaGen® in children with T1DM. It is currently anticipated that it will be conducted at 2-3 centers in Europe and 1-2 centers in the USA.

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

- A screening period from Day -30 to Day -3
- A treatment period, from Day 0 to Day 1 (day of randomization and single dosing with trial product). Unblinded trial personnel will do the handling, preparation and administration of trial product. All trial assessments will be done by blinded trial personnel
- A follow-up visits at Day 28 (the end-of-trial visit)

Figure 7-1 Overview of the Trial Design



Abbreviation: PK=pharmacokinetic

The schedule of assessments (Table 2-1) gives an overview of the trial procedures. Patients should attend all visits on the designated day or as close to it as possible.

7.2 Discussion of trial design and choice of control groups

The trial will be randomized and double-blind to increase trial validity and to reduce bias during evaluation of assessments with the treatments. Since the 3 trial products are not identical in appearance, (dasiglucagon and placebo are liquid formulations and GlucaGen® is available as a powder for reconstitution), unblinded trial personnel, who will not be involved in other trial procedures and assessments, will do the handling, preparation and administration of trial product. Blinded trial personnel will do all trial assessments performed at the trial center.

Children with T1DM will be randomized 2:1:1 in order to evaluate the efficacy and safety of dasiglucagon compared to placebo and GlucaGen® and PK/PD parameters. The randomized, double-blind, parallel arm design with administration of a single dose of randomized trial product (dasiglucagon, placebo or GlucaGen®) will allow a relative comparison between the 3 treatment arms.

Dasiglucagon will be administered at fixed doses independent of body weight because this is the intended therapeutic dosing regimen in the emergency treatment of hypoglycemia. The selected dose of 1 mg GlucaGen® is the recommended dose for treatment of severe hypoglycemia (in accordance with the label, children below 25 kg body weight will be administered 0.5 mg GlucaGen®). Based on pre-clinical and clinical studies in adult patients, it has been demonstrated that 0.6 mg of dasiglucagon results in an initial PD response (i.e. acute glucose mobilization) comparable to 1 mg GlucaGen® (see also Section 5.1).

7.3 Selection of trial population

Dasiglucagon is indicated for treatment of severe hypoglycemia in patients with T1DM. As this condition also affects children and there is a therapeutic need for them to get access to safe and efficacious emergency treatment, the present trial aims to evaluate the efficacy and safety of a single dose of SC dasiglucagon compared to placebo and GlucaGen® in children with T1DM in experimentally induced hypoglycemia in a hypoglycemic clamp procedure.

The trial will enroll patients in centers in the EU and in the USA.

7.3.1 Inclusion criteria

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

1. Following receipt of verbal and written information about the trial, patient, parent(s) or guardian(s) of the patient must provide signed informed consent before any trial-related activity is carried out*
2. Female or male patients with T1DM for at least 1 year, diagnostic criteria as defined by the American Diabetes Association; and receiving daily insulin
3. At least 6.0 years of age (inclusive) and less than 18.0 years
4. Body weight ≥20 kg
5. Female patients must meet one of the following criteria:
    a. Participant is of childbearing potential and agrees to use one of the accepted contraceptive regimens throughout the entire duration of the trial from screening until last follow-up visit. An acceptable method of contraception includes at least one of the following:
        i. Abstinence from heterosexual intercourse
        ii. Systemic contraceptives (birth control pills, injectable/implant/ insertable hormonal birth control products, transdermal patch); if the participant is using systemic contraceptives, she must use an additional form of acceptable contraception (iii or iv, below)
        iii. Intrauterine device (with and without hormones)
        iv. Condom with spermicide

or

b. Participant is of non-childbearing potential due to pre-puberty status or a medical condition confirmed by the investigator

6. Male patients must meet the following criteria: If sexually active, uses condom and partner practices contraception during the trial from screening and until last follow-up visit
7. Willingness to adhere to the protocol requirements

* Informed consent signatures must be obtained according to local regulations.

7.3.2 Exclusion criteria

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

1. Females who are pregnant according to a positive urine pregnancy test, actively attempting to get pregnant, or are lactating
2. Known or suspected allergy to trial product(s) or related products
3. History of anaphylaxis or symptoms of severe systemic allergy (such as angioedema)
4. Previous randomization in this trial
5. History of an episode of severe hypoglycemia that required a third party assistance within a month prior to screening visit
6. History of hypoglycemic events associated with seizures or hypoglycemia unawareness in the last year prior to screening
7. History of epilepsy or seizure disorder
8. Receipt of any investigational drug within 3 months prior to screening
9. Active malignancy within the last 5 years
10. Congestive heart failure, New York Heart Association class II-IV
11. Current bleeding disorder, including anti-coagulant treatment
12. Known presence or history of pheochromocytoma (i.e. adrenal gland tumor) or insulinoma (i.e. insulin secreting pancreas tumor)
13. Use of a daily systemic beta-blocker drug, indomethacin, warfarin or anticholinergic drugs in the previous 28 days before Day 1 of this trial
14. 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, or altered electrolyte values of clinical relevance for cardiac conduction, as judged by the investigator
15. Clinically significant abnormal electrocardiogram (ECG) at screening as judged by the investigator
16. Clinically significant illness within 4 weeks before screening, as judged by the investigator
17. Surgery or trauma with significant blood loss within the last 2 months prior to screening
18. Patients 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
19. Any condition interfering with trial participation or evaluation or that could be hazardous to the patient
20. The use of prescription or non-prescription medications known to cause QT prolongation

In addition, the following exclusion criteria at clinic admission on Visit 2, day 0 apply at the time of admission to the clinic, which is the day before clamp procedure:

Patients who meet one or more of the following exclusion criteria at the time of admission to the clinic will be excluded from the dosing visit, however, the visit can be rescheduled 1-7 days later. The dosing visit can only be rescheduled once.

1. Atypically strenuous exercise within 4 days prior to dosing, as judged by the investigator. Exercise during the trial should follow patient's typical routine, and should not exceed a near maximum intensity for more than 20 minutes per day, or moderate intensity for more than 90 minutes per day
2. Clinically significant illness within 4 weeks before dosing, as judged by the investigator
3. Consumption of alcohol within 24 hours prior to dosing visit, determined by positive results from an alcohol breath test
4. Not fasting from 22:00 hours the evening prior to dosing, apart from water.
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 72 hours prior to dosing; or use of other long-acting insulins (e.g. insulin glargine U100 or insulin detemir) within 48 hours prior to dosing; or use of neutral protamine Hagedorn (NPH) insulin within 22 hours prior to dosing
7. Use of any short acting (bolus) insulin within 12 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 admission to the clinic

7.3.3 Premature withdrawal from trial

Participation in the trial is strictly voluntary. A patient has the right to withdraw from the trial at any time and for any reason. If the child chooses to withdraw or his/her parents or legal guardians choose to have the child withdrawn, the investigator must be informed immediately. The investigator has the right to terminate participation of any patient at any time if the investigator deems it in the patient's best interest. The reason and circumstances for withdrawal will be documented in the electronic case report form (eCRF).

7.3.3.1 Possible reasons for patient withdrawal

A patient will be withdrawn if the following applies:

- If a protocol deviation occurs which, in the clinical judgment of the investigator, can invalidate the trial endpoints, the patient will be withdrawn by the investigator
- AEs that are considered unacceptable by the patient or the investigator

If withdrawal occurs following administration of any trial product, the patient will be asked to return and participate in the complete follow-up visit at trial Day 28. In this case, withdrawal relates only to blood sampling but not to the safety assessments. Patients should be followed for AEs for the same duration as those who are not withdrawn.

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

If the patient meets any of the exclusion criteria 1-9 at the time of admission to the clinic at V2, he/she will be excluded from the dosing visit but may be rescheduled once 1-7 days later.

7.3.3.2 Center discontinuation

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

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

However, even in case of center discontinuation the center should be kept open as long as previously enrolled patients are ongoing. Patients already enrolled should be followed and documented for their entire individual trial duration before actually discontinuing the center.

7.3.3.3 Trial termination

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

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

7.3.4 Replacement of patients

Patients who are dosed will not be replaced.

7.4 Investigational medicinal product(s)

7.4.1 Identity of investigational medicinal product(s)

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

Table 7-1: Identity of Investigational Products

	Test product	Placebo	Reference product
Name	Dasiglucagon	Placebo	GlucaGen®
Active substance	ZP4207	Not applicable	Recombinant glucagon hydrochloride
Formulation	Liquid formulation, 0.6 mL	Liquid formulation, 0.6 mL	Powder and solvent for reconstitution as 1 mL solution for injection
Strength	1 mg/mL	Not applicable	1 mg/mL
Container	Single use pre-filled syringe	Single use pre-filled syringe	Powder and solvent for reconstitution packed together in a plastic box. A “hypo-kit”
Manufacturer	Zealand Pharma A/S, Glostrup (Copenhagen), Denmark	Zealand Pharma A/S, Glostrup (Copenhagen), Denmark	Novo Nordisk A/S, Bagsværd, Denmark
Storage requirements	Store between 2 and 8°C	Store between 2 and 8°C	Store between 2 and 8°C

7.4.2 Treatments administered

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

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

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

7.4.3 Packaging and labelling

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

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

Labelling will be performed according to Annex 13 of the Good Manufacturing Practice guidelines of the European Commission, International Council for Harmonisation (ICH) guidelines on Good Clinical Practice (GCP), and local law.

7.4.4 Storage of trial product

The investigator must ensure the availability of proper storage conditions. All trial product supplies provided for this trial will be stored in the fridge, in a secure area with restricted access at the trial center. The trial products ready for use should be separated from any other clinical supplies, to avoid product 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 unblinded person responsible for trial product handling must contact the unblinded monitor in case of temperature deviations outside the acceptable range.

Please see the trial materials manual for additional information on handling trial product.

7.4.5 Investigational treatment and dosing conditions

7.4.5.1 Changes to diabetes management prior to dosing visit

At the screening visit, patients will be instructed by the investigator about the changes in their diabetes treatment leading up to and immediately prior to the dosing visits. There should not be any changes in their diabetes management until 3 days (72 hours) prior to the planned trial product administration except to switch to NPH insulin. The investigator will provide individual dosing instructions for this change. Within 72 hours prior to dosing, the use of insulin degludec or insulin glargine U300 is prohibited. Within 48 hours prior to dosing, the use of long-acting insulin (e.g. insulin glargine U100 or insulin detemir) is prohibited. Within 22 hours prior to dosing, the use of insulin NPH is prohibited. Within 12 hours prior to dosing, the use of any short acting (bolus) insulin, except insulin glulisine (Apidra®) is prohibited. During the insulin-induced hypoglycemic procedure, continuous SC insulin infusion must be stopped.

7.4.5.2 Dosing visit

Patients will be admitted to the clinic with their parents in the evening of the day before dosing (Day 0). Their eligibility for the trial will be checked, and only those still eligible will continue and remain in the clinic overnight.

If the patient meets any of the exclusion criteria at clinic admission on Visit 2, day 0, he/she will be excluded from the dosing visit but may be rescheduled once 1-7 days later.

An infusion catheter will be inserted into each arm for the manual glucose clamp procedure, with the glucose infusion in one arm and the insulin infusion in the opposite arm preferably, but the same infusion catheter can also be used. Another IV catheter, separated from infusion catheter(s), for blood sampling will be placed in the morning before trial product administration. The hand of this arm will be warmed (55-65 ºC) to arterialize venous blood. If there are issues with blood sampling from e.g. the metacarpal vein for the purpose of glucose measurements, the investigator may use a new and more proximal IV access.

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. They may consume water ad libitum. The patients must not consume any alcohol or smoke within 24 hours prior to dosing.

7.4.5.2.1 Diabetes management

Last injection or bolus administration via continuous SC insulin infusion of any insulin medication should not take place within the 12 hours before dosing. Patients normally using insulin aspart will be switched to human soluble insulin or glulisine for the period between 12 and 10 hours prior to dosing, if needed. Patients using continuous SC insulin infusion will have their pump switched off at 22:00 hours.

To achieve a target glucose level of 90-160 mg/dL (5.0-8.9 mmol/L) in the morning of dosing, patients may be administered insulin glulisine and/or glucose at the discretion of the investigator by IV infusion using the following glucose infusion rate and insulin infusion rate as a guidance. Deviation from the guidance is allowed at the discretion of the investigator. Plasma glucose levels will be checked overnight at regular intervals in order to achieve and maintain the target plasma glucose level of 90-160 mg/dL (5.0-8.9 mmol/L).

A general guidance for administration of insulin IV and/or glucose IV at the dosing visit

Fluid infusion rate:

If plasma glucose < 300 mg/dL, then infusion of isotonic solution with 5% glucose:

Age 6–10 years: 80 mL/kg/24 h

Age >10 years: 60 mL/kg/24 h

If plasma glucose >300 mg/dL the glucose infusion will be substituted with fluid volume of normal saline (0.9% NaCl) at same infusion rate as above.

Infusion of insulin:

Insulin infusions should be performed with 0.5 IU insulin glulisine (Apidra®) per kg body weight in 48 mL NaCl 0.9% according to the following recommendations:

Glucose	Infusion rate	Insulin dose (IU/kg BW/h)
>200 mg/dL	10.0 mL/h	0.1
150–200 mg/dL	5.0 mL/h	0.05
80-150 mg/dL	2.5 mL/h	0.025
<80 mg/dL	Insulin infusion stop!	0

Abbreviation: BW=Body weight

7.4.5.2.2 Hypoglycemic clamp procedure and dosing

At the time of admission to the clinic, which is the day before clamp procedure, the patient's eligibility must be checked. If the patient meets any of the exclusion criteria at the time of admission to the clinic at V2, he/she will be excluded from the dosing visit but may be rescheduled once 1-7 days later.

Eligible patients will be randomized to either dasiglucagon, placebo or GlucaGen® in the morning of the dosing visit. That morning after 7 o'clock am, the infusion of IV glucose will be stopped. This should be at least 30 minutes prior to planned trial product administration. Insulin infusion rate and insulin dose are at the discretion of the investigator or can follow the protocol general guidance (see above). The investigator may deviate from the guidance. Insulin IV infusion will be stopped when glucose level declines to below 80 mg/dL.

During insulin-induced hypoglycemia, plasma glucose levels will be monitored closely at the site. After the start of insulin infusion, plasma glucose should be checked approximately every 10 minutes while plasma glucose is above 110 mg/dL, approximately every 5 minutes once plasma glucose is at or below 110 mg/dL, until after dosing, when the patient's plasma glucose returns to ≥70 mg/dL (3.9 mmol/L). From that time on, 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, Ohio, USA or the Super GL analyzer, Dr. Müller Gerätebau GmbH, Freital, Germany.

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

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

In case of persistent post-treatment hypoglycemia, patients will receive rescue treatment with an IV glucose infusion (see Section 7.4.5.2.3 for details). Blood samples for PD and PK assessments should still be taken at the specified time points.

Once the glucose concentration declines to <80 mg/dL, blood samples for baseline assessment of plasma glucose and dasiglucagon/GlucaGen PK will be collected 5 minutes later. The samples are the baseline samples and should be collected within 2-5 minutes before trial product administration. If plasma glucose is ≥54 mg/dL and <80 mg/dL (3.0-4.4 mmol/L), trial product will be administered.

If plasma glucose is <54 mg/dL (3.0 mmol/L), IV glucose solution will be administered sufficient to raise plasma glucose to within the 54-80 mg/dL 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/GlucaGen PK, will be collected. In this case, glucose should not be infused within 10 minutes before trial product administration. If plasma glucose is not within the target range after the second attempt, the patient should be rescheduled for a new treatment visit within 7 days (+ 2 days).

After 60 minutes, patients will be allowed to eat and drink moderately (appropriate to their body size, with a maximum of 50gr carbohydrates) to minimize discomfort in terms of potential nausea. The amount and type of food and drink consumed will be recorded. Patients should remain in bed until completion of the test procedure 300 minutes after dosing (bathroom visits are allowed).

7.4.5.2.3 Rescue provisions for hypoglycemia

During insulin-induced hypoglycemia, plasma glucose levels as well as the patient status will be monitored closely throughout the procedure. After administration of trial product, if their glucose levels become too low, patients may receive post-treatment IV glucose infusion to ameliorate hypoglycemia, as follows:

1. The IV glucose infusion should be initiated if a patient experiences escalating symptoms of hypoglycemia (e.g. start of moderate symptoms of hypoglycemia) at any time during the test procedure. Glucose infusion should then be initiated targeting a plasma glucose level >70 mg/dL
2. If plasma glucose is <54 mg/dL between t=8 and t=44 minutes, glucose infusion (e.g. 1-2 mg/kg administered over 5-10 seconds) should be initiated to maintain plasma glucose between 55 mg/dL and 70 mg/dL. Pause glucose infusion if plasma glucose is >70 mg/dL
3. If plasma glucose is <70 mg/dL at t≥45 minutes, glucose infusion (e.g. 2-3 mg/kg administered over 5-10 seconds) should be initiated to maintain plasma glucose between 70 mg/dL and 90 mg/dL. Pause glucose infusion if plasma glucose is >80 mg/dL

7.4.6 Selection of doses in the trial

The selected dose of 1 mg GlucaGen® is the recommended dose for treatment of severe hypoglycemia (in accordance with the label, children below 25 kg body weight will be administered 0.5 mg GlucaGen®). Pre-clinical and adult clinical studies demonstrated that 0.6 mg of dasiglucagon results in an initial PD response (i.e. acute glucose mobilization) comparable to 1 mg GlucaGen®.

The estimation of the dasiglucagon dose in the pediatric trial is based on a PK and PD approach[6] where a previously developed population PK/PD model[3] for dasiglucagon was extended with an allometric scaling component for the PK model[4]. The PD response was assumed to be similar in pediatric and adult patients as organ maturation for patients above 4 years is assumed not to have a meaningful impact on dasiglucagon PD. The pediatric PK component was developed by allometric scaling of clearance (Cl) and volume (V) by body weight from published glucagon PK data obtained from 2 clinical trials following intramuscular administration to pediatric (4 to 17 years) and adult T1DM patients[11,9].

The conclusion of the modelling was that a dose of 0.6 mg appears appropriate to ensure a fast rescue from hypoglycemia for adult patients and children down to a weight of 25 kg. Although the dasiglucagon C_{max} and AUC is expected to be higher in children, it does not exceed the total C_{max} and AUC obtained in adults previously administered 2 mg dasiglucagon. The higher total drug exposure (AUC and C_{max}) at lower weight is partially compensated by the shorter $t_{½}$ and saturated PD response. The resulting total time of drug exposure and PD effect (AEU and CE_{max}) is similar throughout the weight range.

7.4.7 Collection of blood samples

The total blood volume to be obtained from any individual child will be about 43 mL. The maximum amount per visit is 12 to 17 mL. These values are within the recommended scope provided by the EU[5].

The following blood volume will be taken for each sample at each visit:

Sample type	Visit 1 (mL)	Visit 2 (mL)	Visit 3 (mL)	
Biochemistry/pregnancy	5.00	-	5.00	
Hematology	2.00	-	2.00	
Coagulation	2.00	-	2.00	
HbA1c	2.00	-	-	
ADA	3.00	-	3.00	
PK	-	4.00	-	
PD	-	12.00	-	
Onsite-PG	-	1.00	-	
Sum for each visit	14.00	17.00	12.00	
Total				43.00

Abbreviations: ADA=Anti-drug antibodies; HbA1c=Hemoglobin A1c; Onsite-PG=Onsite monitoring of plasma glucose; PD=Pharmacodynamics; PK=Pharmacokinetics

7.4.8 Treatment compliance

Unblinded trial personnel will handle, prepare and administer all trial products.

7.4.9 Method of assigning patients to treatments

Forty patients will be randomized in a 2:1:1 ratio (dasiglucagon/placebo/GlucaGen®) into the 3 treatment arms and stratified in 2 age groups: 6 to ≤12 years and 12 to ≤18 years and by injection site (abdomen/thigh). A minimum of 16 patients will be enrolled into each age group, including a minimum of 8 patients in either of age groups on dasiglucagon treatment arm.

Patients who meet the inclusion and exclusion criteria will be randomized into one of the following treatment arms:

- Dasiglucagon, liquid formulation, 1 mg/mL, 0.6 mL

- Placebo treatment: Placebo, liquid formulation, 0.6 mL
- Active control treatment: Recombinant glucagon hydrochloride, 1 mg lyophilized powder for reconstitution (GlucaGen®, Novo Nordisk) in 1 mL sterile water (in accordance with the label, children below 25 kg body weight will be administered 0.5 mg GlucaGen®)

Randomization will be performed using a central, dynamic variance minimization randomization method using an interactive web response system (IWRS) that will randomize a patient to one of the 3 aforementioned treatment arms and then assign dispensing unit numbers to that patient.

An unblinded statistician/programmer who is separate from the trial team will generate the randomized kit list before the initiation of the trial.

Treatment assignment will be kept strictly confidential and accessible only to authorized persons until after data base lock. However, the investigators can perform an emergency unblinding within the IWRS in case of an emergency, see Section 7.4.10.

7.4.10 Blinding and breaking the blind

This is a double-blind trial. Dasiglucagon is available as a liquid formulation and GlucaGen® is available as a powder for reconstitution; they are therefore not identical in appearance. Unblinded trial personnel will be responsible for handling, preparing (according to the prescription from the IWRS), and administering the trial product, and the syringes used for administration will be wrapped in aluminum foil to maintain the blinding at bedside. Parents being in the room with their child must be instructed not to look while the unblinded staff is administering the injection. To maintain double-blind conditions, blinded trial personnel not involved in the administration of trial products will perform all trial assessments at the trial center and is responsible for keeping the records strictly confidential and accessible only to the unblinded staff. However, unblinded personnel at the specialty laboratories will perform the exposure assessments and ADA assessments to make sure that dasiglucagon or GlucaGen® administration is matched with the applicable bioanalytical assay.

Treatment assignment will be kept strictly confidential and accessible only to authorized persons until after the time of unblinding (database lock). 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 patient may be broken in a medical emergency if knowing the identity of the treatment allocation would influence the treatment of the patient. 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 a patient should not unblind the trial personnel to the treatment information of other patients. The person performing the unblinding should inform as few people as possible about the result of the unblinding. All persons unblinded for a specific patient should be documented.

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

7.4.11 Drug accountability and disposal

Unblinded trial personnel will do the handling, preparation and administration of trial product. Each center will keep accurate records of the trial supplies received, stored, and dispensed, using appropriate forms. The trial supplies will be stored under appropriate conditions, locked and with restricted access. The unblinded monitor will perform the drug accountability to ensure compliance.

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

7.4.12 Prior and concomitant therapy

All concomitant medications will be recorded in the eCRF at each visit.

Prior to the start of the clamp procedure, the patient's eligibility must be checked. If the patient has taken any prohibited medication, he/she will be excluded from the dosing visit but may be rescheduled 1-7 days later. Each patient may only have their dosing visit rescheduled once. See Section 7.3.3.1 for possible reasons for patient discontinuation.

7.4.13 Treatment after end of trial

After the end of their trial participation, patients should return to the standard of care that they received prior to enrollment in the trial. The treating physician will be responsible for supervising patients after the end of the trial.

7.5 Assessments and schedule of measurements

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

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

7.5.1 Screening visit

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

- Informed consent
- Inclusion/exclusion criteria
- Demography
- Body measurements
- Diabetes diagnosis, and current diabetes treatment
- Medical history including concomitant illnesses
- Prior and concomitant medications
- History of alcohol/drug abuse
- Physical examination
- Vital signs
- 12-lead ECG
- AEs
- Biochemistry, hematology, coagulation, hemoglobin A1c (at Visit 1 only)
- Serum pregnancy test (female patients of childbearing potential only)
- Urinalysis
- Antibodies against dasiglucagon/GlucaGen®

7.5.2 Dosing visit

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

The following assessments will take place:

- Inclusion/exclusion criteria (prior to the start of the insulin-induced hypoglycemic procedure. Only check of exclusion criteria at clinic admission on Visit 2, day 0 and changes between screening visit and Visit 2)
- Concomitant medications (prior to the start of the insulin-induced hypoglycemic procedure)
- Current diabetes treatment
- Randomization (prior to the start of the insulin-induced hypoglycemic procedure)
- Exclusion criteria at clinic admission on Visit 2, day 0 (prior to the start of the insulin-induced hypoglycemic procedure)
- 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)
- Alcohol breath test (prior to the start of the insulin-induced hypoglycemic procedure)
- Physical examination (prior to the start of the insulin-induced hypoglycemic procedure)
- Insulin-induced hypoglycemia
- Vital signs (prior to the start of the insulin-induced hypoglycemic procedure (within 30 minutes), and at 30, 60 and 300 minutes after dosing. The actual time of the assessment should not deviate from the nominal time by more than ±10 minutes)
- 12-lead ECG (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)
- Local tolerability (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)
- AEs
- Plasma dasiglucagon/GlucaGen® (pre-dose, and at 10, 20, 30, 40, 60, 90, 140, 220, and 300 minutes after dosing. The actual time of blood sampling should not deviate from the nominal time by more than ±1 minute between t=5 minutes and t=90 minutes, ±5 minutes between t=150 minutes and t=240 minutes. Pre-dose is defined as within 2 minutes prior to dosing)
- Plasma glucose (pre-dose, and at 4, 6, 8, 10, 12, 15, 17, 20, 30 and 45 minutes (and as well at 60 minutes if the patient´s body weight is ≥21 kg) after dosing. The actual time of blood sampling should not deviate from the nominal time by more than ±30 seconds until the 20-minute collection time point, and by more than ±1 minute for the subsequent collection time points. Pre-dose is defined as within 2 minutes prior to dosing
- Administration of trial product (during hypoglycemic clamp procedure)

7.5.3 Follow-up visit

At 28 (+ 5) days after the hypoglycemic clamp procedure, the follow-up visit is scheduled to collect safety data. The following assessments will take place:

- Concomitant medications
- Current diabetes treatment
- Physical examination
- Vital signs

- 12-lead ECG
- Local tolerability
- AEs
- Biochemistry, hematology, coagulation, HbA1c (HbA1c at Visit 1 only)
- Urine stick pregnancy test (women of childbearing potential only)
- Urinalysis
- Antibodies against dasiglucagon/GlucaGen® (any treatment-induced or treatment-boosted [titer increase above 5 fold] ADA-positive patients will be monitored until the ADA levels return to baseline levels)

7.5.4 Final examination at the end of the trial

The final visit of the trial is Visit 3 (Day 28[+5] of the follow-up period; end-of-trial visit). See Section 7.5.3 for further details.

7.5.5 Additional (safety) examinations

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

7.5.6 Efficacy measurements

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.

Pharmacokinetic measurements

The exposure to trial medication (dasiglucagon or GlucaGen®) for evaluation of PK will be assessed based on plasma concentration data ($AUC_{0\text{-}90min}$, C_{max}, t_{max}) from samples collected at the dosing visit (Visit 2). The sampling procedure is described in Section 7.5.2.

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. The sampling procedure is described in Section 7.5.2.

7.5.7 Safety and tolerability measurements

7.5.7.1 Safety laboratory tests

Routine safety laboratory tests will be performed centrally. Samples for safety laboratory parameters will be collected at Visits 1 and 3. Samples for urinalysis will be collected at Visits 1, 2, and 3. The following parameters will be determined:

- Clinical chemistry: sodium, potassium, calcium, glucose, urea, creatinine, total bilirubin, AST, ALT, gamma-glutamyltransferase, alkaline phosphatase, total protein, C-reactive protein, HbA1c, C-peptide
- Hematology: hemoglobin, red blood cell count (erythrocytes), hematocrit, platelet count (thrombocytes), total white blood cell count (leucocytes)

- Coagulation: international normalized ratio, fibrinogen (at screening visit only)
- Urinalysis: pH, blood (leukocytes and erythrocytes), protein, glucose, ketones, nitrite
- Immunogenicity:
  Serum samples for assessment of antibodies against dasiglucagon/GlucaGen® will be measured at screening Visit 1 and at follow-up Visit 3 by the special laboratory, York Bioanalytical Solutions (York, United Kingdom). A description of the sample handling and sample processing, storage and shipment at the center will be included in the laboratory manual. Immunogenicity samples will be analyzed after the last patient last visit.

  The clinical ADA assays, one specific for dasiglucagon and another for GlucaGen®, have been validated in accordance with existing guidelines and recommendations.

  Confirmed positive anti-dasiglucagon antibody samples from treatment- induced or treatment-boosted (titer increase above 5-fold) ADA-positive patients will be evaluated for binding titer, neutralizing potential and neutralizing titer as well as cross-reactivity towards endogenous glucagon. Any treatment-induced or treatment-boosted ADA-positive patients will be monitored until the ADA levels return to baseline levels.

  The *in vitro* neutralizing effect of the antibodies will be measured using an assay based on glucagon receptor expression in transfected human embryonic kidney cells. The calculated sensitivity in previous studies was about 51.8 ng/mL. The assay was also validated for recombinant glucagon with similar results. 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, North Carolina, USA.

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

  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.

A pregnancy test will be performed at screening and follow-up (Visits 1, 2 and 3) for female patients of childbearing potential only.

An alcohol breath test will be performed at the dosing visit (Visit 2).

For further details, please refer to the laboratory manual.

7.5.7.2 Safety examinations

Physical examination is performed at screening (Visit 1), dosing (Visit 2) and end-of-trial (Visit 3).

AEs are assessed at all visits. Local tolerability is assessed at dosing visit and follow-up visit (Visits 2 and 3). ECG and vital signs are assessed at screening, dosing and follow-up visit (Visit 1, 2 and 3).

- Physical examination includes examination of the following body systems: head, ears, eyes, nose, throat, including the thyroid gland; heart, lung, chest; abdomen; skin; musculoskeletal system; nervous system; lymph nodes
- Vital signs include: pulse rate and blood pressure after 5 minutes in sitting position, body temperature
- Local tolerability: skin reactions will be reported as AEs (see Section 8)
- 12-lead ECG: Details from ECG assessments will be recorded, including heart rate, PR, QRS, QT and QTc corrected by Fridericia´s formula intervals

8. Adverse events

8.1 Definitions

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

Adverse event

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

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

AEs include:

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

The following should not be recorded as AEs, if recorded at screening (on Medical History form or eCRF):

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

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

Serious adverse event

An SAE is any untoward medical occurrence that at any dose results 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 defect
- Is medically important
  Medical judgement must be exercised in deciding whether an AE is believed to be ‘medically important’. A medically important event may not be immediately life-threatening or result in

death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above.

Other important events:

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 > 3 x ULN and total bilirubin > 2 x ULN, where no alternative etiology exist
- Suspicion of transmission of infectious agents via the trial product
- Overdose of the trial product
- Suspected abuse or misuse of the trial product
- Medication error involving the trial product
- Inadvertent or accidental exposure to the trial product.

Severity of an adverse event

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

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

Causality relationship to trial product

- Probable: Good reason and sufficient documentation to assume a causal relationship
- Possible: A causal relationship is conceivable and cannot be dismissed
- Unlikely: The event is most likely related to etiology other than the trial product

Outcome of an adverse event

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

Suspected unexpected serious adverse reactions

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

Adverse event of special interest (AESI)

An AESI is an event that, in the evaluation of safety, has a special focus (e.g. required by health authorities). In this trial, hemodynamic changes are considered AESIs that are defined as follows:

- 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.

AESIs must be reported to the sponsor in the same way and with the same timelines as SAEs (see Sections 8.2 and 8.3).

8.2 Collection, recording and reporting of adverse events

All AEs, whether serious or non-serious, will be reported from the time a signed and dated informed consent form (ICF) is obtained until the end of the post-treatment follow-up period (which may include contacts for follow-up of safety). In addition, patients 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 center (visit or telephone). Patients 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 product, 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 product. All measures required for AE management must be recorded in the source document and reported according to sponsor instructions.

Each AE must be reported on the AE page of the eCRF.

All AE information should at a minimum include the following:

- Date and time of onset and resolution
- Date and time of investigator's first information on the AE
- Seriousness
- Severity
- Causal relationship with trial product
- Measures taken due to AE
- Date and time of resolution and final outcome

Each AE will be coded using the latest version of the Medical Dictionary for Regulatory Activities.

SAEs and AESIs, including those spontaneously reported to the investigator within 30 days after the last dose of trial product, must be reported to the appropriate sponsor contact person(s) within

24 hours after obtaining knowledge about the event, followed by a complete SAE form as soon as more information is available. For each SAE and AESI, a separate SAE form should be completed.

The investigator should report the SAE and AESI in the electronic data capture (EDC) and the system will generate an email to the sponsor´s Pharmacovigilance Unit (PharmaLex), informing them of the reported SAE.

It is the responsibility of the sponsor´s Pharmacovigilance Unit (PharmaLex) to report all SUSARs that occur in this trial to the respective competent authorities and the institutional review board (IRB), or independent ethics committee (IEC) in accordance with the local requirements in force and ICH guideline for GCP.

8.3 Follow-up of adverse events

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

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

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

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

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

The investigator must record follow-up information in the eCRF for non-serious AE and on the SAE form for SAEs and AESI. Follow-up questions to investigators regarding SAEs are queried directly by safety CRO to the investigator.

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

- SAEs and AESI: All SAEs and AESI 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 that are 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 patient has completed the follow-up period and is expected by the investigator to recover.

  The SAE and AESI follow-up information should only include new (eg, corrections or additions) 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 that are 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 a patient has completed the follow-up period and is expected by the investigator to recover.

If a potential hypersensitivity reaction is observed, additional blood samples, as clinically indicated, 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 worst-case severity and seriousness of an event is kept throughout the trial, ie, if the severity of an AE changes over time then it should be reported as 1 AE with the most severity. A worsening of an unresolved AE must be reported as follow-up with re-assessment of severity and/or seriousness of the event.

If an AE is resolved and re-appears later then it should be reported as a new AE.

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.

8.4 Hypoglycemia

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

Hypoglycemia is defined as a decline in plasma glucose below 3.9 mmol/L (70 mg/dL). Since hypoglycemia will be induced during the dosing visit, in accordance with hypoglycemia clamp procedure described in Section 7.4.5.2.2, **ONLY** plasma glucose below 3.0 mmol/L (54 mg/dL) must be reported as an AE, at the dosing visit (V2), and at incidences that IV glucose is given after trial product administration.

8.5 Pregnancy

Female patients must be instructed to notify the investigator immediately if they become pregnant or if they suspect to be pregnant during the trial. All initial reports of pregnancy in female patients must be reported to the sponsor by the trial center 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 patient becomes pregnant during the trial, a determination regarding the trial product discontinuation must be made by the investigator.

Because the effect of the trial product on sperm is unknown, pregnancies in partners of male patients must be reported by the trial center personnel within 24 hours of their knowledge of the event using the appropriate pregnancy form. Male patients 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 1 month of age. The investigator must report information about the pregnancy, pregnancy outcome, and health of the newborn infant(s), as well as AEs in connection with the pregnancy, and AEs in the fetus and newborn infant.

8.6 Precautions

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

For further information on safety precautions for dasiglucagon and GlucaGen®, please refer to the current version of the Investigator's Brochure[2] and the Summary of Product Characteristics for GlucaGen®[7], respectively.

8.7 Safety committee

An internal Zealand Pharma A/S safety committee is constituted to perform the 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 patients.

The safety committee convenes regularly, every quarter, to review the safety information, including SAEs, AEs and laboratory data. Additional safety committee meeting may be called at the discretion of any safety committee member, should new safety signals occur during this time interval.

9. Data management and quality control

9.1 Electronic case report forms

All the information collected during the trial will be recorded in the eCRFs, which are identified by patient number. Synteract will design suitable eCRFs. The investigator will ensure that the eCRFs are completed correctly. The investigator will sign all data entered in the eCRF electronically, signifying agreement with and responsibility for the recorded data.

9.2 Quality control

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

A monitor from Synteract will supervise the trial. The trial monitor will contact the investigator regularly to discuss the progress of the trial and to check the trial documents including the informed consent forms for completeness and consistency.

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

The eCRFs will be checked for completeness and correctness by the monitor and by the data management department of Synteract according to the Synteract standard operating procedures, and the investigator will resolve any queries.

All of the clinical data will be captured via EDC using a web-based tool. The software Marvin from the company XClinical (www.xclinical.com) is the preferred EDC software. Marvin is compliant with all legislation relevant to EDC (Food and Drug Administration USA, 21 Code of Federal Regulations [CFR] Part 11, GCP).

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

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

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

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

9.3 Data management

Data management will be performed according to Synteract standard operating procedures.

10. Statistical methods and determination of sample size

10.1 Statistical analysis plan

A separate Statistical Analysis Plan will detail the planned statistical analysis and may include necessary adaptations to the planned statistical analysis before unblinding of the data.

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

10.1.1 General considerations

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

Raw data listings and summary tables will be generated using SAS© software Version 9 or higher (SAS Institute, Inc, Cary, North Carolina, USA).

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

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

10.1.2 Classification of patients to subsets

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

The following definitions are applicable:

Safety analysis set (SAS)	all patients who were randomized and received at least 1 dose of trial product
Full analysis set (FAS)	all patients of the SAS
Per protocol set (PPS)	all patients of the FAS for whom no relevant protocol deviations were documented.

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

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

10.1.3 Efficacy endpoints

For the confirmatory analyses, the primary and secondary endpoints for the dasiglucagon 0.6 mg and placebo treated patients 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 patients 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 patients who received rescue IV glucose.

10.1.3.1 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 secondary endpoints will be inferentially evaluated within the FAS in the following order, where inference will proceed at the 2-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
- Secondary 1-4: Plasma glucose recovery within 30 minutes, within 20 minutes, within 15 minutes, and within 10 minutes after study drug injection without administration of rescue IV glucose.
- Secondary 5-8: Plasma glucose changes from baseline within 30 minutes, within 20 minutes, within 15 minutes, and within 10 minutes after study drug injection or at the time of rescue.

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

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

10.1.3.2 Primary efficacy endpoint

The primary endpoint, time to plasma glucose recovery, will be summarized using Kaplan-Meier estimates for each treatment group in total and stratified by age group and injection site. The median time to recovery with 95% confidence interval will be estimated by treatment group, in total and in each stratum.

A stratified log-rank test will be applied to compare the dasiglucagon 0.6 mg treatment group to the placebo group. The same method will be applied to compare dasiglucagon 0.6 mg with GlucaGen®.

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

A further sensitivity analysis will be done by censoring data at the actual time when the patients received glucose IV.

10.1.3.3 Secondary efficacy endpoints

The secondary efficacy endpoints are plasma glucose recovery within 10, 15, 20 and 30 minutes after trial product injection, i.e. achieving a ≥20 mg/dL increase in plasma glucose from baseline within 0 to 10, 15, 20 and 30 minutes. If a patient has received an IV glucose treatment before recovery, the patient is set to 'not recovered' in the analysis of the 4 endpoints, corresponding to censoring in the time-to-recovery analysis for the primary endpoint. The 10-, 15-, 20- and 30-minute recovery rates of 2 treatment groups will be compared by a Cochran-Mantel-Haenszel test stratified by age group and injection site. The treatment and age group responder rates with the 95% confidence intervals will be presented for the 10-, 15-, 20- and 30-minute endpoint.

If due to small or zero cell counts the Cochran-Mantel- Haenszel test fails, non-stratified Fisher's exact tests will be applied instead.

Plasma glucose changes from baseline at 10, 15, 20 and 30 minutes after trial product injection will be analyzed in an analysis of variance with factors treatment (3 levels) age group (2 levels) and injection site (2 levels) for each endpoint. If the rescue IV glucose was administered before 10, 15, 20 or 30 minutes, respectively, the patient's plasma glucose changes from baseline will be determined from the value at the time of rescue IV glucose administration. Adjusted treatment means will be presented with their 95% confidence intervals. The 4 analysis of variance will also be used to test the difference between treatments adjusted for age group and injection site.

10.1.4 Safety endpoints

Clinical laboratory data

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

Adverse events

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

Other safety data

Time to first IV glucose infusion, after IMP administration (N.B. IV glucose infusion prior to IMP administration should not be included, as it is part of hypoglycemic clamp procedure) will be described with descriptive statistics. No statistical tests will be performed.

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

Immunogenicity

Occurrence of ADA will be analyzed descriptively per treatment group. No statistical tests are planned.

10.1.5 Pharmacokinetic endpoints

Plasma dasiglucagon and glucagon concentrations will be described and the following parameters are calculated and summarized with descriptive statistics:

- Area under the plasma dasiglucagon or GlucaGen® concentration versus time curve from 0 to 30 minutes post-dose ($AUC_{0\text{-}30min}$)
- Area under the plasma dasiglucagon or GlucaGen® concentration versus time curve from 0 to 300 minutes post-dose ($AUC_{0\text{-}300min}$)
- Area under the plasma dasiglucagon or GlucaGen® concentration versus time curve from 0 to infinitely post-dose ($AUC_{0\text{-}inf}$)
- Maximum of all valid plasma dasiglucagon or GlucaGen® concentration measurements from 0 to 300 minutes post-dose (C_{max})
- Time to maximum of plasma dasiglucagon or GlucaGen® concentration measurements (t_{max})
- Terminal elimination rate constant of plasma dasiglucagon or GlucaGen® (λ_z)
- Terminal plasma elimination half-life of dasiglucagon or GlucaGen® ($t_{½}$)
- Total body clearance of plasma dasiglucagon or GlucaGen® (CL/f)
- Volume of distribution of plasma dasiglucagon or GlucaGen® (V_z/f)
- Mean residence time of plasma dasiglucagon or GlucaGen® (MRT)

10.1.6 Pharmacodynamic endpoints

Plasma glucose response as area under the effect curve above baseline from time 0 to 30 minutes, $AUE_{0\text{-}30min}$, will be summarized with descriptive statistics.

10.1.7 Further data

Baseline and demographic data will be summarized using descriptive statistics.

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

10.1.8 Withdrawals, drop-outs and missing data

Only valid cases will be analyzed, i.e. no imputation technique like last observation carried forward will be applied.

10.1.9 Baseline and center comparisons

Demographic and other baseline characteristics will be compared.

10.1.10 Subgroup analysis

No subgroup analysis is currently planned.

10.1.11 Interim analysis

No interim analysis is currently planned.

10.2 Determination of sample size

The primary comparison is between the dasiglucagon and placebo treatment arms. From phase 2 results, the median time to an increase of 20 mg/dL of the 0.6 mg dose was approximately 10 minutes. For placebo-treated patients, the median time to recovery is assumed to be more than 50 minutes. Assuming an exponential time-to-recovery distribution with median times of 10 and 50 minutes, a 2-sided log-rank test will be able to detect a difference between dasiglucagon 0.6 mg and placebo with 90% power with a follow-up time of 45 minutes at a 5% significance level with 20 patients randomized to the dasiglucagon arm and 10 patients to placebo.

GlucaGen® is included as a reference to compare the responses and AE profile to dasiglucagon with those to a marketed product. It is expected that 10 patients in the GlucaGen® group will suffice for the comparison.

11. Ethics and regulations

11.1 Independent ethics committees and competent authorities

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

The CA and the IEC/IRB will be notified about the end of the trial and a report summarizing the trial results will be sent to the CA and the IEC within 1 year after the end of the trial. If the trial is terminated early, the CA and the EC will be notified within 15 days.

The IECs and/or IRBs meet the requirements of the ICH Harmonised Tripartite Guideline for GCP and local legislation. They also meet the requirements of 21 CFR 312.3.

11.2 Ethical conduct of the trial

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

11.3 Patient information and consent

Written informed consent will be obtained from the parents/legal representatives of all patients prior to enrollment into the trial. Additionally, the children will be informed about the trial with age-appropriate information materials, and their assent will be obtained in accordance with local regulations. The investigator will explain to each patient and their parents/legal representatives orally and in writing (patient information sheet) the nature, significance, risks and implications of the trial before inclusion. In particular, the patients will be informed about the following:

- The possibility of withdrawing from the clinical trial at any time by revoking the consent and without any resulting disadvantage
- How personal and health-related data will be collected and used during the trial
- Confidentiality, i.e. medical records may be examined by authorized monitors or Clinical Quality Assurance auditors appointed by the sponsor, by appropriate IEC/IRB members, and by inspectors from regulatory authorities

All parents/legal representatives and the children will receive a copy of the respective patient information sheet and a copy of their signed and dated informed consent/assent form.

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

11.4 Legal and regulatory requirements

This trial will be carried out in accordance with:

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

12. Trial administration

12.1 Responsibilities

Zealand Pharma A/S is the sponsor of this trial. Synteract, a contract research organization, will organize the performance of this trial.

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

12.2 Protocol deviations

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

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

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

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

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

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

12.3 Protocol changes

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

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

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

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

12.4 Reports and publications

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

No confidential information will be disclosed to others without prior written consent from Zealand Pharma A/S. Such information will 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 product, if deemed necessary by Zealand Pharma A/S. Provided that certain conditions are fulfilled, Zealand Pharma A/S may grant access to information obtained during this trial to researchers who require access for research projects studying the same disease and/or trial product 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.

Communication of results

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

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

Zealand Pharma A/S 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 A/S. Zealand Pharma A/S 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 A/S 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.

12.5 Retention of trial records

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

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

After trial completion at sites in the US, the investigator will retain and preserve 1 copy of all data generated in the course of the trial, specifically including, but not limited to, those defined by GCP as essential until:

- At least 2 years after the last marketing authorization for the trial product has been approved or the sponsor has discontinued its research with the trial product, or
- At least 2 years have elapsed since the formal discontinuation of clinical development of the trial product

However, these documents should be retained for a longer period if required by the applicable regulatory requirement(s) or if needed by the sponsor.

At the end of such period, the investigator will notify the sponsor in writing of his or her intent to destroy all such material. The sponsor will have 30 days to respond to the investigator's notice, and the sponsor will have a further opportunity to retain such materials at the sponsor's expense.

After trial completion at sites in Europe, the sponsor will receive a copy of their data in electronic format (e.g. CD) and retain them for at least 25 years.

One copy will remain with the investigator. The investigator will arrange for the retention of the patient identification codes, patient files, and other source data until at least 5 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 until at least 2 years have elapsed since the formal discontinuation of the clinical development of the product. These documents need to be retained for a longer period of time if required by applicable regulatory authorities or by agreement with the sponsor.

The investigator will keep copies of these trial records (and all trial-related documents, including source data) for the maximum period of time permitted by the hospital, institution, or private practice.

13. References

1. American Diabetes Association. Standards of medical care in diabetes - 2016. Diabetes Care 2016; 39 (Suppl. 1):S1-S112.
2. Dasiglucagon Investigator Brochure, Edition 4, 25 Oct 2017. Zealand Pharma A/S.
3. Diderichsen PM. Population PK and population PKPD modeling and simulation of dasiglucagon in patients with type I diabetes mellitus. 2016 Zealand Pharma report 16-147.
4. Diderichsen PM. Model-based simulation of paediatric dasiglucagon exposure and glucose response. Zealand Pharma report 17-094. 2017
5. European Commission. Ethical considerations for clinical trials on medicinal products conducted with minors Revision 1. 18 Sep 2017. Available at: https://ec.europa.eu/health/sites/health/files/files/eudralex/vol-10/2017_09_18_ethical_consid_ct_with_minors.pdf
6. Holst JJ, Albrechtsen NJW, Pedersen J, Knop FK. Glucagon and amino acids are linked in mutual feedback cycle: The liver-α-cell axis. Diabetes 2017; 66(2): 235-40.
7. Novo Nordisk. Summary of Product Characteristics. GlucaGen® HypoKit 1 mg powder and solvent for solution for injection. Available at: http://www.medicines.org.uk/emc/medicine/4258/SPC/GlucaGen®+Hypokit+1+mg.
8. Rickels MR, Ruedy KJ, Foster NC, Piché CA, Dulude H, Sherr JL, et al. Intranasal Glucagon for treatment of insulin-induced hypoglycemia in adults with type 1 dabetes: A randomized crossover noninferiority study. Diabetes Care. 2016 Feb;39(2):264-70.
9. Seaquist ER, Anderson J., Childs B, Cryer P, Dagogo-Jack S, Fish L, et al. Hypoglycemia and diabetes: A report of a workgroup of the American Diabetes Association and the Endocrine Society. Diabetes Care 2013; 36(5):1384-95.
10. Sherr JL, Ruedy KJ, Foster NC, Piché CA, Dulude H, Rickels MR, et al. Glucagon nasal powder: A promising alternative to intramuscular glucagon in youth with type 1 Diabetes. Diabetes Care. 2016 Apr; 39(4):555-62.
11. United States. Food and Drug Administration. General clinical pharmacology considerations for pediatric studies for drugs and biological products. Guidance for industry. Draft guidance. 2014. Available at: http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm425885.pdf.

APPENDIX 1:

List of names and addresses

Sponsor	Zealand Pharma A/S Smedeland 36 2600 Glostrup, Copenhagen Denmark
Contract Research Organization (CRO)	Synteract Deutschland GmbH Albrechtstr. 14 80636 Munich, Germany
Coordinating investigator	Prof. Dr. med. Thomas Danne Allgemeine Kinderheilkunde Diabetologie, Endokrinologie, Klinische Forschung Diabeteszentrum für Kinder und Jugendliche AUF DER BULT Kinder- und Jugendkrankenhaus Janusz-Korczak-Allee 12 30173 Hannover, Germany
Trial monitor	To be decided Synteract Deutschland GmbH Albrechtstr. 14 80636 Munich, Germany
Statistician	[redacted] Senior Director Biometrics EMEA Synteract Deutschland GmbH Albrechtstr. 14 80636 Munich, Germany
Sponsor representative	[redacted] Clinical Project Director Zealand Pharma A/S Smedeland 36, 2600 Glostrup, Denmark Tel: [redacted] [redacted] E-mail: [redacted]
Sponsor's medical expert	Dr. [redacted] Medical Director Zealand Pharma A/S Smedeland 36, 2600 Glostrup, Denmark Tel: [redacted] E-mail: [redacted]
Sponsor representative for pharmacovigilance	[redacted] PharmaLex A/S Agern Allé 24, 2970 Hørsholm, Denmark Tel: [redacted] (8 a.m. to 4 p.m.) [redacted] (outside 8 a.m. to 4 p.m.) Fax: [redacted] E-mail: drugsafety@lindeq.com
Clinical laboratory	MLM Medical Labs GmbH Dohrweg 63

	41066 Mönchengladbach Germany Phone +49 2161 4642 0 Fax +49 2161 4642 190 E-mail info@mlm-labs.com
Analytical laboratory (ZP4207 PK, ZP4207 ADA, Glucagon ADA)	York Bioanalytical Solutions (YBS) [redacted] (ZP4207 Bioanalysis) [redacted] (ADA analyses) Cedar House Northminster Business Park Northfield Lane York, YO26 6QR United Kingdom E-mail: [redacted] E-mail: [redacted]
(ZP4207 and Glucagon Nab)	[redacted] BioAgilytix Labs 2300 Englert Drive Durham, NC 27713 USA E-mail: [redacted]

Abbreviations: ADA=Anti-drug antibody; Nab=Neutralizing antibody; PK=Pharmacokinetics

Clinical Trial Protocol

A phase 3, randomized, double-blind, placebo- and active-controlled, parallel-arm trial to assess the efficacy, safety, and pharmacokinetics of dasiglucagon relative to placebo and GlucaGen® when administered as a rescue therapy for severe hypoglycemia in children with T1DM treated with insulin

Sponsor code: ZP4207-17086
Synteract: ZEA-DNK-02170
EudraCT number: 2018-000892-33

Coordinating investigator: Prof. Dr. med. Thomas Danne

Allgemeine Kinderheilkunde

Diabetologie, Endokrinologie, Klinische Forschung
Diabeteszentrum für Kinder und Jugendliche AUF DER BULT

Kinder- und Jugendkrankenhaus

Janusz-Korczak-Allee 12

30173 Hannover

Germany

Sponsor: Zealand Pharma A/S

Smedeland 36

2600 Glostrup, Copenhagen

Denmark

Version: final version 3.0

Date: 08 January 2019

GCP statement

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

1. Signatures and agreement with protocol

Title: A phase 3, randomized, double-blind, placebo- and active-controlled, parallel-arm trial to assess the efficacy, safety, and pharmacokinetics of dasiglucagon relative to placebo and GlucaGen® when administered as a rescue therapy for severe hypoglycemia in children with T1DM treated with insulin

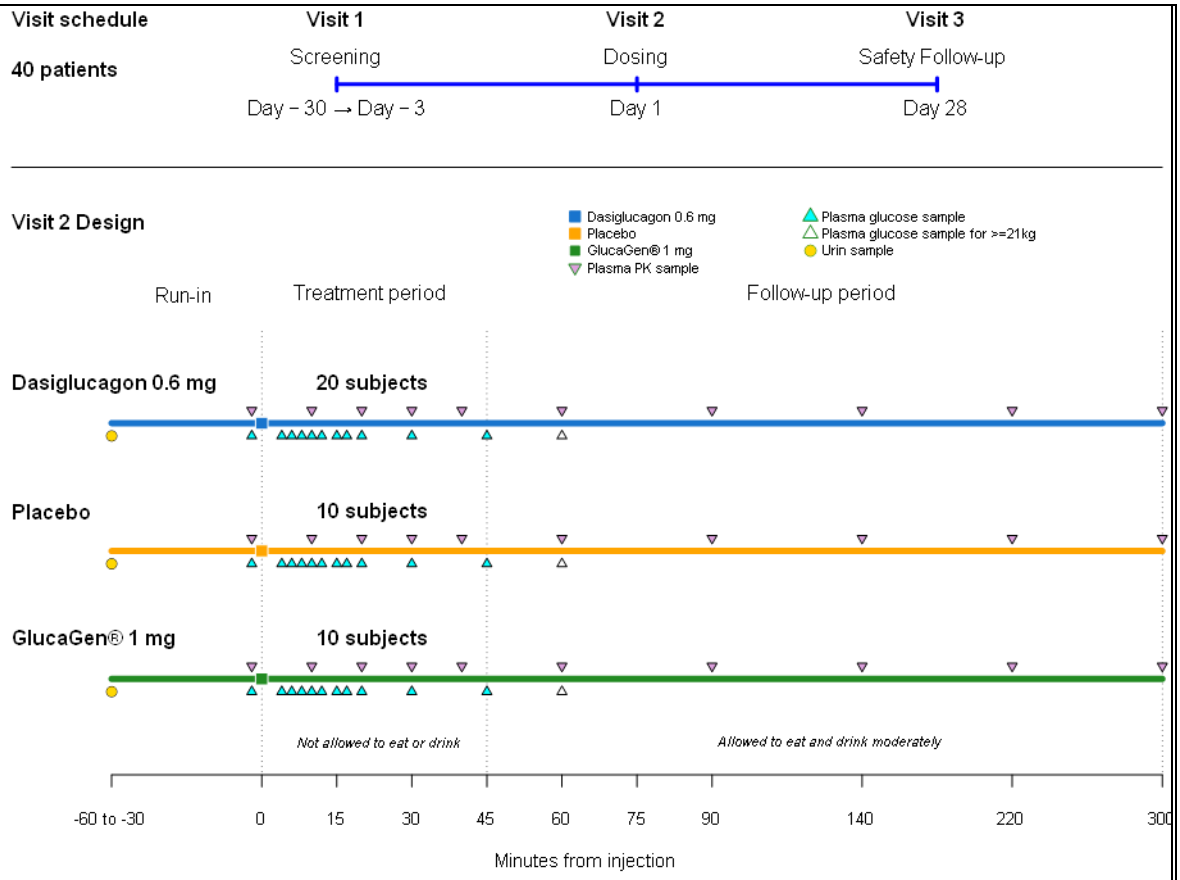
We, the undersigned, agree to conduct this trial according to the Trial Protocol.

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

Coordinating investigator	Prof. Dr. med. Thomas Danne	[redacted]
Statistician	[redacted] Senior Director Biometrics Synteract Deutschland GmbH Albrechtstr. 14 80636 Munich, Germany	[redacted]
Sponsor's representative	[redacted] Clinical Project Director Zealand Pharma A/S Smedeland 36 2600 Glostrup, Denmark	[redacted]
	Dr. [redacted] Medical Director Zealand Pharma A/S Smedeland 36 2600 Glostrup, Denmark	[redacted]

2. Trial synopsis

Title of the trial: A phase 3, randomized, double-blind, placebo- and active-controlled, parallel-arm trial to assess the efficacy, safety, and pharmacokinetics of dasiglucagon relative to placebo and GlucaGen® when administered as a rescue therapy for severe hypoglycemia in children with T1DM treated with insulin	
EudraCT number: 2018-000892-33	**Protocol codes:** Sponsor: ZP4207-17086 Synteract: ZEA-DNK-02170
Sponsor or sponsor's representative in the European Union: Zealand Pharma A/S, Smedeland 36, 2600 Glostrup (Copenhagen), Denmark	
Coordinating investigator: Prof. Dr. med. Thomas Danne, Kinder- und Jugendkrankenhaus AUF DER BULT, Janusz-Korczak-Allee 12, 30173 Hannover, Germany	
Trial center(s): 2-3 centers in the EU (Germany, Slovenia) and 1-2 centers in the USA	
Planned trial period: First Patient First Visit: September 2018 Last Patient Last Visit: Second quarter 2019	**Phase of development:** Phase 3
Objectives: The primary objective is to demonstrate that dasiglucagon is superior to placebo following a single injection of 0.6 mg of dasiglucagon in treating hypoglycemia in children with type 1 diabetes mellitus (T1DM). Secondary objectives are: • To confirm that a single dose of dasiglucagon [0.6 mg] is comparable to a single dose of GlucaGen® [1 mg/mL] in treating hypoglycemia in children with T1DM, (in accordance with the label, children below 25 kg body weight will be administered 0.5 mg GlucaGen®), • To assess safety profile of dasiglucagon in children with T1DM, • To assess pharmacokinetic (PK) profile of dasiglucagon in children with T1DM.	
Trial design: This is a phase 3, randomized, double-blind, placebo- and active-controlled, parallel-arm trial designed to assess efficacy, safety and PK of dasiglucagon vs. placebo and vs. GlucaGen® in children with diabetes mellitus type 1 (T1DM). Patients will receive a single subcutaneous injection of the investigational product during a hypoglycemic clamp procedure. Handling, preparation and administration of trial product will be done by unblinded trial personnel. All trial assessments will be done by blinded trial personnel. The trial design is illustrated in the figure below.	



Abbreviation: PK=pharmacokinetic

Planned number of patients:

At least 40 children ≥6 years and <18 years of age with T1DM will be randomized into the trial (2:1:1 dasiglucagon/placebo/GlucaGen®) and stratified by age intervals: 6 years to <12 years, and 12 years to <18 years and by injection site (abdomen/thigh). A minimum of 16 patients will be enrolled into each age group, including a minimum of 8 patients in either of age groups on dasiglucagon treatment arm.

Patients who are dosed will not be replaced.

Medical condition or disease under investigation:

This is the first dedicated efficacy and safety trial in children; thus, the target population for this trial is children with T1DM ≥6 to <18 years of age. Dasiglucagon is in development for treatment of severe hypoglycemia.

Inclusion criteria:

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

1. Following receipt of verbal and written information about the trial, patient, parent(s) or guardian(s) of the patient must provide signed informed consent before any trial-related activity is carried out*
2. Female or male patients with T1DM for at least 1 year, diagnostic criteria as defined by the American Diabetes Association, and receiving daily insulin and in good and stable medical condition
3. At least 6.0 years of age (inclusive) and less than 18.0 years
   Germany only: A “staggered approach” will be applied, whereby a positive safety assessment needs to be available for at least 10 patients who have completed the dosing visit in the overall trial before younger patients (6 to 11 years of age) may be enrolled.
4. Body weight ≥20 kg
5. Female patients must meet one of the following criteria:

a. Participant is of childbearing potential and agrees to use one of the accepted contraceptive regimens throughout the entire duration of the trial from screening until last follow-up visit. An acceptable method of contraception includes at least one of the following:
   i. Abstinence from heterosexual intercourse
   ii. Systemic contraceptives (birth control pills, injectable/implant/ insertable hormonal birth control products, transdermal patch); if the participant is using systemic contraceptives, she must use an additional form of acceptable contraception (iii or iv, below)
   iii. Intrauterine device (with and without hormones)
   iv. Condom with spermicide

or

b. Participant is of non-childbearing potential due to pre-puberty status or a medical condition confirmed by the investigator

6. Male patients must meet the following criteria: If sexually active, uses condom and partner practices contraception during the trial from screening and until last follow-up visit
7. Willingness to adhere to the protocol requirements

* Informed consent signatures must be obtained according to local regulations.

Exclusion criteria:

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

1. Females who are pregnant according to a positive urine pregnancy test, actively attempting to get pregnant, or are lactating
2. Known or suspected allergy to trial product(s) or related products
3. History of anaphylaxis or symptoms of severe systemic allergy (such as angioedema)
4. Previous randomization in this trial
5. History of an episode of severe hypoglycemia that required a third party assistance within a month prior to screening visit
6. History of hypoglycemic events associated with seizures or hypoglycemia unawareness in the last year prior to screening
7. History of epilepsy or seizure disorder
8. Receipt of any investigational drug within 3 months prior to screening
9. Active malignancy within the last 5 years
10. Congestive heart failure, New York Heart Association class II-IV
11. Current bleeding disorder, including anti-coagulant treatment
12. Known presence or history of pheochromocytoma (i.e. adrenal gland tumor) or insulinoma (i.e. insulin secreting pancreas tumor)
13. Use of a daily systemic beta-blocker drug, indomethacin, warfarin or anticholinergic drugs in the previous 28 days before Day 1 of this trial
14. 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, or altered electrolyte values of clinical relevance for cardiac conduction, as judged by the investigator
15. Clinically significant abnormal electrocardiogram (ECG) at screening as judged by the investigator
16. Clinically significant illness within 4 weeks before screening as judged by the investigator
17. Surgery or trauma with significant blood loss within the last 2 months prior to screening
18. Patients 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
19. Any condition interfering with trial participation or evaluation or that could be hazardous to the patient
20. The use of prescription or non-prescription medications known to cause QT prolongation

In addition, the following exclusion criteria at clinic admission on Visit 2, day 0 apply at the time of admission to the clinic, which is the day before clamp procedure:

Patients who meet one or more of the following exclusion criteria at the time of admission to the clinic will be excluded from the dosing visit, however, the visit can be rescheduled 1-7 days later. The dosing visit can only be rescheduled once.

1. Atypically strenuous exercise within 4 days prior to dosing, as judged by the investigator. Exercise during the trial should follow patient's typical routine, and should not exceed a near maximum intensity for more than 20 minutes per day, or moderate intensity for more than 90 minutes per day
2. Clinically significant illness within 4 weeks before dosing, as judged by the investigator
3. Consumption of alcohol within 24 hours prior to dosing visit, determined by positive results from an alcohol breath test
4. Not fasting from 22:00 hours the evening prior to dosing, apart from water.
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 72 hours prior to dosing; or use of other long-acting insulins (e.g. insulin glargine U100 or insulin detemir) within 48 hours prior to dosing; or use of neutral protamine Hagedorn insulin NPH within 22 hours prior to dosing
7. Use of any short acting (bolus) insulin within 12 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 investigatorPlasma 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 admission to the clinic.

Test product, dose and mode of administration:
Dasiglucagon, liquid formulation, 1 mg/mL, 0.6 mL

Reference product, dose and mode of administration:
Placebo for dasiglucagon (hereafter referred to as placebo): Placebo, liquid formulation, 0.6 mL
Active control: Recombinant glucagon hydrochloride, 1 mg lyophilized powder for reconstitution (GlucaGen®, Novo Nordisk) in 1 mL sterile water (hereafter referred to as GlucaGen®). In accordance with the label, children below 25 kg body weight will be administered 0.5 mg GlucaGen®

Duration of treatment:
Patients will receive a single subcutaneous dose of investigational product. Patients will be subjected to a hypoglycemic clamp procedure and have to be within a plasma glucose target range of 54-80 mg/dL at dosing. The total individual trial duration will be a maximum of 63 days (up to 30 days screening, 1 dosing, 28 [+5] days of follow-up).

Criteria for evaluation:

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.

Secondary endpoints

Secondary efficacy endpoints:

- Plasma glucose recovery within 30 minutes, within 20 minutes, within 15 minutes, and within 10 minutes after trial product injection without administration of IV glucose

- Plasma glucose changes from baseline within 30 minutes, within 20 minutes, within 15 minutes, and within 10 minutes after trial product injection or at the time of rescue IV glucose

Safety endpoints:

- Adverse events (AEs)
- Clinical laboratory assessments (biochemistry, hematology, coagulation, urinalysis)
- Vital signs
- Physical examination
- Clinically significant changes in the electrocardiogram
- Local tolerability
- Administration of IV glucose infusion during the hypoglycemic clamp procedure
- Time to first IV glucose infusion, after trial product administration. (N.B. IV glucose infusion prior to trial product administration should not be included, as it is part of hypoglycemic clamp procedure)
- Immunogenicity endpoint: occurrence of anti-drug antibodies

Pharmacokinetic endpoints

PK endpoints will be derived from plasma dasiglucagon and GlucaGen® profiles from 0 to 300 minutes:

- Area under the plasma dasiglucagon or GlucaGen® concentration versus time curve from 0 to 30 minutes post-dose ($AUC_{0\text{-}30min}$)
- Area under the plasma dasiglucagon or GlucaGen® concentration versus time curve from 0 to 300 minutes post-dose ($AUC_{0\text{-}300min}$)
- Area under the plasma dasiglucagon or GlucaGen® concentration versus time curve from 0 to infinitely post-dose ($AUC_{0\text{-}inf}$)
- Maximum of all valid plasma dasiglucagon or GlucaGen® concentration measurements from 0 to 300 minutes post-dose (C_{max})
- Time to maximum of plasma dasiglucagon or GlucaGen® concentration measurements (t_{max})
- Terminal elimination rate constant of plasma dasiglucagon or GlucaGen® (λ_z)
- Terminal plasma elimination half-life of dasiglucagon or GlucaGen® ($t_{½}$)
- Total body clearance of plasma dasiglucagon or GlucaGen® (CL/f)
- Volume of distribution of plasma dasiglucagon or GlucaGen® (V_z/f)
- Mean residence time of plasma dasiglucagon or GlucaGen® (MRT)

Pharmacodynamic endpoint

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

Statistical methods:

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

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

Other summaries (e.g. quartiles, 95% confidence intervals) may be used as appropriate. Categorical efficacy and safety variables will be summarized by counts and by percentage of patients in corresponding categories. The primary endpoint, time to recovery, will be summarized using Kaplan-Meier estimates for each treatment group in total and stratified by age intervals and injection site. The median time to recovery with 95% confidence interval will be estimated by treatment group.

Log-rank tests will be applied to compare the 2 treatment groups to the placebo group.

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

All safety data will be analyzed with descriptive methods.

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

Plasma dasiglucagon and glucagon concentrations will be described and PK metrics determined.

$AUE_{0\text{-}30min}$ will be summarized with descriptive statistics.

Sample size calculation:
The primary comparison is between the dasiglucagon and placebo treatment arms. From phase 2 results, the median time to an increase of 20 mg/dL of the 0.6 mg dose was approximately 10 minutes. For placebo-treated patients, the median time to recovery is assumed to be more than 50 minutes. Assuming an exponential time-to-recovery distribution with median times of 10 and 50 minutes, a 2-sided log-rank test will be able to detect a difference between dasiglucagon 0.6 mg and placebo with 90% power with a follow-up time of 45 minutes at a 5% significance level with 20 patients randomized to the dasiglucagon arm and 10 patients to placebo. GlucaGen® is included as a reference to compare the responses and AE profile to dasiglucagon with those to a marketed product. It is expected that 10 patients in the GlucaGen® group will suffice for the comparison.

Log-rank tests will be applied to compare the 2 treatment groups to the placebo group.

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

All safety data will be analyzed with descriptive methods.

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

Plasma dasiglucagon and glucagon concentrations will be described and PK metrics determined.

$AUE_{0\text{-}30min}$ will be summarized with descriptive statistics.

Sample size calculation:
The primary comparison is between the dasiglucagon and placebo treatment arms. From phase 2 results, the median time to an increase of 20 mg/dL of the 0.6 mg dose was approximately 10 minutes. For placebo-treated patients, the median time to recovery is assumed to be more than 50 minutes. Assuming an exponential time-to-recovery distribution with median times of 10 and 50 minutes, a 2-sided log-rank test will be able to detect a difference between dasiglucagon 0.6 mg and placebo with 90% power with a follow-up time of 45 minutes at a 5% significance level with 20 patients randomized to the dasiglucagon arm and 10 patients to placebo. GlucaGen® is included as a reference to compare the responses and AE profile to dasiglucagon with those to a marketed product. It is expected that 10 patients in the GlucaGen® group will suffice for the comparison.

Table 2-1: Schedule of Assessments

Visit number	V1	V2	V3	Vx
Trial day	-3	0 and 1	28	Unscheduled[1]
Visit type	Screening	Dosing	Follow-up	Unscheduled[1]
Window	-30 to -3	–	+5 days	Unscheduled[1]
Patient related information/assessments				
Informed consent	x	–	–	–
Inclusion/exclusion criteria	x	x[2,3]	–	–
Demography	x	–	–	–
Body measurements	x	–	–	–
Diabetes diagnosis and current diabetes treatment	x	x	x	x
Medical history including concomitant illnesses	x	–	–	–
Concomitant medications	x	x[2]	x	–
Randomization	–	x[2]	–	–
Exclusion criteria at clinic admission on Visit 2, day 0	–	x[2]	–	–
Insulin-induced hypoglycemia	–	x	–	–
Safety assessments				
Physical examination	x	x	x	x
Vital signs	x	x[4]	x	x
12-lead ECG	x	x[5]	x	–
Local tolerability	–	x[6]	x	–
Adverse events	x	x	x	x
Laboratory				
Biochemistry, hematology, coagulation, HbA1c (HbA1c at Visit 1 only)	x	–	x	–
Pregnancy test (women of childbearing potential only)	x[7]	x[2,8]	x[8]	–
Urinalysis	x	x[2]	x	–
Alcohol breath test	–	x[2]	–	–
PK/Clinical efficacy				
Plasma dasiglucagon/GlucaGen®	–	x[9]	–	–
Plasma glucose	–	x[10]	–	–
Other assessments				

Visit number	V1	V2	V3	Vx
Trial day	-3	0 and 1	28	Unscheduled[1]
Visit type	Screening	Dosing	Follow-up	Unscheduled[1]
Window	-30 to -3	–	+5 days	Unscheduled[1]
Antibodies against dasiglucagon/GlucaGen®	x	–	x[11]	x
Trial material				
Administration of trial product (during hypoglycemic clamp procedure)	–	x	–	–
End of trial status	–	–	x	–

Abbreviations: ADA=Anti-drug antibody; ECG=Electrocardiogram, HbA1c=Hemoglobin A1c, PK=Pharmacokinetics

[1]For ADA-positive patients only.
[2]Prior to the start of the insulin-induced hypoglycemic procedure.
[3]Only check exclusion criteria at clinic admission on Visit 2, day 0 and changes between screening visit and Visit 2.
[4]Prior to the start of the insulin-induced hypoglycemic procedure (within 30 minutes), and at 30, 60 and 300 minutes after dosing. The actual time of the assessment should not deviate from the nominal time by more than ±10 minutes.
[5]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.
[6]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.
[7]Serum pregnancy test.
[8]Urine stick pregnancy test.
[9]Pre-dose, and at 10, 20, 30, 40, 60, 90, 140, 220, and 300 minutes after dosing. The actual time of blood sampling should not deviate from the nominal time by more than ±1 minute between t=5 minutes and t=90 minutes, ±5 minutes between t=150 minutes and t=300 minutes.
Pre-dose is defined as within 2 minutes prior to dosing.
[10]Pre-dose, and at 4, 6, 8, 10, 12, 15, 17, 20, 30 and 45 minutes (as well as 60 minutes if the patient´s body weight is ≥21 kg) after dosing. The actual time of blood sampling should not deviate from the nominal time by more than ±30 seconds until the 20-minute collection time point, and by more than ±1 minute for the subsequent collection time points. Pre-dose is defined as within 2 minutes prior to dosing.
[11]Antibodies against dasiglucagon/GlucaGen® (any treatment-induced or treatment-boosted [titer increase above 5 fold] ADA-positive patients will be monitored until the ADA levels return to baseline levels).

3. Table of contents

Appendices:

(1) List of names and addresses

Index of in-text tables

Index of in-text figures

4. List of abbreviations and definition of terms

4.1 Abbreviations

ADA	anti-drug antibody
AE	adverse event
AESI	adverse event of special interest
ALT	alanine aminotransferase
AST	aspartate aminotransferase
CA	competent authority
CDISC	Clinical Data Interchange Standards Consortium
CFR	Code of Federal Regulations
CRO	contract research organization
ECG	electrocardiogram
eCRF	electronic case report form
EDC	electronic data capture
FAS	full analysis set
GCP	Good Clinical Practice
ICH	International Council for Harmonisation
IEC	independent ethics committee
IMP	investigational medicinal product
IRB	institutional review board
IV	intravenous(ly)
IWRS	interactive web response system
MRT	mean residence time
NPH	neutral protamine Hagedorn
ODM	OpenDocument Master
PD	pharmacodynamic(s)
PK	pharmacokinetic(s)
PPS	per protocol set
PR	PR interval; i.e. the measure of the time between the start of the p wave and the end of the r wave in the heart's electrical cycle
PT	preferred term
QRS	QRS interval; i.e. the measure of the time between the start of the q wave and the end of the s wave in the heart's electrical cycle
QT	QT interval; i.e. the measure of the time between the start of the q wave and the end of the t wave in the heart's electrical cycle
SAE	serious adverse event
SAS	safety analysis set

SC	subcutaneous(ly)
SOC	system organ class
SUSAR	suspected unexpected serious adverse reaction
T1DM	type 1 diabetes mellitus
ULN	upper limit of normal

Plasma concentrations of dasiglucagon/GlucaGen

$AUC_{0\text{-}30min}$	Area under the plasma concentration versus time curve from 0 to 30 minutes post-dose
$AUC_{0\text{-}300min}$	Area under the plasma concentration versus time curve from 0 to 300 minutes post-dose
$AUC_{0\text{-}inf}$	Area under the plasma concentration versus time curve from 0 to infinitely post-dose
C_{max}	Maximum of all valid plasma concentration measurements from 0 to 300 minutes post-dose
t_{max}	Time to maximum of plasma concentration measurements
λ_z	Terminal elimination rate constant
$t_{½}$	Terminal plasma elimination half-life
CL/f	Total body clearance
V_z/f	Volume of distribution
MRT	Mean residence time

Plasma glucose concentrations

AUE0-30min	Plasma glucose response as area under the effect curve above baseline from time 0 to 30 minutes

4.2 Definitions of terms

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

5. Introduction

5.1 Background of the trial

Hypoglycemia

Hypoglycemia in patients with diabetes is defined as episodes of an abnormally low plasma glucose concentration.[9] This is a common, unpredictable, and potentially dangerous side effect of treatment of diabetes mellitus with especially insulin or sulfonylureas. It is more frequent in patients with profound endogenous insulin deficiency, such as occurs in type 1 diabetes mellitus (T1DM) and advanced type 2 diabetes mellitus. Treatment of type 2 diabetes mellitus with insulin causes hypoglycemia progressively and more frequently over time, whereas in T1DM, hypoglycemia is experienced throughout the course of established disease.

Symptoms and signs of hypoglycemia are not specific. Patients undergoing a hypoglycemic episode experience unpleasant symptoms such as anxiety, sweating, hunger, tremors, palpitations, paresthesia, nausea and pallor. Depending on its severity, the hypoglycemia may lead to mild confusion, behavioral changes, loss of consciousness, seizures, coma, and death.[1]

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

Glucagon

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

Insulin decreases blood glucose levels and cases of hypoglycemia can be reversed by glucagon. Therefore, glucagon is indicated for the treatment of severe hypoglycemia.

Dasiglucagon

Dasiglucagon (ZP4207) is a stable peptide analog of human glucagon, available in a ready-to-use liquid formulation and is in development for the treatment of severe hypoglycemia in insulin dependent patients with diabetes mellitus. Dasiglucagon is a specific and full glucagon receptor agonist designed to mimic the effects of glucagon, having a fast absorption and elimination (minutes). It is a peptide of 29 amino acids, with 7 amino acid substitutions compared to native glucagon. The main purpose of the substitutions is to increase the physical and chemical stability of the glucagon analog compared to marketed glucagon products such as Lilly´s Glucagon or GlucaGen®. Dasiglucagon exhibits improved physical and chemical stability and is available in an aqueous solution at neutral pH.[2]

Five clinical trials have been completed with dasiglucagon: A first human dose trial (ZP4207-14013), a multiple-dose dose-escalation trial (ZP4207-15007) to evaluate the safety, tolerability, pharmacokinetics (PK) and pharmacodynamics (PD) of dasiglucagon, a phase 2 crossover trial to assess the PK and PD of a single dose of an optimized formulation of dasiglucagon administered subcutaneously (SC) in patients with T1DM (ZP4207-15126), and a feasibility trial testing the bionic pancreas with dasiglucagon was completed (ZP4207-16051).[2] Finally, a cross-over trial (ZP4207-16098) assessing PK and PD responses after micro-doses of dasiglucagon administered SC to patients with type 1 diabetes mellitus under euglycemic and hypoglycemic conditions, compared to marketed glucagon, was completed.

Pharmacokinetics and pharmacodynamics of dasiglucagon

The results of the phase 1 and 2 clinical trials confirm 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 (C_{max}) was later for dasiglucagon than for GlucaGen® (35 versus 20 minutes). For C_{max}, the results indicated that 0.3 mg dasiglucagon was comparable to 0.5 mg GlucaGen® (90% confidence interval: 0.8167; 1.0068) and 0.6 mg dasiglucagon was comparable to 1.0 mg GlucaGen® (90% confidence interval: 0.8850; 1.1991).[2] At these dose levels, the total exposures ($AUC_{0\text{-}inf}$) were higher for dasiglucagon compared to GlucaGen®.

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

Safety of dasiglucagon

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

Immunogenicity of dasiglucagon

In adults, anti-drug antibodies (ADA) have been assessed in the completed clinical trials. In one of the 5 completed clinical trials (ZP4207-16098) 1 transient ADA incidence have been detected in one T1DM patient. The antibodies were able to bind to dasiglucagon and glucagon confirming the cross-reactivity potential found in the non-clinical toxicity studies (IND 135869 Section 2.4.4.6). The antibodies were also capable of neutralizing the in vitro activity of both dasiglucagon and glucagon, but could not be associated with any AEs or effects on PK or PD in the patient. The trial was a multiple dose trial and completers had received a total of 11 investigational drug administrations (8 injections of dasiglucagon and 3 injections of recombinant glucagon) (IND 135869 Section 2.5.6.3) and indicated that increased exposure will likely increase the risk of ADA formation. However, due to the crossover trial design, the induction of ADA cannot be decisively attributed to either dasiglucagon or glucagon treatment. The ADA incidence in that trial was 4.3% and considered low. From the long-term non-clinical toxicology studies, the detection of antibodies was not associated with a change in toxicity profile compared to ADA negative animals. In the 13- and 26-week rat studies, an increase in exposure was observed in dose groups ≥8 mg/kg/day, which correlates with the dose groups with the highest frequency of ADA-positive animals. So far, these findings have not been observed in humans.

The consequences of an immune response towards endogenous glucagon is not fully known. In a recent review summarizing the current physiology of glucagon and learnings from recent glucagon antagonist research, it was concluded that although glucagon is an important hormone for controlling postprandial glucose levels, it was not essential for the maintenance of fasting glucose and glucagon antagonism did not cause hypoglycemia.[5] Rather, the lack of glucagon signaling induced imbalance in amino acid metabolism leading to elevated amino acid plasma

levels. As excess levels of amino acids can be excreted by the kidneys and the detected ADA incidence have been transient, the impact is not considered a major risk for the trial population.

The overall immunogenicity risk of dasiglucagon in a clinical context is therefore considered low and the potential effects of induced ADAs judged to be of limited clinical consequence.

5.2 Trial rationale

The SC bolus dose of 0.6 mg dasiglucagon that is the most appropriate dosing to ensure rapid rescue from hypoglycemia in adults appears to be appropriate also in children 6 years old and above.

To simulate the potential PK/PD response in children, a previously developed population PK/PD model for dasiglucagon was updated with an allometric PK component derived from published glucagon pediatric data from weight groups of 25.4±5.2, 43.2±8.9, and 61.2±13.8 kg (mean±SD) and adults. The simulations suggested that a 0.3 mg SC bolus dose of dasiglucagon would result in a slightly slower increase of blood glucose compared to the 0.6 mg dose in the 3 pediatric body weight groups. Considering the intended clinical indication of treating patients for severe hypoglycemia where time to PD response is considered essential, the 0.3 mg dose appears less attractive than the 0.6 mg dose.

The higher total drug exposure (AUC and C_{max}) predicted in children at lower body weight, relative to that observed in adults at a dose of 0.6 mg, is within the tolerable exposure achieved in adults administered 2.0 mg dasiglucagon, and the duration of exposure is truncated due to a shorter $t_{½}$ in children. In addition, rapid saturation of the PD response results in similar total and maximum glucose response (AUE and CE_{max}) throughout the body weight range from 25 to 77 kg.

Based on all data generated to date across 4 clinical trials performed in adults receiving dasiglucagon at different dose levels, no clear dose-response for nausea and vomiting was observed above a certain threshold. It is therefore not expected that the intensity and frequency of nausea and vomiting at a dose of 0.6 mg will differ between adults and children/adolescents. This expectation is further consistent with results of the recently conducted trial ZP4207-16098 in adults with T1DM, which indicated a similar degree of nausea and vomiting at SC bolus doses of 0.2 and 0.6 mg dasiglucagon.

5.3 Assessment of anticipated benefits and risks

Glucagon emergency kits are often underutilized in the treatment of patients experiencing severe hypoglycemia. Considering the seriousness and potential complications that can arise from severe hypoglycemic episodes, it is essential that parents and caregivers receive adequate, hands on training for use of glucagon rescue kits to ensure safe, timely, and effective administration. Given the challenges with parent/caregiver training and use of current glucagon products, Zealand Pharma A/S is developing dasiglucagon as a ready to use rescue treatment for severe hypoglycemia. The development of dasiglucagon will provide parents and caregivers of children with diabetes mellitus a more efficient alternative for the treatment of severe hypoglycemia. The ready-to-use kit addresses an unmet need and may potentially prove to be life-saving. The trial may also provide a direct benefit to patients and their parent(s)/caregiver by allowing patients to experience signs and symptoms of hypoglycemia in a highly specialized treatment center where they will be under continuous supervision and medical monitoring. This will help the patient and their parent(s)/caregiver recognize the signs and symptoms of hypoglycemia and start treatment before the event becomes more serious.

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

Treatment with an investigational medicinal product may result in undesired effects or complaints. Undesired effects and complaints such as nausea and vomiting are known AEs occurring with glucagon administration. Similar AEs have also been observed to a limited degree in the 5 clinical

studies conducted with dasiglucagon. As with every novel drug substance, new and yet unknown side effects also may occur.

There are limited data available to assess the immunogenic potential of glucagon products available on the market, but the data available indicate that marketed glucagon only has a small immunogenic potential. Based on the 5 clinical studies completed with dasiglucagon to date (see Section 5.2), no anti-dasiglucagon or anti-glucagon antibodies have been detected, except for trial ZP4207-16098, in which there was one transient low titer ADA incidence showing reactivity towards both dasiglucagon and glucagon. The patient 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 patient was also 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 this trial, the induction of ADAs could not be associated with a specific treatment. 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 patient was negative for anti-dasiglucagon antibodies. There was no evidence for altered PK, PD, or safety profile for this patient.

Administration of dasiglucagon may be associated with a risk of allergic reactions similar to those observed for other therapeutic peptides or proteins. Patients with known or suspected allergies to the trial products 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 4 clinical trials conducted with dasiglucagon. Direct access to resuscitation equipment is ensured at the clinical trial centers.

The results of the trial may contribute to the future use of dasiglucagon in patients with diabetes mellitus experiencing severe hypoglycemic reactions.

Overall, the benefit to risk ratio for patients entering the ZP4207-17086 trial is considered acceptable. The trial design subjects the patients concerned to as little burden and other foreseeable risks as possible.

Germany only: With the exception of medical examinations, a patient participating in this trial is not likely to derive any personal health-related benefits. The results of the trial may contribute to the future use of dasiglucagon in patients with diabetes mellitus experiencing severe hypoglycemic reactions. The perceived risks and burden of the trial will be discussed with the patients and their parent(s)/guardian(s) prior to the trial, and it is therefore expected that a given patient will not be enrolled if there are concerns about the risks or burden of the trial. A patient can be withdrawn if the burden of trial procedures is considered unacceptable by the patient, their parent(s)/guardian(s) or the investigator; see Section 7.3.3.1. Therefore, the investigator will need to monitor the burden of the trial activities for each patient.

To minimize the burden for the pediatric participants, the trial will be conducted in highly specialized centers for clinical research studies in patients with T1DM having specific experience and expertise in the conduct of pediatric trials. In Germany, this trial will be conducted at the Diabetes Center for Children and Adolescents, Children's Hospital AUF DER BULT, Hannover under supervision of Professor Thomas Danne. All trial nurses, trial coordinators and sub-investigators are specially trained in conducting pediatric trials and in the management of diabetes in children. All patients that will be included in the trial are familiar with the site and staff. Following diagnosis of T1DM, they have all attended information meetings about their illness and training sessions about controlling their diabetes, measuring blood glucose levels, injecting insulin and treating hypoglycemia (based on a monitored provocation) at the trial site. This trial gives another opportunity to the patients and their parents to learn about hypoglycemia signs and symptoms in a well-controlled environment and to learn how to treat hypoglycemia in a real-life emergency situation.

Facilities at the site allow for overnight stays with 2 patients (and their parents) in a room. The patients will be allocated to rooms based on age and their relationships with other patients. Age-appropriate entertainment will be provided for all patients. The participating patients will be investigated with two being present in the same room as this has proven to distract the participants from the burden of the trial and allows for interaction with peers, which makes the experience much more pleasurable. As only otherwise healthy participants are eligible for inclusion in the trial, the risk of transmitting an infection from one participant to another is negligible. The participating patients will be separated from other in-patients at the hospital to ensure a minimum degree of risk to the trial patients from potential infections as well as any bias this may cause in the outcome of the trial.

The interaction between the trial staff and the other patients during the overnight stay helps reduce the stress/anxiety felt by the patients prior to the procedure. Parents and patients also have the opportunity to discuss day-to-day issues with other participants or staff. In addition, the staff can control the patients' blood glucose values during the night and ensure the patients are ready to participate in the trial. Following the procedures, the patients will remain under observation in their rooms for 5 hours. Age-appropriate entertainment will be provided for all patients during this period of observation, which is similar to that occurring after an event of hypoglycemia.

Currently, there is no plan to include any additional sites in Germany. Should any other sites be included, the sponsor will ensure that the same standards and qualifications are applied to these sites as well.

The Kinder- und Jugendkrankenhaus AUF DER BULT is an institution which is dedicated to the care of sick and handicapped children, and was founded for this purpose more than 150 years ago. The mission statement of the institution expressively states the importance of research for pediatric care with focus on creating an acceptable environment for children and their families. For example, the patient's age and personal preference is considered when appointments are made, as patients often know each other from the outpatient ward or diabetes camps and choose to do studies at the same time as others. Age-appropriate entertainment and leisure activities are available to reduce the stress of being treated as an in-patient and lying in a bed. The trial personnel are experienced in pediatric medical procedures. For example, all patients are offered a local anesthetic (EMLA®) to reduce the potential pain of venous punctures.

The patients will be examined prior to the trial visit to ensure that patients with health issues are identified in time and no health issues are overlooked, which could be aggravated through participation in the trial procedures.

The trial protocol has been adapted to be appropriate for children between 6 and 17 years of age. The trial patients will be exposed to minimal invasive procedures, which include insertion of two pediatric catheters for a clamp procedure and blood draw. To ensure a minimum of burden to the children, the number of catheters has been reduced compared with the adult trials. The site offers use of topical anesthetic as part of their standard of care when placing catheters. The use of catheters allows injection of insulin/glucose to quickly control blood glucose levels and blood samples can be drawn without the need for finger pricks or other sampling methods.

The number and volume of blood samples do not exceed what is required for a successful trial and do not exceed the limits permitted by Health Authorities. In accordance with guidance and directive of the European Medicines Agency (EMA), blood sampling should not exceed 1% of the total blood volume at each visit (i.e. approximately 18 mL for a patient with a body weight of 20 kg), and should not exceed 3% of the total blood volume during a period of four weeks (i.e. approximately 54 mL for a 20 kg patient).

Insulin induced-hypoglycemia is commonly used to assess the safety and efficacy of glucagon or similar products in this class. Compared with the trials conducted with dasiglucagon in adults, the children in the ZP4207-17086 trial will have a higher target plasma glucose concentration prior to treatment with dasiglucagon and a lower rate of insulin infusion. Both of these measures increase

the level of control and reduce the risk for plasma glucose concentrations below the expected range (54-80 mg/dL; 3.0-4.4 mmol/L) prior to treatment.

Patients will be in a controlled setting with access to immediate intervention, should this be required. If any child reacts differently than expected, rescue provisions based on the type of reaction and the timing relative to the clamp procedure are available (see Section 7.4.5.2.3).

With the close monitoring of patients at the clinical site, the considerable experience with the insulin induction technique at the clinical site and the pre-defined thresholds for administering IV glucose described in the protocol, the risk of the insulin induction technique to patients is considered to be minimal and well controlled.

The burden associated with nausea and vomiting is considered minimal (slight and temporary impairment of the health of the patient) and any discomfort is temporary. The trial was designed based on a large amount of data generated in adults, thereby limiting the number of pediatric patients needed to be dosed to provide adequate and conclusive data on the safety and efficacy of dasiglucagon in this age group. It is not possible to treat the patients prophylactically with anti-emetics, as this will bias the tolerability profiles of the treatments. However, treatment of any adverse events will be provided when it is needed. In order to reduce the effects of nausea, the children will be given a small meal 1 hour after the clamp procedure is completed.

The potential risks associated with allergic reactions are similar to those for other therapeutic peptides or proteins. Patients with known or suspected allergies to the trial products or related products will be excluded from the trial. No severe acute hypersensitivity reactions have been observed in the clinical trials conducted to date with dasiglucagon and the risk of such an event is assessed as low. However, direct access to resuscitation equipment is ensured at the clinical trial sites.

ADAs have been assessed in all completed clinical trials in adults (n=448). Only one transient ADA incidence has been detected in one patient with T1DM in one of the completed clinical trials (ZP4207-16098). The trial was a multiple-dose trial and completers had received a total of 11 investigational drug doses (8 injections of dasiglucagon and 3 injections of recombinant glucagon). The antibodies were able to bind to dasiglucagon and glucagon and were also capable of neutralizing the *in vitro* activity of both dasiglucagon and glucagon, but were not associated with any AEs or effects on PK or PD in the patient. Due to the cross-over trial design, the induction of ADA could not be decisively attributed to either dasiglucagon or glucagon treatment. Since the potential of dasiglucagon to induce ADAs at clinically relevant doses is considered to be low, the sponsor considers the risk of developing ADA in this trial as minimal. To ensure minimal burden on the trial population ADA assessment will only be performed at the screening and the end of trial visit.

In balancing the risk and benefit of the trial, it has to be taken into consideration that this age group is at highest risk of severe hypoglycemia due to unplanned physical activities and the eating behaviors frequently encountered in pediatric patients.

6. Trial objectives

Primary objective

- To demonstrate that dasiglucagon is superior to placebo following a single injection of 0.6 mg of dasiglucagon in treating hypoglycemia in children with T1DM

Secondary objectives

- To confirm that a single dose of dasiglucagon [0.6 mg] is comparable to a single dose of GlucaGen® [1 mg/mL] in treating hypoglycemia in children with T1DM (in accordance with the label, children below 25 kg body weight will be administered 0.5 mg GlucaGen®)
- To assess safety profile of dasiglucagon in children with T1DM
- To assess PK profile of dasiglucagon in children with T1DM

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

Secondary endpoints

Secondary efficacy endpoints

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

Safety endpoints

- AEs
- Clinical laboratory assessments (biochemistry, hematology, coagulation, urinalysis)
- Vital signs
- Physical examination
- Clinically significant changes in the electrocardiogram
- Local tolerability
- Administration of rescue IV glucose infusion after trial product injection
- Time to first IV glucose infusion, after trial product administration (N.B. IV glucose infusion prior to trial product administration should not be included, as it is part of hypoglycemic clamp procedure)
- Immunogenicity endpoint: occurrence of ADAs

Pharmacokinetic endpoints

PK endpoints will be derived from plasma dasiglucagon and GlucaGen® profiles from 0 to 300 minutes:

- Area under the plasma dasiglucagon or GlucaGen® concentration versus time curve from 0 to 30 minutes post-dose ($AUC_{0\text{-}30min}$)

- Area under the plasma dasiglucagon or GlucaGen® concentration versus time curve from 0 to 300 minutes post-dose ($AUC_{0\text{-}300min}$)
- Area under the plasma dasiglucagon or GlucaGen® concentration versus time curve from 0 to infinitely post-dose ($AUC_{0\text{-}inf}$)
- Maximum of all valid plasma dasiglucagon or GlucaGen® concentration measurements from 0 to 300 minutes post-dose (C_{max})
- Time to maximum of plasma dasiglucagon or GlucaGen® concentration measurements (t_{max})
- Terminal elimination rate constant of plasma dasiglucagon or GlucaGen® (λ_z)
- Terminal plasma elimination half-life of dasiglucagon or GlucaGen® ($t_{½}$)
- Total body clearance of plasma dasiglucagon or GlucaGen® (CL/f)
- Volume of distribution of plasma dasiglucagon or GlucaGen® (V_z/f)
- Mean residence time of plasma dasiglucagon or GlucaGen® (MRT)

Pharmacodynamics endpoint

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

7. Investigational plan

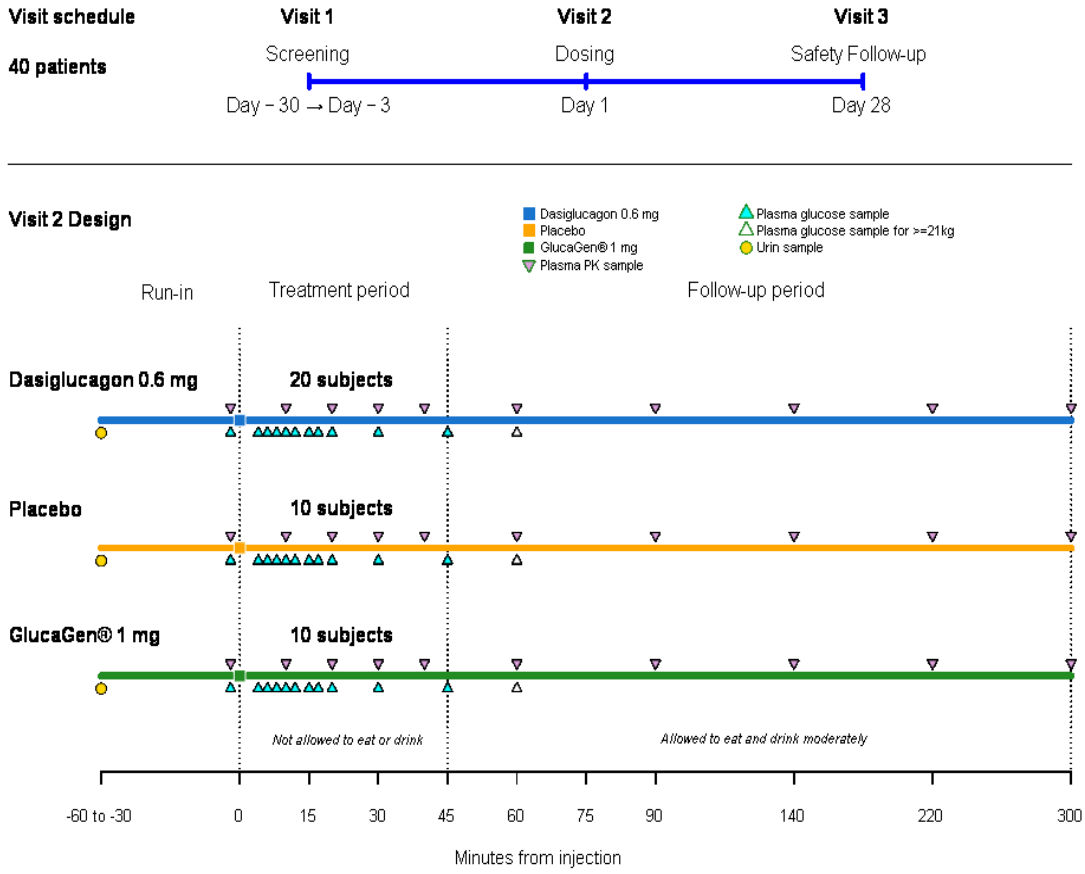
7.1 Overall trial design and plan

This is a phase 3, randomized, double-blind, placebo- and active-controlled, parallel-arm trial designed to assess efficacy, safety and PK of dasiglucagon vs. placebo and vs. GlucaGen® in children with T1DM. It is currently anticipated that it will be conducted at 2-3 centers in Europe and 1-2 centers in the USA.

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

- A screening period from Day -30 to Day -3
- A treatment period, from Day 0 to Day 1 (day of randomization and single dosing with trial product). Unblinded trial personnel will do the handling, preparation and administration of trial product. All trial assessments will be done by blinded trial personnel
- A follow-up visits at Day 28 (the end-of-trial visit)

Figure 7-1 Overview of the Trial Design



Abbreviation: PK=pharmacokinetic

The schedule of assessments (Table 2-1) gives an overview of the trial procedures. Patients should attend all visits on the designated day or as close to it as possible.

7.2 Discussion of trial design and choice of control groups

The trial will be randomized and double-blind to increase trial validity and to reduce bias during evaluation of assessments with the treatments. Since the 3 trial products are not identical in appearance, (dasiglucagon and placebo are liquid formulations and GlucaGen® is available as a powder for reconstitution), unblinded trial personnel, who will not be involved in other trial procedures and assessments, will do the handling, preparation and administration of trial product. Blinded trial personnel will do all trial assessments performed at the trial center.

Children with T1DM will be randomized 2:1:1 in order to evaluate the efficacy and safety of dasiglucagon compared to placebo and GlucaGen® and PK/PD parameters. The randomized, double-blind, parallel arm design with administration of a single dose of randomized trial product (dasiglucagon, placebo or GlucaGen®) will allow a relative comparison between the 3 treatment arms.

Dasiglucagon will be administered at fixed doses independent of body weight because this is the intended therapeutic dosing regimen in the emergency treatment of hypoglycemia. The selected dose of 1 mg GlucaGen® is the recommended dose for treatment of severe hypoglycemia (in accordance with the label, children below 25 kg body weight will be administered 0.5 mg GlucaGen®). Based on pre-clinical and clinical studies in adult patients, it has been demonstrated that 0.6 mg of dasiglucagon results in an initial PD response (i.e. acute glucose mobilization) comparable to 1 mg GlucaGen® (see also Section 5.1).

7.3 Selection of trial population

Dasiglucagon is indicated for treatment of severe hypoglycemia in patients with T1DM. As this condition also affects children and there is a therapeutic need for them to get access to safe and efficacious emergency treatment, the present trial aims to evaluate the efficacy and safety of a single dose of SC dasiglucagon compared to placebo and GlucaGen® in children with T1DM in experimentally induced hypoglycemia in a hypoglycemic clamp procedure.

The trial will enroll patients in centers in the EU and in the USA.

7.3.1 Inclusion criteria

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

1. Following receipt of verbal and written information about the trial, patient, parent(s) or guardian(s) of the patient must provide signed informed consent before any trial-related activity is carried out*
2. Female or male patients with T1DM for at least 1 year, diagnostic criteria as defined by the American Diabetes Association, and receiving daily insulin and in good and stable medical condition
3. At least 6.0 years of age (inclusive) and less than 18.0 years
   Germany only: A “staggered approach” will be applied, whereby a positive safety assessment needs to be available for at least 10 patients who have completed the dosing visit in the overall trial before younger patients (6 to 11 years of age) may be enrolled.
4. Body weight ≥20 kg
5. Female patients must meet one of the following criteria:
   a. Participant is of childbearing potential and agrees to use one of the accepted contraceptive regimens throughout the entire duration of the trial from screening until last follow-up visit. An acceptable method of contraception includes at least one of the following:
      i. Abstinence from heterosexual intercourse
      ii. Systemic contraceptives (birth control pills, injectable/implant/ insertable hormonal birth control products, transdermal patch); if the participant is using

systemic contraceptives, she must use an additional form of acceptable contraception (iii or iv, below)

iii. Intrauterine device (with and without hormones)

iv. Condom with spermicide

or

b. Participant is of non-childbearing potential due to pre-puberty status or a medical condition confirmed by the investigator

6. Male patients must meet the following criteria: If sexually active, uses condom and partner practices contraception during the trial from screening and until last follow-up visit
7. Willingness to adhere to the protocol requirements

* Informed consent signatures must be obtained according to local regulations.

7.3.2 Exclusion criteria

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

1. Females who are pregnant according to a positive urine pregnancy test, actively attempting to get pregnant, or are lactating
2. Known or suspected allergy to trial product(s) or related products
3. History of anaphylaxis or symptoms of severe systemic allergy (such as angioedema)
4. Previous randomization in this trial
5. History of an episode of severe hypoglycemia that required a third party assistance within a month prior to screening visit
6. History of hypoglycemic events associated with seizures or hypoglycemia unawareness in the last year prior to screening
7. History of epilepsy or seizure disorder
8. Receipt of any investigational drug within 3 months prior to screening
9. Active malignancy within the last 5 years
10. Congestive heart failure, New York Heart Association class II-IV
11. Current bleeding disorder, including anti-coagulant treatment
12. Known presence or history of pheochromocytoma (i.e. adrenal gland tumor) or insulinoma (i.e. insulin secreting pancreas tumor)
13. Use of a daily systemic beta-blocker drug, indomethacin, warfarin or anticholinergic drugs in the previous 28 days before Day 1 of this trial
14. 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, or altered electrolyte values of clinical relevance for cardiac conduction, as judged by the investigator
15. Clinically significant abnormal electrocardiogram (ECG) at screening as judged by the investigator
16. Clinically significant illness within 4 weeks before screening, as judged by the investigator
17. Surgery or trauma with significant blood loss within the last 2 months prior to screening
18. Patients 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
19. Any condition interfering with trial participation or evaluation or that could be hazardous to the patient
20. The use of prescription or non-prescription medications known to cause QT prolongation

In addition, the following exclusion criteria at clinic admission on Visit 2, day 0 apply at the time of admission to the clinic, which is the day before clamp procedure:

Patients who meet one or more of the following exclusion criteria at the time of admission to the clinic will be excluded from the dosing visit, however, the visit can be rescheduled 1-7 days later. The dosing visit can only be rescheduled once.

1. Atypically strenuous exercise within 4 days prior to dosing, as judged by the investigator. Exercise during the trial should follow patient's typical routine, and should not exceed a near maximum intensity for more than 20 minutes per day, or moderate intensity for more than 90 minutes per day
2. Clinically significant illness within 4 weeks before dosing, as judged by the investigator
3. Consumption of alcohol within 24 hours prior to dosing visit, determined by positive results from an alcohol breath test
4. Not fasting from 22:00 hours the evening prior to dosing, apart from water.
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 72 hours prior to dosing; or use of other long-acting insulins (e.g. insulin glargine U100 or insulin detemir) within 48 hours prior to dosing; or use of neutral protamine Hagedorn (NPH) insulin within 22 hours prior to dosing
7. Use of any short acting (bolus) insulin within 12 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 admission to the clinic

7.3.3 Premature withdrawal from trial

Participation in the trial is strictly voluntary. A patient has the right to withdraw from the trial at any time and for any reason. If the child chooses to withdraw or his/her parents or legal guardians choose to have the child withdrawn, the investigator must be informed immediately. The investigator has the right to terminate participation of any patient at any time if the investigator deems it in the patient's best interest. The reason and circumstances for withdrawal will be documented in the electronic case report form (eCRF).

7.3.3.1 Possible reasons for patient withdrawal

A patient will be withdrawn if the following applies:

- If a protocol deviation occurs which, in the clinical judgment of the investigator, can invalidate the trial endpoints, the patient will be withdrawn by the investigator
- AEs that are considered unacceptable by the patient or the investigator
- If the burden of study procedures is considered unacceptable by the patient, their parent(s) or the investigator

If withdrawal occurs following administration of any trial product, the patient will be asked to return and participate in the complete follow-up visit at trial Day 28. In this case, withdrawal relates only to blood sampling but not to the safety assessments. Patients should be followed for AEs for the same duration as those who are not withdrawn.

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

If the patient meets any of the exclusion criteria 1-9 at the time of admission to the clinic at V2, he/she will be excluded from the dosing visit but may be rescheduled once 1-7 days later.

7.3.3.2 Center discontinuation

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

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

However, even in case of center discontinuation the center should be kept open as long as previously enrolled patients are ongoing. Patients already enrolled should be followed and documented for their entire individual trial duration before actually discontinuing the center.

7.3.3.3 Trial termination

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

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

7.3.4 Replacement of patients

Patients who are dosed will not be replaced.

7.4 Investigational medicinal product(s)

7.4.1 Identity of investigational medicinal product(s)

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

Table 7-1: Identity of Investigational Products

	Test product	Placebo	Reference product
Name	Dasiglucagon	Placebo	GlucaGen®
Active substance	ZP4207	Not applicable	Recombinant glucagon hydrochloride
Formulation	Liquid formulation, 0.6 mL	Liquid formulation, 0.6 mL	Powder and solvent for reconstitution as 1 mL solution for injection
Strength	1 mg/mL	Not applicable	1 mg/mL
Container	Single use pre-filled syringe	Single use pre-filled syringe	Powder and solvent for reconstitution packed together in a plastic box. A “hypo-kit”
Manufacturer	Zealand Pharma A/S, Glostrup (Copenhagen), Denmark	Zealand Pharma A/S, Glostrup (Copenhagen), Denmark	Novo Nordisk A/S, Bagsværd, Denmark
Storage requirements	Store between 2 and 8°C	Store between 2 and 8°C	Store between 2 and 8°C

7.4.2 Treatments administered

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

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

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

7.4.3 Packaging and labelling

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

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

Labelling will be performed according to Annex 13 of the Good Manufacturing Practice guidelines of the European Commission, International Council for Harmonisation (ICH) guidelines on Good Clinical Practice (GCP), and local law.

7.4.4 Storage of trial product

The investigator must ensure the availability of proper storage conditions. All trial product supplies provided for this trial will be stored in the fridge, in a secure area with restricted access at the trial center. The trial products ready for use should be separated from any other clinical supplies, to avoid product 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 unblinded person responsible for trial product handling must contact the unblinded monitor in case of temperature deviations outside the acceptable range.

Please see the trial materials manual for additional information on handling trial product.

7.4.5 Investigational treatment and dosing conditions

7.4.5.1 Changes to diabetes management prior to dosing visit

At the screening visit, patients will be instructed by the investigator about the changes in their diabetes treatment leading up to and immediately prior to the dosing visits. There should not be any changes in their diabetes management until 3 days (72 hours) prior to the planned trial product administration except to switch to NPH insulin. The investigator will provide individual dosing instructions for this change. Within 72 hours prior to dosing, the use of insulin degludec or insulin glargine U300 is prohibited. Within 48 hours prior to dosing, the use of long-acting insulin (e.g. insulin glargine U100 or insulin detemir) is prohibited. Within 22 hours prior to dosing, the use of insulin NPH is prohibited. Within 12 hours prior to dosing, the use of any short acting (bolus) insulin, except insulin glulisine (Apidra®) is prohibited. During the insulin-induced hypoglycemic procedure, continuous SC insulin infusion must be stopped.

7.4.5.2 Dosing visit

Patients will be admitted to the clinic with their parents in the evening of the day before dosing (Day 0). Their eligibility for the trial will be checked, and only those still eligible will continue and remain in the clinic overnight.

If the patient meets any of the exclusion criteria at clinic admission on Visit 2, day 0, he/she will be excluded from the dosing visit but may be rescheduled once 1-7 days later.

An infusion catheter will be inserted into each arm for the manual glucose clamp procedure, with the glucose infusion in one arm and the insulin infusion in the opposite arm preferably, but the same infusion catheter can also be used. Another IV catheter, separated from infusion catheter(s), for blood sampling will be placed in the morning before trial product administration. The hand of this arm will be warmed (55-65 ºC) to arterialize venous blood. If there are issues with blood sampling from e.g. the metacarpal vein for the purpose of glucose measurements, the investigator may use a new and more proximal IV access.

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. They may consume water ad libitum. The patients must not consume any alcohol or smoke within 24 hours prior to dosing.

7.4.5.2.1 Diabetes management

Last injection or bolus administration via continuous SC insulin infusion of any insulin medication should not take place within the 12 hours before dosing. Patients normally using insulin aspart will be switched to human soluble insulin or glulisine for the period between 12 and 10 hours prior to dosing, if needed. Patients using continuous SC insulin infusion will have their pump switched off at 22:00 hours.

To achieve a target glucose level of 90-160 mg/dL (5.0-8.9 mmol/L) in the morning of dosing, patients may be administered insulin glulisine and/or glucose at the discretion of the investigator by IV infusion using the following glucose infusion rate and insulin infusion rate as a guidance. Deviation from the guidance is allowed at the discretion of the investigator. Plasma glucose levels will be checked overnight at regular intervals in order to achieve and maintain the target plasma glucose level of 90-160 mg/dL (5.0-8.9 mmol/L).

A general guidance for administration of insulin IV and/or glucose IV at the dosing visit

Fluid infusion rate:

If plasma glucose < 300 mg/dL, then infusion of isotonic solution with 5% glucose:

Age 6–10 years: 80 mL/kg/24 h

Age >10 years: 60 mL/kg/24 h

If plasma glucose >300 mg/dL the glucose infusion will be substituted with fluid volume of normal saline (0.9% NaCl) at same infusion rate as above.

Infusion of insulin:

Insulin infusions should be performed with 0.5 IU insulin glulisine (Apidra®) per kg body weight in 48 mL NaCl 0.9% according to the following recommendations:

Glucose	Infusion rate	Insulin dose (IU/kg BW/h)
>200 mg/dL	10.0 mL/h	0.1
150–200 mg/dL	5.0 mL/h	0.05
80-150 mg/dL	2.5 mL/h	0.025
<80 mg/dL	Insulin infusion stop!	0

Abbreviation: BW=Body weight

7.4.5.2.2 Hypoglycemic clamp procedure and dosing

At the time of admission to the clinic, which is the day before clamp procedure, the patient's eligibility must be checked. If the patient meets any of the exclusion criteria at the time of admission to the clinic at V2, he/she will be excluded from the dosing visit but may be rescheduled once 1-7 days later.

Eligible patients will be randomized to either dasiglucagon, placebo or GlucaGen® in the morning of the dosing visit. That morning after 7 o'clock am, the infusion of IV glucose will be stopped. This should be at least 30 minutes prior to planned trial product administration. Insulin infusion rate and insulin dose are at the discretion of the investigator or can follow the protocol general guidance (see above). The investigator may deviate from the guidance. Insulin IV infusion will be stopped when glucose level declines to below 80 mg/dL.

During insulin-induced hypoglycemia, plasma glucose levels will be monitored closely at the site. After the start of insulin infusion, plasma glucose should be checked approximately every 10 minutes while plasma glucose is above 110 mg/dL, approximately every 5 minutes once plasma glucose is at or below 110 mg/dL, until after dosing, when the patient's plasma glucose returns to ≥70 mg/dL (3.9 mmol/L). From that time on, 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, Ohio, USA or the Super GL analyzer, Dr. Müller Gerätebau GmbH, Freital, Germany.

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

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

In case of persistent post-treatment hypoglycemia, patients will receive rescue treatment with an IV glucose infusion (see Section 7.4.5.2.3 for details). Blood samples for PD and PK assessments should still be taken at the specified time points.

Once the glucose concentration declines to <80 mg/dL, blood samples for baseline assessment of plasma glucose and dasiglucagon/GlucaGen PK will be collected 5 minutes later. The samples are the baseline samples and should be collected within 2-5 minutes before trial product administration. If plasma glucose is ≥54 mg/dL and <80 mg/dL (3.0-4.4 mmol/L), trial product will be administered.

If plasma glucose is <54 mg/dL (3.0 mmol/L), IV glucose solution will be administered sufficient to raise plasma glucose to within the 54-80 mg/dL 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/GlucaGen PK, will be collected. In this case, glucose should not be infused within 10 minutes before trial product administration. If plasma glucose is not within the target range after the second attempt, the patient should be rescheduled for a new treatment visit within 7 days (+ 2 days).

After 60 minutes, patients will be allowed to eat and drink moderately (appropriate to their body size, with a maximum of 50gr carbohydrates) to minimize discomfort in terms of potential nausea. The amount and type of food and drink consumed will be recorded. Patients should remain in bed until completion of the test procedure 300 minutes after dosing (bathroom visits are allowed).

Germany only: Trial staff will be instructed on pediatric aspects of hypoglycemia according to the Clinical Practice Consensus Guidelines of the International Society for Pediatric and Adolescent Diabetes (ISPAD) 2018 (https://www.ispad.org/page/ISPADGuidelines2018). As the blood glucose falls, the initial symptoms result from activation of the autonomic nervous system and include shakiness, sweating, pallor and palpitation. In healthy individuals with no diabetes, these symptoms occur at a blood glucose level of approximately 70 mg/dL (3.9 mmol/L) in children and 54 mg/dL (3.0 mmol/L) in adults. However, this threshold in individuals with diabetes will depend on their glycemic control, with an adaptive shift of the glycemic threshold for symptom onset to a higher glucose level with chronic hyperglycemia and lower glucose level with chronic hypoglycemia. Neuroglycopenic symptoms result from brain glucose deprivation and include headache, difficulty concentrating, blurred vision, difficulty hearing, slurred speech and confusion. Behavioral changes such as irritability, agitation, quietness, stubbornness and tantrums may be the prominent symptom particularly for the preschool child, and may result from a combination of neuroglycopenic and autonomic responses. The patient's blood glucose values will be monitored via Point of Care plasma calibrated blood glucose measurements every 10 minutes or per investigator discretion from the start of the hypoglycemia induction protocol. Frequency of glucose testing will be according to investigator discretion based on the patient's condition, the glucose values and their rate of change.
The investigator must terminate the experiment immediately if he/she feels at any point that a patient's burden is not acceptable or safety may be at risk.

7.4.5.2.3 Rescue provisions for hypoglycemia

During insulin-induced hypoglycemia, plasma glucose levels as well as the patient status will be monitored closely throughout the procedure. After administration of trial product, if their glucose

levels become too low, patients may receive post-treatment IV glucose infusion to ameliorate hypoglycemia, as follows:

1. The IV glucose infusion should be initiated if a patient experiences escalating symptoms of hypoglycemia (e.g. start of moderate symptoms of hypoglycemia) at any time during the test procedure. Glucose infusion should then be initiated targeting a plasma glucose level >70 mg/dL
2. If plasma glucose is <54 mg/dL between t=8 and t=44 minutes, glucose infusion (e.g. 1-2 mg/kg administered over 5-10 seconds) should be initiated to maintain plasma glucose between 55 mg/dL and 70 mg/dL. Pause glucose infusion if plasma glucose is >70 mg/dL
3. If plasma glucose is <70 mg/dL at t≥45 minutes, glucose infusion (e.g. 2-3 mg/kg administered over 5-10 seconds) should be initiated to maintain plasma glucose between 70 mg/dL and 90 mg/dL. Pause glucose infusion if plasma glucose is >80 mg/dL

7.4.6 Selection of doses in the trial

The selected dose of 1 mg GlucaGen® is the recommended dose for treatment of severe hypoglycemia (in accordance with the label, children below 25 kg body weight will be administered 0.5 mg GlucaGen®). Pre-clinical and adult clinical studies demonstrated that 0.6 mg of dasiglucagon results in an initial PD response (i.e. acute glucose mobilization) comparable to 1 mg GlucaGen®.

The estimation of the dasiglucagon dose in the pediatric trial is based on a PK and PD approach[6] where a previously developed population PK/PD model[3] for dasiglucagon was extended with an allometric scaling component for the PK model[4]. The PD response was assumed to be similar in pediatric and adult patients as organ maturation for patients above 4 years is assumed not to have a meaningful impact on dasiglucagon PD. The pediatric PK component was developed by allometric scaling of clearance (Cl) and volume (V) by body weight from published glucagon PK data obtained from 2 clinical trials following intramuscular administration to pediatric (4 to 17 years) and adult T1DM patients[11,9].

The conclusion of the modelling was that a dose of 0.6 mg appears appropriate to ensure a fast rescue from hypoglycemia for adult patients and children down to a weight of 25 kg. Although the dasiglucagon C_{max} and AUC is expected to be higher in children, it does not exceed the total C_{max} and AUC obtained in adults previously administered 2 mg dasiglucagon. The higher total drug exposure (AUC and C_{max}) at lower weight is partially compensated by the shorter $t_{½}$ and saturated PD response. The resulting total time of drug exposure and PD effect (AEU and CE_{max}) is similar throughout the weight range.

7.4.7 Collection of blood samples

The total blood volume to be obtained from any individual child will be about 43 mL. The maximum amount per visit is 12 to 17 mL. These values are within the recommended scope provided by the EU[5].

The following blood volume will be taken for each sample at each visit:

Sample type	Visit 1 (mL)	Visit 2 (mL)	Visit 3 (mL)	
Biochemistry/pregnancy	5.00	-	5.00	
Hematology	2.00	-	2.00	
Coagulation	2.00	-	2.00	
HbA1c	2.00	-	-	
ADA	3.00	-	3.00	
PK	-	4.00	-	
PD	-	12.00	-	
Onsite-PG	-	1.00	-	
Sum for each visit	14.00	17.00	12.00	
Total				43.00

Abbreviations: ADA=Anti-drug antibodies; HbA1c=Hemoglobin A1c; Onsite-PG=Onsite monitoring of plasma glucose; PD=Pharmacodynamics; PK=Pharmacokinetics

7.4.8 Treatment compliance

Unblinded trial personnel will handle, prepare and administer all trial products.

7.4.9 Method of assigning patients to treatments

Forty patients will be randomized in a 2:1:1 ratio (dasiglucagon/placebo/GlucaGen®) into the 3 treatment arms and stratified in 2 age groups: 6 to ≤12 years and 12 to ≤18 years and by injection site (abdomen/thigh). A minimum of 16 patients will be enrolled into each age group, including a minimum of 8 patients in either of age groups on dasiglucagon treatment arm.

Patients who meet the inclusion and exclusion criteria will be randomized into one of the following treatment arms:

- Dasiglucagon, liquid formulation, 1 mg/mL, 0.6 mL
- Placebo treatment: Placebo, liquid formulation, 0.6 mL
- Active control treatment: Recombinant glucagon hydrochloride, 1 mg lyophilized powder for reconstitution (GlucaGen®, Novo Nordisk) in 1 mL sterile water (in accordance with the label, children below 25 kg body weight will be administered 0.5 mg GlucaGen®)

Randomization will be performed using a central, dynamic variance minimization randomization method using an interactive web response system (IWRS) that will randomize a patient to one of the 3 aforementioned treatment arms and then assign dispensing unit numbers to that patient.

An unblinded statistician/programmer who is separate from the trial team will generate the randomized kit list before the initiation of the trial.

Treatment assignment will be kept strictly confidential and accessible only to authorized persons until after data base lock. However, the investigators can perform an emergency unblinding within the IWRS in case of an emergency, see Section 7.4.10.

7.4.10 Blinding and breaking the blind

This is a double-blind trial. Dasiglucagon is available as a liquid formulation and GlucaGen® is available as a powder for reconstitution; they are therefore not identical in appearance. Unblinded trial personnel will be responsible for handling, preparing (according to the prescription from the IWRS), and administering the trial product, and the syringes used for administration will be wrapped in aluminum foil to maintain the blinding at bedside. Parents being in the room with their child must be instructed not to look while the unblinded staff is administering the injection. To maintain double-blind conditions, blinded trial personnel not involved in the administration of trial

products will perform all trial assessments at the trial center and is responsible for keeping the records strictly confidential and accessible only to the unblinded staff. However, unblinded personnel at the specialty laboratories will perform the exposure assessments and ADA assessments to make sure that dasiglucagon or GlucaGen® administration is matched with the applicable bioanalytical assay.

Treatment assignment will be kept strictly confidential and accessible only to authorized persons until after the time of unblinding (database lock). 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 patient may be broken in a medical emergency if knowing the identity of the treatment allocation would influence the treatment of the patient. 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 a patient should not unblind the trial personnel to the treatment information of other patients. The person performing the unblinding should inform as few people as possible about the result of the unblinding. All persons unblinded for a specific patient should be documented.

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

7.4.11 Drug accountability and disposal

Unblinded trial personnel will do the handling, preparation and administration of trial product. Each center will keep accurate records of the trial supplies received, stored, and dispensed, using appropriate forms. The trial supplies will be stored under appropriate conditions, locked and with restricted access. The unblinded monitor will perform the drug accountability to ensure compliance.

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

7.4.12 Prior and concomitant therapy

All concomitant medications will be recorded in the eCRF at each visit.

Prior to the start of the clamp procedure, the patient's eligibility must be checked. If the patient has taken any prohibited medication, he/she will be excluded from the dosing visit but may be rescheduled 1-7 days later. Each patient may only have their dosing visit rescheduled once. See Section 7.3.3.1 for possible reasons for patient discontinuation.

7.4.13 Treatment after end of trial

After the end of their trial participation, patients should return to the standard of care that they received prior to enrollment in the trial. The treating physician will be responsible for supervising patients after the end of the trial.

7.5 Assessments and schedule of measurements

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

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

7.5.1 Screening visit

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

- Informed consent
- Inclusion/exclusion criteria
- Demography
- Body measurements
- Diabetes diagnosis, and current diabetes treatment
- Medical history including concomitant illnesses
- Prior and concomitant medications
- History of alcohol/drug abuse
- Physical examination
- Vital signs
- 12-lead ECG
- AEs
- Biochemistry, hematology, coagulation, hemoglobin A1c (at Visit 1 only)
- Serum pregnancy test (female patients of childbearing potential only)
- Urinalysis
- Antibodies against dasiglucagon/GlucaGen®

7.5.2 Dosing visit

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

The following assessments will take place:

- Inclusion/exclusion criteria (prior to the start of the insulin-induced hypoglycemic procedure. Only check of exclusion criteria at clinic admission on Visit 2, day 0 and changes between screening visit and Visit 2)
- Concomitant medications (prior to the start of the insulin-induced hypoglycemic procedure)
- Current diabetes treatment
- Randomization (prior to the start of the insulin-induced hypoglycemic procedure)
- Exclusion criteria at clinic admission on Visit 2, day 0 (prior to the start of the insulin-induced hypoglycemic procedure)
- 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)
- Alcohol breath test (prior to the start of the insulin-induced hypoglycemic procedure)
- Physical examination (prior to the start of the insulin-induced hypoglycemic procedure)
- Insulin-induced hypoglycemia

- Vital signs (prior to the start of the insulin-induced hypoglycemic procedure (within 30 minutes), and at 30, 60 and 300 minutes after dosing. The actual time of the assessment should not deviate from the nominal time by more than ±10 minutes)
- 12-lead ECG (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)
- Local tolerability (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)
- AEs
- Plasma dasiglucagon/GlucaGen® (pre-dose, and at 10, 20, 30, 40, 60, 90, 140, 220, and 300 minutes after dosing. The actual time of blood sampling should not deviate from the nominal time by more than ±1 minute between t=5 minutes and t=90 minutes, ±5 minutes between t=150 minutes and t=240 minutes. Pre-dose is defined as within 2 minutes prior to dosing)
- Plasma glucose (pre-dose, and at 4, 6, 8, 10, 12, 15, 17, 20, 30 and 45 minutes (and as well at 60 minutes if the patient´s body weight is ≥21 kg) after dosing. The actual time of blood sampling should not deviate from the nominal time by more than ±30 seconds until the 20-minute collection time point, and by more than ±1 minute for the subsequent collection time points. Pre-dose is defined as within 2 minutes prior to dosing
- Administration of trial product (during hypoglycemic clamp procedure)

7.5.3 Follow-up visit

At 28 (+ 5) days after the hypoglycemic clamp procedure, the follow-up visit is scheduled to collect safety data. The following assessments will take place:

- Concomitant medications
- Current diabetes treatment
- Physical examination
- Vital signs
- 12-lead ECG
- Local tolerability
- AEs
- Biochemistry, hematology, coagulation, HbA1c (HbA1c at Visit 1 only)
- Urine stick pregnancy test (women of childbearing potential only)
- Urinalysis
- Antibodies against dasiglucagon/GlucaGen® (any treatment-induced or treatment-boosted [titer increase above 5 fold] ADA-positive patients will be monitored until the ADA levels return to baseline levels)

7.5.4 Final examination at the end of the trial

The final visit of the trial is Visit 3 (Day 28[+5] of the follow-up period; end-of-trial visit). See Section 7.5.3 for further details.

7.5.5 Additional (safety) examinations

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

7.5.6 Efficacy measurements

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.

Pharmacokinetic measurements

The exposure to trial medication (dasiglucagon or GlucaGen®) for evaluation of PK will be assessed based on plasma concentration data ($AUC_{0\text{-}90min}$, C_{max}, t_{max}) from samples collected at the dosing visit (Visit 2). The sampling procedure is described in Section 7.5.2.

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. The sampling procedure is described in Section 7.5.2.

7.5.7 Safety and tolerability measurements

7.5.7.1 Safety laboratory tests

Routine safety laboratory tests will be performed centrally. Samples for safety laboratory parameters will be collected at Visits 1 and 3. Samples for urinalysis will be collected at Visits 1, 2, and 3. The following parameters will be determined:

- Clinical chemistry: sodium, potassium, calcium, glucose, urea, creatinine, total bilirubin, AST, ALT, gamma-glutamyltransferase, alkaline phosphatase, total protein, C-reactive protein, HbA1c, C-peptide
- Hematology: hemoglobin, red blood cell count (erythrocytes), hematocrit, platelet count (thrombocytes), total white blood cell count (leucocytes)
- Coagulation: international normalized ratio, fibrinogen (at screening visit only)
- Urinalysis: pH, blood (leukocytes and erythrocytes), protein, glucose, ketones, nitrite
- Immunogenicity:
  Serum samples for assessment of antibodies against dasiglucagon/GlucaGen® will be measured at screening Visit 1 and at follow-up Visit 3 by the special laboratory, York Bioanalytical Solutions (York, United Kingdom). A description of the sample handling and sample processing, storage and shipment at the center will be included in the laboratory manual. Immunogenicity samples will be analyzed after the last patient last visit.

  The clinical ADA assays, one specific for dasiglucagon and another for GlucaGen®, have been validated in accordance with existing guidelines and recommendations.

  Confirmed positive anti-dasiglucagon antibody samples from treatment- induced or treatment-boosted (titer increase above 5-fold) ADA-positive patients will be evaluated for binding titer, neutralizing potential and neutralizing titer as well as cross-reactivity towards endogenous glucagon. Any treatment-induced or treatment-boosted ADA-positive patients will be monitored until the ADA levels return to baseline levels.

The *in vitro* neutralizing effect of the antibodies will be measured using an assay based on glucagon receptor expression in transfected human embryonic kidney cells. The calculated sensitivity in previous studies was about 51.8 ng/mL. The assay was also validated for recombinant glucagon with similar results. 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, North Carolina, USA.

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

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.

A pregnancy test will be performed at screening and follow-up (Visits 1, 2 and 3) for female patients of childbearing potential only.

An alcohol breath test will be performed at the dosing visit (Visit 2).

For further details, please refer to the laboratory manual.

7.5.7.2 Safety examinations

Physical examination is performed at screening (Visit 1), dosing (Visit 2) and end-of-trial (Visit 3).

AEs are assessed at all visits. Local tolerability is assessed at dosing visit and follow-up visit (Visits 2 and 3). ECG and vital signs are assessed at screening, dosing and follow-up visit (Visit 1, 2 and 3).

- Physical examination includes examination of the following body systems: head, ears, eyes, nose, throat, including the thyroid gland; heart, lung, chest; abdomen; skin; musculoskeletal system; nervous system; lymph nodes
- Vital signs include: pulse rate and blood pressure after 5 minutes in sitting position, body temperature
- Local tolerability: skin reactions will be reported as AEs (see Section 8)
- 12-lead ECG: Details from ECG assessments will be recorded, including heart rate, PR, QRS, QT and QTc corrected by Fridericia´s formula intervals

8. Adverse events

8.1 Definitions

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

Adverse event

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

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

AEs include:

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

The following should not be recorded as AEs, if recorded at screening (on Medical History form or eCRF):

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

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

Serious adverse event

An SAE is any untoward medical occurrence that at any dose results 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 defect
- Is medically important
  Medical judgement must be exercised in deciding whether an AE is believed to be ‘medically important’. A medically important event may not be immediately life-threatening or result in

death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above.

Other important events:

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 > 3 x ULN and total bilirubin > 2 x ULN, where no alternative etiology exist
- Suspicion of transmission of infectious agents via the trial product
- Overdose of the trial product
- Suspected abuse or misuse of the trial product
- Medication error involving the trial product
- Inadvertent or accidental exposure to the trial product.

Severity of an adverse event

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

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

Causality relationship to trial product

- Probable: Good reason and sufficient documentation to assume a causal relationship
- Possible: A causal relationship is conceivable and cannot be dismissed
- Unlikely: The event is most likely related to etiology other than the trial product

Outcome of an adverse event

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

Suspected unexpected serious adverse reactions

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

Adverse event of special interest (AESI)

An AESI is an event that, in the evaluation of safety, has a special focus (e.g. required by health authorities). In this trial, hemodynamic changes are considered AESIs that are defined as follows:

- 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.

AESIs must be reported to the sponsor in the same way and with the same timelines as SAEs (see Sections 8.2 and 8.3).

8.2 Collection, recording and reporting of adverse events

All AEs, whether serious or non-serious, will be reported from the time a signed and dated informed consent form (ICF) is obtained until the end of the post-treatment follow-up period (which may include contacts for follow-up of safety). In addition, patients 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 center (visit or telephone). Patients 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 product, 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 product. All measures required for AE management must be recorded in the source document and reported according to sponsor instructions.

Each AE must be reported on the AE page of the eCRF.

All AE information should at a minimum include the following:

- Date and time of onset and resolution
- Date and time of investigator's first information on the AE
- Seriousness
- Severity
- Causal relationship with trial product
- Measures taken due to AE
- Date and time of resolution and final outcome

Each AE will be coded using the latest version of the Medical Dictionary for Regulatory Activities.

SAEs and AESIs, including those spontaneously reported to the investigator within 30 days after the last dose of trial product, must be reported to the appropriate sponsor contact person(s) within

24 hours after obtaining knowledge about the event, followed by a complete SAE form as soon as more information is available. For each SAE and AESI, a separate SAE form should be completed.

The investigator should report the SAE and AESI in the electronic data capture (EDC) and the system will generate an email to the sponsor´s Pharmacovigilance Unit (PharmaLex), informing them of the reported SAE.

It is the responsibility of the sponsor´s Pharmacovigilance Unit (PharmaLex) to report all SUSARs that occur in this trial to the respective competent authorities and the institutional review board (IRB), or independent ethics committee (IEC) in accordance with the local requirements in force and ICH guideline for GCP.

8.3 Follow-up of adverse events

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

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

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

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

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

The investigator must record follow-up information in the eCRF for non-serious AE and on the SAE form for SAEs and AESI. Follow-up questions to investigators regarding SAEs are queried directly by safety CRO to the investigator.

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

- SAEs and AESI: All SAEs and AESI 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 that are 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 patient has completed the follow-up period and is expected by the investigator to recover.

  The SAE and AESI follow-up information should only include new (eg, corrections or additions) 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 that are 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 a patient has completed the follow-up period and is expected by the investigator to recover.

If a potential hypersensitivity reaction is observed, additional blood samples, as clinically indicated, 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 worst-case severity and seriousness of an event is kept throughout the trial, ie, if the severity of an AE changes over time then it should be reported as 1 AE with the most severity. A worsening of an unresolved AE must be reported as follow-up with re-assessment of severity and/or seriousness of the event.

If an AE is resolved and re-appears later then it should be reported as a new AE.

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.

8.4 Hypoglycemia

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

Hypoglycemia is defined as a decline in plasma glucose below 3.9 mmol/L (70 mg/dL). Since hypoglycemia will be induced during the dosing visit, in accordance with hypoglycemia clamp procedure described in Section 7.4.5.2.2, **ONLY** plasma glucose below 3.0 mmol/L (54 mg/dL) must be reported as an AE, at the dosing visit (V2), and at incidences that IV glucose is given after trial product administration.

8.5 Pregnancy

Female patients must be instructed to notify the investigator immediately if they become pregnant or if they suspect to be pregnant during the trial. All initial reports of pregnancy in female patients must be reported to the sponsor by the trial center 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 patient becomes pregnant during the trial, a determination regarding the trial product discontinuation must be made by the investigator.

Because the effect of the trial product on sperm is unknown, pregnancies in partners of male patients must be reported by the trial center personnel within 24 hours of their knowledge of the event using the appropriate pregnancy form. Male patients 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 1 month of age. The investigator must report information about the pregnancy, pregnancy outcome, and health of the newborn infant(s), as well as AEs in connection with the pregnancy, and AEs in the fetus and newborn infant.

8.6 Precautions

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

For further information on safety precautions for dasiglucagon and GlucaGen®, please refer to the current version of the Investigator's Brochure[2] and the Summary of Product Characteristics for GlucaGen®[7], respectively.

8.7 Safety committee

An internal Zealand Pharma A/S safety committee is constituted to perform the 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 patients.

The safety committee convenes regularly, every quarter, to review the safety information, including SAEs, AEs and laboratory data. Additional safety committee meeting may be called at the discretion of any safety committee member, should new safety signals occur during this time interval.

9. Data management and quality control

9.1 Electronic case report forms

All the information collected during the trial will be recorded in the eCRFs, which are identified by patient number. Synteract will design suitable eCRFs. The investigator will ensure that the eCRFs are completed correctly. The investigator will sign all data entered in the eCRF electronically, signifying agreement with and responsibility for the recorded data.

9.2 Quality control

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

A monitor from Synteract will supervise the trial. The trial monitor will contact the investigator regularly to discuss the progress of the trial and to check the trial documents including the informed consent forms for completeness and consistency.

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

The eCRFs will be checked for completeness and correctness by the monitor and by the data management department of Synteract according to the Synteract standard operating procedures, and the investigator will resolve any queries.

All of the clinical data will be captured via EDC using a web-based tool. The software Marvin from the company XClinical (www.xclinical.com) is the preferred EDC software. Marvin is compliant with all legislation relevant to EDC (Food and Drug Administration USA, 21 Code of Federal Regulations [CFR] Part 11, GCP).

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

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

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

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

9.3 Data management

Data management will be performed according to Synteract standard operating procedures.

10. Statistical methods and determination of sample size

10.1 Statistical analysis plan

A separate Statistical Analysis Plan will detail the planned statistical analysis and may include necessary adaptations to the planned statistical analysis before unblinding of the data.

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

10.1.1 General considerations

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

Raw data listings and summary tables will be generated using SAS© software Version 9 or higher (SAS Institute, Inc, Cary, North Carolina, USA).

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

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

10.1.2 Classification of patients to subsets

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

The following definitions are applicable:

Safety analysis set (SAS)	all patients who were randomized and received at least 1 dose of trial product
Full analysis set (FAS)	all patients of the SAS
Per protocol set (PPS)	all patients of the FAS for whom no relevant protocol deviations were documented.

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

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

10.1.3 Efficacy endpoints

For the confirmatory analyses, the primary and secondary endpoints for the dasiglucagon 0.6 mg and placebo treated patients 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 patients 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 patients who received rescue IV glucose.

10.1.3.1 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 secondary endpoints will be inferentially evaluated within the FAS in the following order, where inference will proceed at the 2-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
- Secondary 1-4: Plasma glucose recovery within 30 minutes, within 20 minutes, within 15 minutes, and within 10 minutes after study drug injection without administration of rescue IV glucose.
- Secondary 5-8: Plasma glucose changes from baseline within 30 minutes, within 20 minutes, within 15 minutes, and within 10 minutes after study drug injection or at the time of rescue.

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

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

10.1.3.2 Primary efficacy endpoint

The primary endpoint, time to plasma glucose recovery, will be summarized using Kaplan-Meier estimates for each treatment group in total and stratified by age group and injection site. The median time to recovery with 95% confidence interval will be estimated by treatment group, in total and in each stratum.

A stratified log-rank test will be applied to compare the dasiglucagon 0.6 mg treatment group to the placebo group. The same method will be applied to compare dasiglucagon 0.6 mg with GlucaGen®.

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

A further sensitivity analysis will be done by censoring data at the actual time when the patients received glucose IV.

10.1.3.3 Secondary efficacy endpoints

The secondary efficacy endpoints are plasma glucose recovery within 10, 15, 20 and 30 minutes after trial product injection, i.e. achieving a ≥20 mg/dL increase in plasma glucose from baseline within 0 to 10, 15, 20 and 30 minutes. If a patient has received an IV glucose treatment before recovery, the patient is set to 'not recovered' in the analysis of the 4 endpoints, corresponding to censoring in the time-to-recovery analysis for the primary endpoint. The 10-, 15-, 20- and 30-minute recovery rates of 2 treatment groups will be compared by a Cochran-Mantel-Haenszel test stratified by age group and injection site. The treatment and age group responder rates with the 95% confidence intervals will be presented for the 10-, 15-, 20- and 30-minute endpoint.

If due to small or zero cell counts the Cochran-Mantel- Haenszel test fails, non-stratified Fisher's exact tests will be applied instead.

Plasma glucose changes from baseline at 10, 15, 20 and 30 minutes after trial product injection will be analyzed in an analysis of variance with factors treatment (3 levels) age group (2 levels) and injection site (2 levels) for each endpoint. If the rescue IV glucose was administered before 10, 15, 20 or 30 minutes, respectively, the patient's plasma glucose changes from baseline will be determined from the value at the time of rescue IV glucose administration. Adjusted treatment means will be presented with their 95% confidence intervals. The 4 analysis of variance will also be used to test the difference between treatments adjusted for age group and injection site.

10.1.4 Safety endpoints

Clinical laboratory data

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

Adverse events

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

Other safety data

Time to first IV glucose infusion, after IMP administration (N.B. IV glucose infusion prior to IMP administration should not be included, as it is part of hypoglycemic clamp procedure) will be described with descriptive statistics. No statistical tests will be performed.

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

Immunogenicity

Occurrence of ADA will be analyzed descriptively per treatment group. No statistical tests are planned.

10.1.5 Pharmacokinetic endpoints

Plasma dasiglucagon and glucagon concentrations will be described and the following parameters are calculated and summarized with descriptive statistics:

- Area under the plasma dasiglucagon or GlucaGen® concentration versus time curve from 0 to 30 minutes post-dose ($AUC_{0\text{-}30min}$)
- Area under the plasma dasiglucagon or GlucaGen® concentration versus time curve from 0 to 300 minutes post-dose ($AUC_{0\text{-}300min}$)
- Area under the plasma dasiglucagon or GlucaGen® concentration versus time curve from 0 to infinitely post-dose ($AUC_{0\text{-}inf}$)
- Maximum of all valid plasma dasiglucagon or GlucaGen® concentration measurements from 0 to 300 minutes post-dose (C_{max})
- Time to maximum of plasma dasiglucagon or GlucaGen® concentration measurements (t_{max})
- Terminal elimination rate constant of plasma dasiglucagon or GlucaGen® (λ_z)
- Terminal plasma elimination half-life of dasiglucagon or GlucaGen® ($t_{½}$)
- Total body clearance of plasma dasiglucagon or GlucaGen® (CL/f)
- Volume of distribution of plasma dasiglucagon or GlucaGen® (V_z/f)
- Mean residence time of plasma dasiglucagon or GlucaGen® (MRT)

10.1.6 Pharmacodynamic endpoints

Plasma glucose response as area under the effect curve above baseline from time 0 to 30 minutes, $AUE_{0\text{-}30min}$, will be summarized with descriptive statistics.

10.1.7 Further data

Baseline and demographic data will be summarized using descriptive statistics.

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

10.1.8 Withdrawals, drop-outs and missing data

Only valid cases will be analyzed, i.e. no imputation technique like last observation carried forward will be applied.

10.1.9 Baseline and center comparisons

Demographic and other baseline characteristics will be compared.

10.1.10 Subgroup analysis

No subgroup analysis is currently planned.

10.1.11 Interim analysis

No interim analysis is currently planned.

10.2 Determination of sample size

The primary comparison is between the dasiglucagon and placebo treatment arms. From phase 2 results, the median time to an increase of 20 mg/dL of the 0.6 mg dose was approximately 10 minutes. For placebo-treated patients, the median time to recovery is assumed to be more than 50 minutes. Assuming an exponential time-to-recovery distribution with median times of 10 and 50 minutes, a 2-sided log-rank test will be able to detect a difference between dasiglucagon 0.6 mg and placebo with 90% power with a follow-up time of 45 minutes at a 5% significance level with 20 patients randomized to the dasiglucagon arm and 10 patients to placebo.

GlucaGen® is included as a reference to compare the responses and AE profile to dasiglucagon with those to a marketed product. It is expected that 10 patients in the GlucaGen® group will suffice for the comparison.

11. Ethics and regulations

11.1 Independent ethics committees and competent authorities

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

The CA and the IEC/IRB will be notified about the end of the trial and a report summarizing the trial results will be sent to the CA and the IEC within 1 year after the end of the trial. If the trial is terminated early, the CA and the EC will be notified within 15 days.

The IECs and/or IRBs meet the requirements of the ICH Harmonised Tripartite Guideline for GCP and local legislation. They also meet the requirements of 21 CFR 312.3.

11.2 Ethical conduct of the trial

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

11.3 Patient information and consent

Written informed consent will be obtained from the parents/legal representatives of all patients prior to enrollment into the trial. Additionally, the children will be informed about the trial with age-appropriate information materials, and their assent will be obtained in accordance with local regulations. The investigator will explain to each patient and their parents/legal representatives orally and in writing (patient information sheet) the nature, significance, risks and implications of the trial before inclusion. In particular, the patients will be informed about the following:

- The possibility of withdrawing from the clinical trial at any time by revoking the consent and without any resulting disadvantage
- How personal and health-related data will be collected and used during the trial
- Confidentiality, i.e. medical records may be examined by authorized monitors or Clinical Quality Assurance auditors appointed by the sponsor, by appropriate IEC/IRB members, and by inspectors from regulatory authorities

All parents/legal representatives and the children will receive a copy of the respective patient information sheet and a copy of their signed and dated informed consent/assent form.

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

11.4 Legal and regulatory requirements

This trial will be carried out in accordance with:

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

12. Trial administration

12.1 Responsibilities

Zealand Pharma A/S is the sponsor of this trial. Synteract, a contract research organization, will organize the performance of this trial.

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

12.2 Protocol deviations

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

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

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

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

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

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

12.3 Protocol changes

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

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

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

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

12.4 Reports and publications

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

No confidential information will be disclosed to others without prior written consent from Zealand Pharma A/S. Such information will 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 product, if deemed necessary by Zealand Pharma A/S. Provided that certain conditions are fulfilled, Zealand Pharma A/S may grant access to information obtained during this trial to researchers who require access for research projects studying the same disease and/or trial product 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.

Communication of results

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

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

Zealand Pharma A/S 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 A/S. Zealand Pharma A/S 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 A/S 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.

12.5 Retention of trial records

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

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

After trial completion at sites in the US, the investigator will retain and preserve 1 copy of all data generated in the course of the trial, specifically including, but not limited to, those defined by GCP as essential until:

- At least 2 years after the last marketing authorization for the trial product has been approved or the sponsor has discontinued its research with the trial product, or
- At least 2 years have elapsed since the formal discontinuation of clinical development of the trial product

However, these documents should be retained for a longer period if required by the applicable regulatory requirement(s) or if needed by the sponsor.

At the end of such period, the investigator will notify the sponsor in writing of his or her intent to destroy all such material. The sponsor will have 30 days to respond to the investigator's notice, and the sponsor will have a further opportunity to retain such materials at the sponsor's expense.

After trial completion at sites in Europe, the sponsor will receive a copy of their data in electronic format (e.g. CD) and retain them for at least 25 years.

One copy will remain with the investigator. The investigator will arrange for the retention of the patient identification codes, patient files, and other source data until at least 5 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 until at least 2 years have elapsed since the formal discontinuation of the clinical development of the product. These documents need to be retained for a longer period of time if required by applicable regulatory authorities or by agreement with the sponsor.

The investigator will keep copies of these trial records (and all trial-related documents, including source data) for the maximum period of time permitted by the hospital, institution, or private practice.

13. References

1. American Diabetes Association. Standards of medical care in diabetes - 2016. Diabetes Care 2016; 39 (Suppl. 1):S1-S112.
2. Dasiglucagon Investigator Brochure, Edition 4, 25 Oct 2017. Zealand Pharma A/S.
3. Diderichsen PM. Population PK and population PKPD modeling and simulation of dasiglucagon in patients with type I diabetes mellitus. 2016 Zealand Pharma report 16-147.
4. Diderichsen PM. Model-based simulation of paediatric dasiglucagon exposure and glucose response. Zealand Pharma report 17-094. 2017
5. European Commission. Ethical considerations for clinical trials on medicinal products conducted with minors Revision 1. 18 Sep 2017. Available at: https://ec.europa.eu/health/sites/health/files/files/eudralex/vol-10/2017_09_18_ethical_consid_ct_with_minors.pdf
6. Holst JJ, Albrechtsen NJW, Pedersen J, Knop FK. Glucagon and amino acids are linked in mutual feedback cycle: The liver-α-cell axis. Diabetes 2017; 66(2): 235-40.
7. Novo Nordisk. Summary of Product Characteristics. GlucaGen® HypoKit 1 mg powder and solvent for solution for injection. Available at: http://www.medicines.org.uk/emc/medicine/4258/SPC/GlucaGen®+Hypokit+1+mg.
8. Rickels MR, Ruedy KJ, Foster NC, Piché CA, Dulude H, Sherr JL, et al. Intranasal Glucagon for treatment of insulin-induced hypoglycemia in adults with type 1 dabetes: A randomized crossover noninferiority study. Diabetes Care. 2016 Feb;39(2):264-70.
9. Seaquist ER, Anderson J., Childs B, Cryer P, Dagogo-Jack S, Fish L, et al. Hypoglycemia and diabetes: A report of a workgroup of the American Diabetes Association and the Endocrine Society. Diabetes Care 2013; 36(5):1384-95.
10. Sherr JL, Ruedy KJ, Foster NC, Piché CA, Dulude H, Rickels MR, et al. Glucagon nasal powder: A promising alternative to intramuscular glucagon in youth with type 1 Diabetes. Diabetes Care. 2016 Apr; 39(4):555-62.
11. United States. Food and Drug Administration. General clinical pharmacology considerations for pediatric studies for drugs and biological products. Guidance for industry. Draft guidance. 2014. Available at: http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm425885.pdf.

APPENDIX 1:

List of names and addresses

Sponsor	Zealand Pharma A/S Smedeland 36 2600 Glostrup, Copenhagen Denmark
Contract Research Organization (CRO)	Synteract Deutschland GmbH Albrechtstr. 14 80636 Munich, Germany
Coordinating investigator	Prof. Dr. med. Thomas Danne Allgemeine Kinderheilkunde Diabetologie, Endokrinologie, Klinische Forschung Diabeteszentrum für Kinder und Jugendliche AUF DER BULT Kinder- und Jugendkrankenhaus Janusz-Korczak-Allee 12 30173 Hannover, Germany
Trial monitor	To be decided Synteract Deutschland GmbH Albrechtstr. 14 80636 Munich, Germany
Statistician	[redacted] Senior Director Biometrics EMEA Synteract Deutschland GmbH Albrechtstr. 14 80636 Munich, Germany
Sponsor representative	[redacted] Clinical Project Director Zealand Pharma A/S Smedeland 36, 2600 Glostrup, Denmark Tel: [redacted] E-mail: [redacted]
Sponsor's medical expert	Dr. [redacted] Medical Director Zealand Pharma A/S Smedeland 36, 2600 Glostrup, Denmark Tel: [redacted] E-mail: [redacted]
Sponsor representative for pharmacovigilance	[redacted] PharmaLex A/S Agern Allé 24, 2970 Hørsholm, Denmark Tel: [redacted] (8 a.m. to 4 p.m.) [redacted] (outside 8 a.m. to 4 p.m.) Fax: [redacted] E-mail: drugsafety@lindeq.com
Clinical laboratory	MLM Medical Labs GmbH Dohrweg 63

	41066 Mönchengladbach Germany Phone +49 2161 4642 0 Fax +49 2161 4642 190 E-mail info@mlm-labs.com
Analytical laboratory (ZP4207 PK, ZP4207 ADA, Glucagon ADA)	York Bioanalytical Solutions (YBS) [redacted] (ZP4207 Bioanalysis) [redacted] (ADA analyses) Cedar House Northminster Business Park Northfield Lane York, YO26 6QR United Kingdom E-mail: [redacted] E-mail: [redacted]
(ZP4207 and Glucagon Nab)	[redacted] BioAgilytix Labs 2300 Englert Drive Durham, NC 27713 USA E-mail: [redacted]

Abbreviations: ADA=Anti-drug antibody; Nab=Neutralizing antibody; PK=Pharmacokinetics