Title Page

Protocol Title: A Phase 3, single-administration, open-label trial to assess the efficacy, safety, pharmacokinetics, and pharmacodynamics of dasiglucagon when administered as a rescue therapy for severe hypoglycemia in pediatric patients below 6 years of age with Type 1 Diabetes (T1D)

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Trial Phase: Phase 3

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1 PROTOCOL SUMMARY

1.1 Synopsis

Protocol Title:

A Phase 3, single-administration open-label trial to assess the efficacy, safety, pharmacokinetics, and pharmacodynamics of dasiglucagon when administered as a rescue therapy for hypoglycemia in pediatric patients below 6 years of age with Type 1 Diabetes (T1D).

Rationale:

Dasiglucagon is indicated for treatment of severe hypoglycemia in pediatric and adult patients with diabetes aged 6 years and above. As this condition also affects children below 6 years of age and there is a therapeutic need for them to get access to safe and efficacious emergency treatment, the present trial aims to assess the efficacy and safety of a single subcutaneous (SC) injection of dasiglucagon in children below 6 years of age with T1D. A total of 8 children will be included; 4 children receiving a dose of 0.3 mg and 4 children receiving a dose of 0.6 mg. Of the 4 children receiving 0.3 mg, 2 children should preferably be below 2 years at screening. The remaining 2 children aged 2 years or above receiving 0.3 mg should preferably be below 4 years at screening. For children receiving the 0.6 mg dose, all must be above 2 years at screening. The trial population will include children treated with SC insulin infusion via insulin pump or multiple daily injections in order to include children below 1 year of age. Overall, the eligibility criteria allow for enrollment of a trial population resembling the target population of children below 6 years of age with T1D.

Objectives and Endpoints:

Objectives	Endpoints
To assess the efficacy of dasiglucagon injection in children < 6 years of age with T1D	Primary endpoint is pharmacodynamics (PD): Plasma glucose change from baseline at 30 minutes after IMP injection or at the time of rescue by intravenous (IV) glucose Secondary endpoint is PD*: Plasma glucose change from baseline at 15 minutes after IMP injection or at the time of rescue by IV glucose
To assess PK of dasiglucagon in children with T1D	PK endpoints will be derived from plasma dasiglucagon profiles from 0 to 300 minutes*. • AUC from 0 to 30 minutes post-dose ($AUC_{0\text{-}30min}$) • AUC from 0 to 300 minutes post-dose ($AUC_{0\text{-}300min}$) • AUC from 0 to the last time point with a measured concentration ($AUC_{0\text{-}t}$) • AUC from 0 to infinity post-dose (AUC_{inf})

Objectives	Endpoints
To assess the safety profile of dasiglucagon in children with T1D	• Maximum observed concentration (C_{max}) • Time to C_{max} (T_{max}) • Terminal elimination rate constant of plasma dasiglucagon (λz) • Terminal plasma elimination half-life of dasiglucagon ($t_{½}$) • Total body clearance of plasma dasiglucagon (Cl/f) • Volume of distribution of plasma dasiglucagon (Vz/f) • Mean residence time of plasma dasiglucagon (MRT) • Adverse events • Administration of rescue IV glucose infusion within 30 minutes of IMP injection • Time to first IV glucose infusion following treatment with dasiglucagon within 30 minutes
Other endpoints	• Anti-drug antibodies

Abbreviations: ADA=anti-drug antibodies; AUC=plasma concentration versus time curve; IMP=investigational medicinal product; IV=intravenous; PD=pharmacodynamics; PK=pharmacokinetics; T1D=Type 1 diabetes

* as the sampling frequency for PK and PD will gradually be reduced with lower weight for children of 10 kg and below (as specified in Table 6-4), these marked endpoints will be assessed to the extent possible based on the samples obtained for the individual child.

Overall Design:

This trial will use a single-administration, open-label trial design to assess the ability of a single SC injection of dasiglucagon to increase plasma glucose in pediatric children with T1D with hypoglycemia. A total of 8 children will be included; 4 children receiving a dose of 0.3 mg and 4 children receiving a dose of 0.6 mg. Of the 4 children receiving 0.3 mg, 2 children should preferably be below 2 years at screening. The remaining 2 children aged 2 years or above receiving 0.3 mg should preferably be below 4 years at screening. For children receiving the 0.6 mg dose, all must be above 2 years at screening. Before an eligible child is dosed, the dose level (0.6 mg or 0.3 mg) must be confirmed by the sponsor. Each child will be dosed after the safety assessment of the preceding child has been completed and assessed by the Trial Safety Group.

The trial will include the following visits:

- A screening visit (Visit 1) in the period from Day -50 to Day -29 (pre-treatment visit)
- A dosing visit (Visit 2), Day 1 (day of single dosing with investigational medicinal product [IMP])
- A Safety follow-up visit (Visit 3) at Day 29 +5 days (the end-of-trial visit)

The primary endpoint of the trial is plasma glucose change from baseline at 30 minutes after IMP injection or at the time of rescue by intravenous glucose. Pharmacodynamics (PD) i.e., plasma glucose will be assessed at baseline and 15 and 30 minutes after dosing, while the glucose levels will be monitored by continuous glucose monitoring and by a plasma glucose analyzer during the dosing visit (Visit 2). Pharmacokinetics (PK) will be assessed throughout a 300-minute period at the dosing visit (Visit 2). Safety will be assessed prior to dosing and throughout a 300-minute period after dosing and again at the follow-up visit.

Brief Summary:

The purpose of this trial is to assess efficacy, safety, PK, and PD of a single SC injection of dasiglucagon as a rescue therapy for hypoglycemia in pediatric children (< 6 years of age) with T1D. Trial details include:

- Trial Duration: Maximum of 84 days
- Treatment Duration: 1 day (dosing visit [Visit 2])
- Total of 3 visits

Number of Patients:

At least 8 children below 6 years (at the screening visit) with T1D will be dosed in the trial. Two of these children should preferably be below 2 years of age at the screening visit and successfully complete Visit 2. Furthermore, the remaining 2 children should preferably be below 4 years.

Intervention Groups and Duration:

All 8 children will be treated with dasiglucagon via a single SC injection into the buttocks at the dosing visit (Visit 2).

Four children in the age range ≥ 2 and < 6 years:

- will receive a single SC injection of 0.6 mg/0.6 mL of dasiglucagon by a prefilled syringe.

Four children, 2 of which should preferably be below 2 years, and the children aged ≥ 2 years and preferably < 4 years:

- will receive a single SC injection of 0.3 mg/0.3 mL dasiglucagon by a syringe

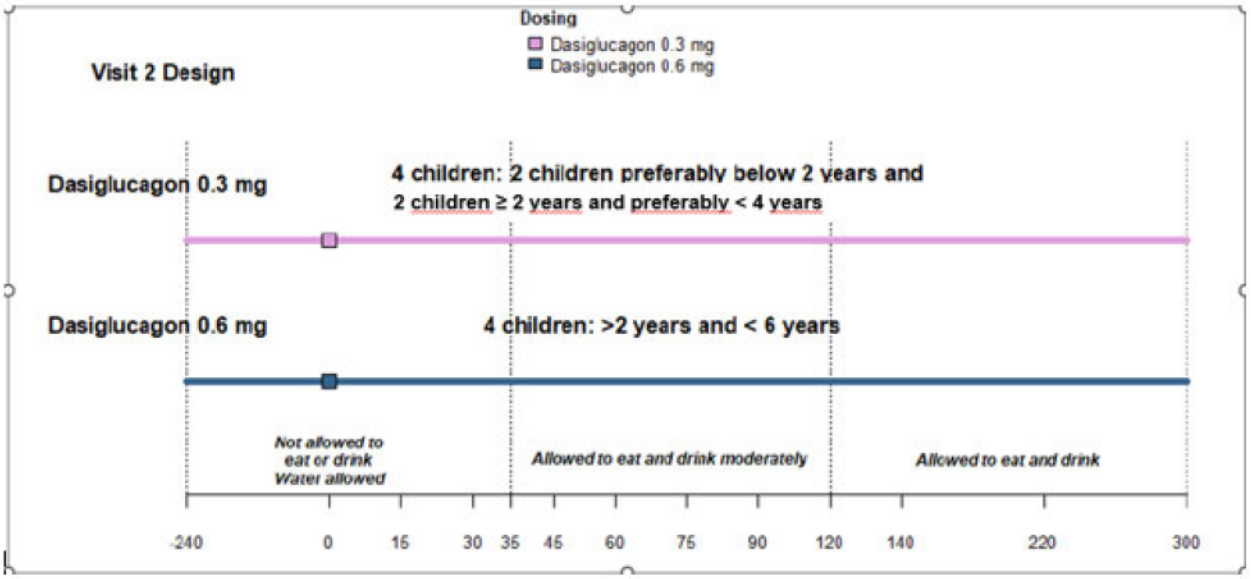
Statistical Methods:

The primary efficacy endpoint will be summarized using descriptive statistics and a 95% confidence interval. Other endpoints will be summarized using descriptive statistics. All data will be listed.

Data Monitoring: Yes, Trial Safety Group.

1.2 Schema


Visit schedule
8 subjects
Visit 1
Screening
Day -50 -> Day -29
Visit 2
Dosing
Day 1
Visit 3
Safety Follow-up
Day 29


Visit 2 Design
Dosing
Dasiglucagon 0.3 mg
Dasiglucagon 0.6 mg
Dasiglucagon 0.3 mg
4 children: 2 children preferably below 2 years and
2 children ≥ 2 years and preferably < 4 years
Dasiglucagon 0.6 mg
4 children: >2 years and < 6 years
Not allowed to eat or drink Water allowed
Allowed to eat and drink moderately
Allowed to eat and drink
-240
0
15
30
35
45
60
75
90
120
140
220
300

1.3 Schedule of Assessments

Table 1-1 Schedule of Assessments

Visit number	Visit 1	Visit 2	Visit 3	Visit x
Trial day	**-29**	**1**	**29**	**Unscheduled[a]**
Visit type	**Screening**	**Dosing**	**Follow-up**	**Unscheduled[a]**
Window	**-50 to -29**	**–**	**+5 days**	**Unscheduled[a]**
Patient-related information/assessments				
Informed consent	X	–	–	–
Inclusion/exclusion criteria	X	X[b,c]	–	–
Demography	X	–	–	–
Body measurements	X	X	–	–
Diabetes diagnosis	X	X	–	–
Medical history including concomitant illnesses	X	–	–	–
Current diabetes treatment	X	X	X	–
Prior/concomitant medications	X	X[d]	X	X
Exclusion criteria at clinic admission	–	X[b]	–	–
Insulin-induced hypoglycemia	–	X	–	–
Record food and drink	–	X[e]	–	–
Safety assessments				
Physical examination	X	X	X	X
Vital signs	X	X[f]	X	X
12-lead electrocardiogram	X	–	–	–
Continuous Glucose Monitoring	–	X	–	–

Visit number	Visit 1	Visit 2	Visit 3	Visit x
Trial day	**-29**	**1**	**29**	**Unscheduled[a]**
Visit type	**Screening**	**Dosing**	**Follow-up**	**Unscheduled[a]**
Window	**-50 to -29**	**–**	**+5 days**	**Unscheduled[a]**
Local tolerability	–	X[g]	X	–
Adverse events	X	X	X	X
Laboratory				
Biochemistry and hematology	X	–	X	–
Anti-drug antibody against dasiglucagon	X	–	X	X[a]
Pharmacokinetics/clinical efficacy				
Plasma dasiglucagon	–	X[h]	–	–
Plasma glucose		X[i]		
Trial material				
Administration of investigational medicinal product	–	X	–	–
Drug Accountability	–	X	–	–
End-of-trial status	–	–	X	–

[a] For anti-drug antibody (ADA)-positive patients only. ADA-positive patients (treatment-induced or treatment-boosted [titer increase above 4-fold]) will be followed as described in Section 8.1.3).

[b] Prior to the start of the insulin-induced hypoglycemic procedure.

[c] Check exclusion criteria at clinic admission on Visit 2 and changes between screening visit (Visit 1) and Visit 2.

[d] Concomitant medication must be checked prior to insulin-induced hypoglycemic procedure, during insulin-induced hypoglycemic procedure, and after treatment with dasiglucagon at Visit 2 (refer to Section 6.8).

[e] Patients will not be allowed to eat or drink (except water) from 4 hours before dosing. After 35 minutes following dosing, patients will be allowed to eat and drink moderately (appropriate to their body size, with a maximum of 50 grams carbohydrates) to minimize discomfort in terms of potential nausea. The amount and type of food and drink consumed will be recorded. After 2 hours following dosing patients can eat and drink their usual meals.

[f] 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.

[g] 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.

[h] The actual time of blood sampling should not deviate from the nominal time by more than ± 1 minute. Pre-dose is defined as within 2 minutes prior to dosing. Refer to Table 6-4 for pharmacokinetic sampling based on body weight at Visit 2.

[i] The actual time of blood sampling should not deviate from the nominal time by more than ± 1 minute for the collection time points. Pre-dose is defined as within 2 minutes prior to dosing. Refer to Table 6-4 for plasma glucose sampling based on body weight at Visit 2.

Due to COVID-19 public health control measures, some clinic visits may be substituted with a combination of telemedicine (a phone call to capture any adverse events, concomitant medication information, and to provide guidance to the patient) and visits from a home-health nurse (for blood sample collection).

2 INTRODUCTION

Dasiglucagon is a peptide analog of human glucagon. Dasiglucagon is approved by the Food and Drug Administration (FDA) for the treatment of severe hypoglycemia in pediatric and adult patients with diabetes aged 6 years and above.[1]

2.1 Trial Rationale

Severe hypoglycemia is a risk in all individuals with diabetes, in particular in those requiring insulin treatment regardless of age. There is a therapeutic need for all individuals at risk for severe hypoglycemia to have access to safe and effective emergency rescue therapies should such an event occur. The present trial aims to assess the efficacy and safety of a single subcutaneous (SC) injection of dasiglucagon (0.3 mg or 0.6 mg) in children with type 1 diabetes (T1D) below 6 years of age. The trial population will include children treated with SC insulin infusion via insulin pump or multiple daily injections (MDI) in order to include children below 1 year of age. To ensure the blood volume collected in the present trial is within an acceptable percentage of total blood volume for the individual child, children must have a body weight above 8 kg (see inclusion criteria Section 5.1). Overall, the eligibility criteria allow for enrollment of a trial population closely approximating the general population of children below 6 years of age with T1D.

2.2 Background

Hypoglycemia

Hypoglycemia in patients with diabetes is defined as episodes of an abnormally low plasma glucose concentration.[2] Hypoglycemia 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 more significant endogenous insulin deficiency, such as occurs in T1D and advanced type 2 diabetes. Treatment of type 2 diabetes with insulin causes hypoglycemia progressively and more frequently over time, whereas in T1D, hypoglycemia is experienced throughout the course of established disease.

Symptoms and signs of hypoglycemia are often non-specific. Patients experiencing a hypoglycemic episode commonly note symptoms such as anxiety, sweating, hunger, tremors, palpitations, paresthesia, nausea, and pallor. Depending on the severity, hypoglycemia may lead to a range of symptoms from mild confusion to more severe symptoms such as behavioral changes, loss of consciousness, seizures, coma, and death.[1]

The occurrence of hypoglycemic events or even the fear of hypoglycemia may influence adherence to prescribed treatment regimens for diabetes. This can contribute to suboptimal glycemic control, which in turn may lead to an increased risk of complications of diabetes.

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 a key counter regulatory hormone – counteracting the effect of insulin on blood glucose levels. Glucagon stimulates hepatic glycogenolysis and gluconeogenesis, thereby raising blood glucose especially during periods of hypoglycemia. Glucagon receptor agonism has also been shown to exert effects on lipid metabolism, energy balance, body adipose tissue mass, and food intake.

Insulin-induced hypoglycemia can be reversed by glucagon administration. Therefore, glucagon is indicated for the treatment of severe hypoglycemia.

Dasiglucagon

Dasiglucagon is a stable peptide analog of human glucagon, available in a ready-to-use liquid formulation and is approved by FDA for the treatment of severe hypoglycemia in pediatric and adult patients with diabetes aged 6 years and above. Dasiglucagon is a specific and full glucagon receptor agonist designed to mimic the effects of glucagon, having a fast absorption and elimination. 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.[3]

The clinical development program to support the indication of treatment of severe hypoglycemia comprises 3 Phase 1 trials, 1 Phase 2 trial and 5 Phase 3 trials. All trials have been completed. The main trials for demonstration of efficacy comprise 2 placebo-controlled trials in adult patients with T1D and a placebo-controlled trial in pediatric patients with T1D. The additional trials comprise 2 initial Phase 1 trials, a dose-finding trial, a dedicated immunogenicity trial, an intravenous (IV)/QTc trial, and a bridging trial comparing 2 dasiglucagon batches.

The pharmacokinetic (PK) profile of 0.6 mg dasiglucagon (the dose level tested in Phase 3 trials) was characterized by a rapid increase in plasma levels following SC administration, with a median T_{max} of approximately 35 minutes (range 30 to 45 minutes across trials) in adults and 21 minutes in pediatric patients. Maximum plasma concentration and total exposure appeared higher in adult versus pediatric patients. Of note for age subgroups of the pediatric population, area under the plasma concentration versus time curve (AUC) and maximum observed concentration (C_{max}) values in children (6 to 11 years of age) were higher than in adolescents (12 to 17 years of age) and both were close to the ranges observed in the trials in adult patients. The above PK differences between adult and pediatric patients did not translate into differences in efficacy between the two populations.[3]

2.3 Benefit/Risk Assessment

Glucagon rescue therapies including glucagon emergency kits are underutilized in the treatment of patients who experience severe hypoglycemia. Considering the seriousness and potential complications that can arise from severe hypoglycemic events, it is essential that all patients at risk for severe hypoglycemia are prescribed rescue therapy and that their caregivers receive adequate, hands-on training for use of glucagon rescue treatments to ensure safe, timely, and effective administration. Given the challenges with parent/caregiver training and use of glucagon kits for reconstitution, Zealand Pharma A/S has developed dasiglucagon as a ready-to-use rescue treatment for severe hypoglycemia. The development of dasiglucagon provides parents and caregivers of children with diabetes mellitus a simpler alternative to rescue kits for the treatment of severe hypoglycemia. The ready-to-use product addresses an unmet need and may potentially prove to be lifesaving.

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 (IMP) may result in undesired effects or complaints. Undesired effects and complaints such as nausea and vomiting are known adverse events (AEs) occurring with glucagon administration. Similar AEs have also been observed in the 9 clinical studies conducted with dasiglucagon. As with every investigational therapy, 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. Immunogenic potential has been investigated in the clinical development program to support the indication of treatment of severe hypoglycemia. In the clinical trials for this indication, 4/498 (<1%) of dasiglucagon treated patients developed treatment-emergent anti-drug antibodies (ADAs). For immunogenic potential in other indications with chronic dasiglucagon treatment, refer to the IB for dasiglucagon.[3]

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 IMP 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 9 clinical trials conducted with dasiglucagon. Direct access to resuscitation equipment is ensured at the clinical trial sites.

With the exception of medical examinations, a patient participating in this trial is not likely to derive any personal health-related benefits. The results of the trial may contribute to the future

use of dasiglucagon in patients with diabetes mellitus experiencing severe hypoglycemic reactions.

Overall, the benefit to risk ratio for patients entering the ZP4207-21052 trial is considered acceptable.

More detailed information about the known and expected benefits and risks and reasonably expected AEs of dasiglucagon may be found in the Investigator's Brochure (IB)[3] and the Zegalogue® label.[1]

3 OBJECTIVES AND ENDPOINTS

Objectives	Endpoints
To assess the efficacy of dasiglucagon injection in children < 6 years of age with T1D	Primary endpoint is pharmacodynamics (PD): Plasma glucose change from baseline at 30 minutes after IMP injection or at the time of rescue by IV glucose Secondary endpoint is PD*: Plasma glucose change from baseline at 15 minutes after IMP injection or at the time of rescue by IV glucose
To assess PK of dasiglucagon in children with T1D	PK endpoints will be derived from plasma dasiglucagon profiles from 0 to 300 minutes*: • Area under the plasma concentration versus time curve from 0 to 30 minutes post-dose ($AUC_{0\text{-}30min}$) • AUC from 0 to 300 minutes post-dose ($AUC_{0\text{-}300min}$) • AUC from 0 to the last time point with a measured concentration ($AUC_{0\text{-}t}$) • AUC from 0 to infinity post-dose (AUC_{inf}) • C_{max} • Time to C_{max} • T_{max} • Terminal elimination rate constant of plasma dasiglucagon (λ_z) • Terminal plasma elimination half-life of dasiglucagon ($t_{½}$) • Total body clearance of plasma dasiglucagon (Cl/f) • Volume of distribution of plasma dasiglucagon (Vz/f) • Mean residence time of plasma dasiglucagon (MRT)
To assess the safety profile of dasiglucagon in children with T1D	• AEs • Administration of rescue IV glucose infusion within 30 minutes of IMP injection • Time to first IV glucose infusion following treatment with dasiglucagon within 30 minutes
Other endpoints	• Anti-drug antibodies

Abbreviations: ADA=anti-drug antibodies; AE=adverse event; AUC=plasma concentration versus time curve; IMP=investigational medicinal product; IV=intravenous; PD=pharmacodynamics; PK=pharmacokinetics; T1D=Type 1 diabetes

* as the sampling frequency for PK and PD will gradually be reduced with lower weight for children of 10 kg and below (as specified in Table 6-4), these marked endpoints will be assessed to the extent possible based on the samples obtained for the individual child.

4 TRIAL DESIGN

4.1 Overall Design

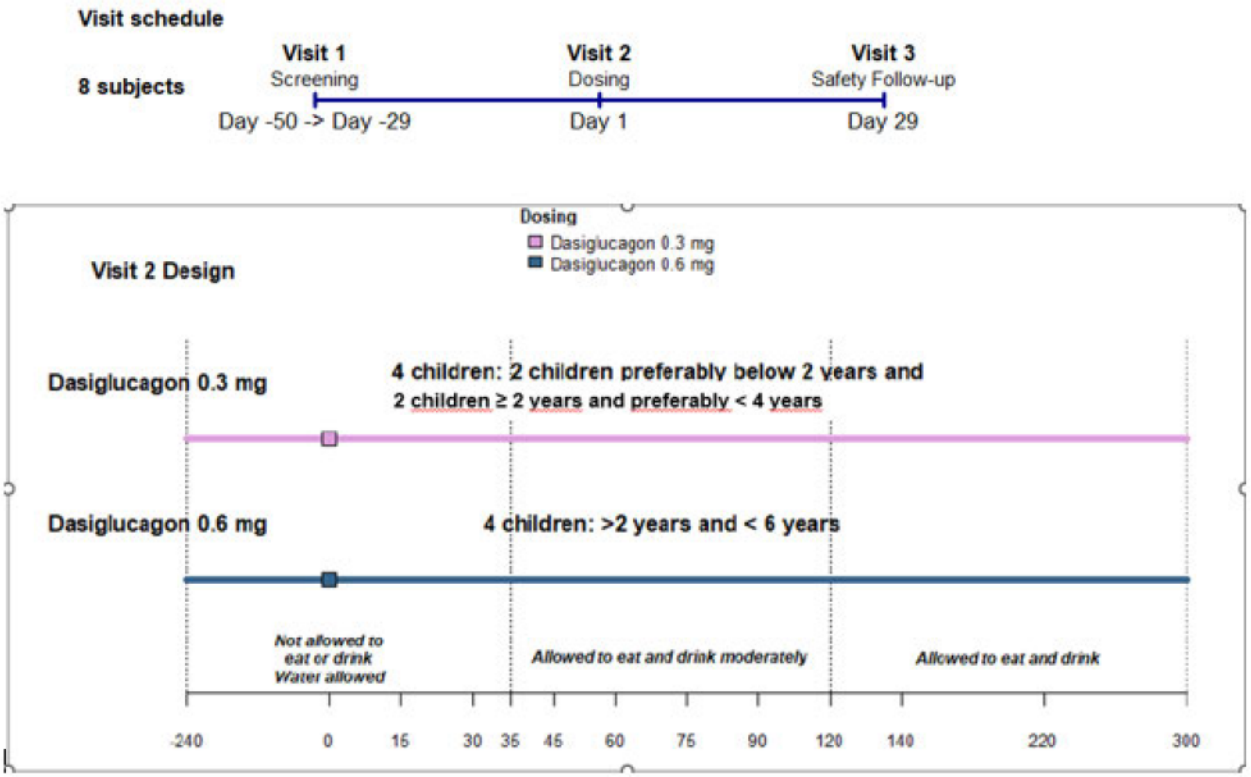
This trial will use a single-administration, open-label trial design to assess the ability of a single SC injection of dasiglucagon to increase plasma glucose in pediatric children with T1D with hypoglycemia. A total of 8 children will be included; 4 children receiving a dose of 0.3 mg and 4 children receiving a dose of 0.6 mg. Of the 4 children receiving 0.3 mg, 2 children should preferably be below 2 years at screening. The remaining 2 children aged 2 years or above receiving 0.3 mg should preferably be below 4 years at screening. For children receiving the 0.6 mg dose, all must be above 2 years at screening. Before an eligible child is dosed, the dose level (0.6 mg or 0.3 mg) must be confirmed by the sponsor. Each child will be dosed after the safety assessment of the preceding child has been completed and assessed by the Trial Safety Group.

The trial will include the following visits (as illustrated in Figure 4-1).

- A screening visit (Visit 1, pre-treatment visit) in the period from Day -50 to Day -29
- A dosing visit (Visit 2), Day 1 (day of single dosing with IMP)
- A Safety follow-up visit (Visit 3) at Day 29 +5 days (the end-of-trial visit)

The primary endpoint of the trial is plasma glucose change from baseline at 30 minutes after IMP injection or at the time of rescue by IV glucose. Pharmacodynamics (PD) i.e., plasma glucose will be assessed at baseline and 15 and 30 minutes after dosing, while the glucose levels will be monitored by CGM and by a plasma glucose analyzer during the dosing visit (Visit 2). PK will be assessed throughout a 300-minute period at the dosing visit (Visit 2). Safety will be assessed prior to dosing and throughout a 300-minute period after dosing and again at the follow-up visit. All data must be logged in the electronic case report form (eCRF).

Figure 4-1 Trial Design



4.2 Scientific Rationale for Trial Design

This is a Phase 3, single-administration open-label trial to assess the efficacy, safety, PK and PD of dasiglucagon when administered as a rescue therapy for hypoglycemia in children below 6 years of age with T1D. A placebo treatment arm was omitted to avoid unnecessary long duration of hypoglycemia in this vulnerable age group.

In the present trial, children with T1D are selected based on the rationale described in Section 2.1. The number of children in the trial is not based on a formal sample size calculation. Considering the lower incidence and prevalence of T1D in this age group, type of indication, and health authorities' feedback, 8 children are believed to be appropriate to assess efficacy and safety.

4.3 Justification for Dose

Dasiglucagon bolus dose of 0.6 mg SC has been approved by FDA for the treatment of severe hypoglycemia in pediatric and adult patients with diabetes aged 6 years and above. Based on population PK model predictions and Pop PK/PD Simulations (Study 20-046), the 0.6 mg dose is expected to be an appropriate dose in an older subset of children < 6 years and ≥ 2 years,

whereas a lower dose of 0.3 mg is expected to be an appropriate dose in a younger subset of children < 6 years. The results from the present trial will be used to determine the appropriate dose(s) for children < 6 years.

The rationale for the suggested split in doses between the 2 groups, which are partially overlapping, is to maximize the efficacy benefit in this vulnerable age group without any increase in untoward effects. Young children are more susceptible to low blood glucose and in addition to this, it has been shown that the frequency of severe hypoglycemia is one of the factors that has a negative impact on specific neuropsychological functions.

4.3.1 Pharmacokinetics

In the population PK report 19-077,[4] 2 models were developed to cover children below 6 years of age: the final population PK model and an alternative population PK model. The final model showed a piece-wise linear relationship between age and relative bioavailability. The alternative model assumed a trial effect of bioavailability for the pediatric trial ZP4207-17086 (age 6 to 17 years) versus adults but was less robust than the final model. The Pop PK/PD Simulations (Study 20-046) used the Pop PD model and both PK models.

Children < 6 years and ≥ 2 years: justification for 0.6 mg dose

The final Pop PK model (Study 19-077) showed a mean C_{max} and AUC that was 1.9 and 1.5 times higher in 2- to 3-year-olds with a 0.6 mg dose compared to the exposure in adults with 0.6 mg, respectively. The alternative model indicated these values to be 4.5 and 3.7 times higher compared to adults. The span of PK predictions based on the final and alternative PK models can be seen as a possible realistic range.

Children preferably < 4 years: justification for 0.3 mg dose

The final Pop PK model (Study 19-077) showed a mean C_{max} and AUC that was 1.4 and 1.1 times higher in 0- to 1-year-olds for a dose of 0.3 mg compared to the exposure in adults with 0.6 mg, respectively. The ratio was 0.9 and 0.7 for C_{max} and AUC in 3- to 4-year-olds for a dose of 0.3 mg compared to the exposure in adults with 0.6 mg, respectively.

The alternative model indicated these values for C_{max} and AUC to be 4.0 and 3.1 times higher in 0- to 1-year-olds and 2.0 and 1.7 times higher in 3- to 4-year-olds compared to adults. The span of PK predictions based on the final and alternative PK models can be seen as a possible realistic range.

4.3.2 Cardiac repolarization and dasiglucagon concentration

To assess the effect of dasiglucagon on cardiac repolarization, trial ZP4207-17144 was conducted in adult healthy volunteers to investigate the effect of IV dasiglucagon up to 1.5 mg on electrocardiogram (ECG) parameters. Dasiglucagon showed no clinically relevant effects on

studied ECG parameters and an effect of dasiglucagon on corrected QT prolongation exceeding 10 ms was excluded within the observed concentration range up to ~30,000 pmol/L.

Children < 6 years and ≥ 2 years: justification for 0.6 mg dose

Since the predicted C_{max} from both PK models (0.6 mg dose: final model: 1,230 to 4,240 pmol/L in children aged 2 to 3 years and alternative model: 3,090 to 10,500 pmol/L in children aged 2 to 3 years) is substantially lower (at least 2.9 times lower) than the observed concentration range, the use of 0.6 mg dasiglucagon is considered to be safe in children < 6 years and ≥ 2 years with regards to the risk of QT prolongation.

Children preferably < 4 years: justification for 0.3 mg dose

Since the predicted C_{max} from both PK models (0.3 mg dose: final model: 926 to 3,660 pmol/L in children aged 0 to 1 years and alternative model: 2,600 to 10,500 pmol/L in children aged 0 to 1 years) is substantially lower (at least 2.9 times lower) than the observed concentration range, the use of 0.3 mg dasiglucagon is considered to be safewith regards to the risk of QT prolongation.

4.3.3 Safety

Overall, the safety profile for dasiglucagon, as determined from the completed development program for treatment of severe hypoglycemia in adults and pediatric patients aged ≥ 6 to ≤ 17 years with a dose of 0.6 mg, is consistent with the well-known class effects associated with glucagon receptor agonism. The most frequently reported AEs were nausea and vomiting, which are known side effects following administration of glucagon.

In the pediatric trial ZP4207-17086, a higher frequency of gastrointestinal events was seen in adolescents than in patients 7 to 11 years of age and no association between nausea/vomiting and exposure (C_{max} and AUC) was detected. In the 4 trials in the development program where a range of dasiglucagon doses was tested in adults, nausea and vomiting were primarily reported with doses of 0.3 mg or higher. The reported nausea and vomiting were mild or moderate and transient. Nausea and vomiting appeared at a similar frequency to marketed glucagon in the pivotal trials in adults. Apart from nausea and vomiting (gastrointestinal AEs), there was no apparent relationship between dasiglucagon dose and the frequency of AEs in any of the trials where a range of doses up to 2 mg SC dasiglucagon was tested.

A single serious AE (SAE) (an event of hypoglycemia in an adult, which was deemed unrelated to investigational product treatment) was reported in the completed development program, and most AEs were events of mild or moderate severity. Antibody formation was observed in less than 1% of patients exposed to dasiglucagon (4/498 patients), and no immunogenicity-related AEs were identified for any of the patients who had anti-dasiglucagon antibodies (3 adults and 1 child).

Taken together, rapid recovery is highly warranted in very young children due to the increased susceptibility and detrimental effects of severe hypoglycemia in this age group. Based on clinical data, no increased safety risks are expected when dosing children < 6 years and ≥ 2 years with 0.6 mg dasiglucagon and children preferably below 4 years with 0.3 mg dasiglucagon even though PK simulations predict a higher exposure compared to adults and children ≥ 6 years. Gastrointestinal events such as nausea and vomiting have been reported throughout the completed clinical program. In the completed pediatric trial, no relationship was seen between dasiglucagon exposure and the occurrence of nausea and vomiting that would indicate any increased risk in younger children. No effect on cardiac repolarization is expected since the maximum predicted dasiglucagon concentration in young children is substantially lower (at least 2.9 times lower) both for children < 6 years and ≥ 2 years receiving 0.6 mg and children preferably below 4 years receiving 0.3 mg dasiglucagon than the dasiglucagon concentration established to have no corrected QT prolongation exceeding 10 mg in adults (trial ZP4207-17144).

4.4 End-of-Trial Definition

The end of the trial is defined as the date of the last visit of the last patient in the trial.

A patient is considered to have completed the trial if he/she has completed all phases of the trial including the last follow-up visit (Visit 3).

5 TRIAL POPULATION

Prospective approval of protocol deviations to recruitment and eligibility criteria, also known as protocol waivers or exemptions, are not permitted.

5.1 Inclusion Criteria

Children are eligible to be enrolled in the trial only if all of the following criteria apply:

Age

1. Child must be < 6 years at the time of screening.

Type of Patient and Disease Characteristics

2. Children who are confirmed as having T1D based on medical history and are receiving daily insulin therapy via insulin pump or MDI.

Informed Consent

3. Following receipt of verbal and written information about the trial, the parent(s) or legal guardian of the child must be capable of giving signed informed consent before any trial-related activity is carried out as described in Appendix 1 which includes compliance with the requirements and restrictions listed in the informed consent form (ICF) and in this protocol.
4. Ability to adhere to the protocol requirements.

Weight

5. Body weight > 8 kg.

5.2 Exclusion Criteria

Patients are excluded from the trial if any of the following criteria apply:

Medical Conditions

1. Known or suspected allergy to the IMP or related products
2. Any condition that in the investigators opinion may result in diminished hepatic glycogen stores (eg, prolonged fasting (more than 24 hours) at Visit 2
3. History of anaphylaxis or symptoms of severe systemic allergy (such as angioedema)
4. History of any hypoglycemic event indicating trial procedures to be high risk for the child, as judged by the investigator
5. History of severe hypoglycemic event (defined as an event associated with a seizure or loss of consciousness or requiring emergency medical personnel, a visit to the emergency department, or a hospital admission) within 3 months prior to screening
6. History of epilepsy or seizure disorder
7. Active malignancy

8. Congenital heart disease
9. Current bleeding disorder, including anti-coagulant treatment
10. Known presence or history of pheochromocytoma (ie, adrenal gland tumor) or insulinoma (ie, insulin secreting pancreas tumor)
11. Surgery or trauma with significant blood loss within the last 2 months prior to screening

Prior/Concomitant Therapy

12. Use of daily systemic beta-blocker, indomethacin, warfarin, or anticholinergic drugs
13. Use of prescription or non-prescription medications known to cause QT prolongation

Prior/Concurrent Clinical Trial Experience

14. Previous participation in this trial. Participation is defined as received treatment with IMP
15. Receipt of any investigational drug within 3 months prior to screening

Diagnostic Assessments

16. Aspartate aminotransferase (AST) or alanine aminotransferase (ALT) >2.5 × the upper limit of the normal range (ULN)
17. Estimated glomerular filtration rate <30 mL/min/1.73 m^2 according to the Modification of Diet in Renal Disease study definition
18. Altered electrolyte values of clinical relevance for cardiac conduction, as judged by the investigator
19. Clinically significant abnormal ECG at screening as judged by the investigator
20. Clinically significant illness within 4 weeks before screening, as judged by the investigator

Other Exclusions

21. Any condition that might jeopardize the child's safety or compliance with the protocol as judged by the investigator

5.3 Lifestyle Considerations

5.3.1 Meals and Dietary Restrictions (Visit 2)

1. Children will not be allowed to eat or drink (except water) from 4 hours before dosing until 35 minutes after dasiglucagon administration.
2. 35 minutes after dosing, children will be allowed to eat and drink moderately (appropriate to their body size, with a maximum of 50 grams carbohydrates) to minimize discomfort in terms of potential nausea. 2 hours after dosing, children can eat and drink their usual meals. The amount and type of food and drink consumed will be recorded.

5.3.2 Activity

3. Children should remain in bed, to the extent possible, from approximately 30 minutes before hypoglycemia induction until completion of the test procedure 300 minutes after dosing (bathroom visits are allowed).

5.4 Screen Failures

Screen failures are defined as patients who consent to participate in the clinical trial but are not subsequently eligible for the trial. A minimal set of screen failure information is required to ensure transparent reporting of screen failure patients to meet the Consolidated Standards of Reporting Trials publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any SAE. The reason for screen failure must be captured in the eCRF.

Individuals who do not meet the criteria for participation in this trial (screen failure) may be rescreened if investigator judges that rescreening is appropriate due to inclusion or exclusion criteria that may have changed. Rescreening may only be performed once.

5.5 Criteria for Temporarily Delaying Enrollment and Administration of Trial Intervention

Each child may have their dosing visit rescheduled within the visit window for Visit 2 with an additional 21 days compared to Visit 1 (thus the maximum time interval between Visit 1 and Visit 2 will be 50 days + 21 days) due to the following circumstances:

- If the patient has taken any prohibited medication prior to the dosing visit (Visit 2)
- If transient illness means that the patient cannot attend the planned dosing visit (Visit 2)
- If the patient cannot attend the planned dosing visit (Visit 2) due to logistical reasons
- If the target plasma glucose cannot be achieved on the dosing day (Visit 2) while inducing hypoglycemia

6 TRIAL INTERVENTION(S) AND CONCOMITANT THERAPY

Trial intervention is defined as any investigational intervention(s), marketed product(s), or medical device(s) intended to be administered to a trial patient according to the trial protocol.

6.1 Trial Intervention(s) Administered

The IMP of 0.6 mg/0.6 mL dasiglucagon, will be supplied by the sponsor and delivered in a pre-filled syringe. Only eligible children may receive dasiglucagon. Dasiglucagon will be prepared for administration by a designated person at the clinical research site and will be administered by authorized site staff as a SC injection into the buttocks.

The pre-filled syringe does not have a scale. Therefore, administration of 0.3 mg/0.3 mL dasiglucagon requires additional preparation, where dasiglucagon will be transferred into a sterile vial and the volume needed in order to administer 0.3 mL will be drawn into a syringe. The needle used for administration will match the needle of the pre-filled syringe (27G, ½ inch). Needle, syringe, and vials will be supplied by Zealand Pharma. Separate Instructions for Use included in the Trial Materials Manual will describe preparation and administration of the 0.3 mg/0.3 mL dose.

The IMP must be visually inspected prior to administration. The solution should appear clear, colorless, and free from particles. If the solution is discolored or contains particulate matter, do not use. The dose (0.3 or 0.6 mg), date and time of each dose administered will be recorded in the source documents.

Table 6-1 Identification of Trial Intervention

	Investigational medicinal product	Background intervention	CGM
Intervention Name	Dasiglucagon	Apidra®	Dexcom 6
Active Substance	Dasiglucagon	insulin glulisine	Not applicable
Type	Drug	Drug	Device
Dose Formulation	Liquid formulation, 0.6 mL	Liquid formulation, dose according to weight (see Section 6.5.1)	Not applicable
Unit Dose Strength(s)	0.6 mg/0.6 mL	100 units/mL	Not applicable
Dosage Level(s)	Single dose	Continuous infusion	Not applicable
Route of Administration	Subcutaneous injection	IV	Not applicable
Use	Experimental	Background intervention	Diagnostic
IMP and NIMP/AxMP	IMP	NIMP	Not applicable
Sourcing	Provided centrally by the sponsor	Provided by investigator	Provided centrally by the sponsor
Packaging and Labeling	Single use pre-filled syringe. Each pre-filled syringe will be labeled as required per country requirement	Apidra®	Dexcom 6 (manufacturer: Dexcom) will be provided by sponsor

Abbreviation: CGM=continuous glucose monitoring; IMP=investigational medicinal product; IV=intravenous; NIMP=non-investigational medicinal product

6.1.1 Medical Devices

- No sponsor manufactured stand-alone medical devices (or devices manufactured for sponsor by a third party) are provided for use in this trial.
- Glucose analyzer: Glucose analyzers available at the sites will be used to sample plasma glucose.
- CGM tracing: Dexcom G6 Continuous Glucose Monitoring System will be used to sample glucose levels and will be provided by the sponsor for use in this trial. Sponsor-provided Dexcom 6 devices must be used for all patients. The CGM device will be fitted at the site after attending Visit 2 and at least 2 hours before the planned hypoglycemia induction. The Dexcom G6 CGM will usually require a 2-hour warm-up period to acclimatize before the sensor reading will start. The hypoglycemia induction should not start until the sensor warm-up period is completed and data is received from sensor. All patients must remain fitted with the CGM until 300 minutes after IMP administration. Patients will be under medical supervision at all times while the device is in use. For the purpose of this trial, the device may be used outside the intended purpose for patients

below 2 years (approval applies to 2 years and older). No mechanical or design changes have been made to the device. The CGM device is used as intended according to the CE mark and the 510K clearance, except for using it in patients below the approved age of 2 years.

- For interstitial glucose data generated by the Dexcom G6 CGM System, the investigator will ensure that CGM data are downloaded from the device at Visit 2.
- Instructions for medical device use are provided by the manufacturer of the medical device. The Dexcom G6 System should be used according to the manufacturer's instructions; it does not require calibration. The contract research organization (CRO) or delegate will handle the sourcing, configuration for use in the trial, procedures for data extraction, service, and return handling for the Dexcom G6 System.
- All technical complaints regarding the dasiglucagon pre-filled syringes shall be documented and reported by the investigator throughout the trial (see Section 8.3.9) and appropriately managed by the sponsor.

6.2 Preparation/Handling/Storage/Accountability

Dasiglucagon must be stored in a refrigerator (36°F to 46°F/2°C to 8°C). The investigator or designee must confirm that appropriate conditions including temperature conditions have been maintained during transit to and storage at site for all dasiglucagon received and any discrepancies are reported and resolved before use of dasiglucagon.

All dasiglucagon must be stored in a secure, environmentally controlled, and monitored (manual or automated) area in accordance with the labeled storage conditions with access limited to the investigator and authorized site staff.

The investigator or designee is responsible for dasiglucagon accountability, reconciliation, and record maintenance (ie, receipt, reconciliation, and final disposition records). Each pre-filled syringe will have a trial unique dispensing unit number (DUN), which is to be used for traceability purposes.

Further guidance and information related to handling and preparation of dasiglucagon including supply ordering, assignment of dasiglucagon, handling of temperature excursions, and final disposition of used/unused dasiglucagon are provided in the Trial Materials Manual (TMM).

6.3 Trial Intervention Compliance

Patients are dosed at the site, they will receive dasiglucagon injection directly from the investigator or designee, under medical supervision. The dose (0.3 or 0.6 mg) and date and time of each dose administered will be recorded in the source documents. The dose of dasiglucagon and trial patient identification will be confirmed at the time of dosing by a member of the trial site staff other than the person administering the trial intervention.

6.4 Dose Modification

No dose modification is planned for this trial.

6.4.1 Retreatment Criteria

No retreatment criteria are planned for this trial.

All patients entered into the trial will be treated at Visit 2. If the target plasma glucose cannot be achieved on the dosing day during the induced hypoglycemia procedure, the patient can be rescheduled as described in Section 5.5.

6.5 Treatment and Dosing Conditions

Patients will be admitted to the clinic with their parent(s)/legal guardian(s) the night before or at early morning of the dosing day (Visit 2). The patient's eligibility will be checked.

The CGM device will be fitted at the site after attending Visit 2 and at least 2 hours before the planned start of the hypoglycemia induction. The Dexcom G6 CGM will usually require a 2-hour warm up period to acclimatize before the sensor reading will start. The hypoglycemia induction should not start until the sensor warm up period is completed and data is received from sensor. All patients must remain fitted with the CGM device until 300 minutes after IMP administration.

For patients on multiple daily injection insulin therapy

For patients on multiple daily injections (MDI), insulin therapy will be adjusted at the discretion of the investigator in the days prior to the dosing visit (Visit 2) with the purpose of having as low as ambient insulin exposure during the intervention period on Day 1 as possible. During the period with adjusted insulin treatment, the safety of the patient will be ensured by frequent plasma glucose monitoring and contact with the clinical site, as needed. Details (insulin, dose level and timing) regarding the patient's regular insulin therapy and the insulin therapy in the days prior to the dosing visit (visit 2) will be captured in the eCRF for the individual patient.

For patients on insulin pump therapy

For patients on insulin pump therapy, the patient's insulin pump is stopped approximately 1 hour prior to dosing in the morning of Day 1, Visit 2.

Details (insulin, dose level and timing) regarding the patient's insulin therapy will be captured in the eCRF for the individual patient.

6.5.1 Hypoglycemic Clamp Procedure and Dosing

Hypoglycemia induction will be initiated using IV insulin to target a plasma glucose level between 70 and 90 mg/dL (between 3.9 and 5 mmol/L).

Insulin (only insulin glulisine [Apidra®] will be used) will be provided by the investigator. Insulin infusion for IV use will be prepared according to the patient's body weight at the dosing visit.

Preparation of the insulin infusion:

- 0.5 international units insulin glulisine (Apidra®) per kg body weight
- 48 mL NaCl 0.9%

Plasma glucose must be checked using glucose analyzer before initiation of induction of hypoglycemia. Hypoglycemia will be induced using insulin IV infusion rates according to plasma glucose levels according to Table 6-2.

Table 6-2 Insulin Infusion Rates

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
90–149 mg/dL	2.5 mL/h	0.025
<90 mg/dL	Insulin infusion stop!	0

Abbreviations: BW=body weight; IU=International units

The procedure to induce hypoglycemia is given as a guidance and any deviation to increase or decrease the insulin rate is allowed at the discretion of the investigator. If the target plasma glucose cannot be achieved on the dosing day, the patient can be rescheduled for another visit as described in Section 5.5.

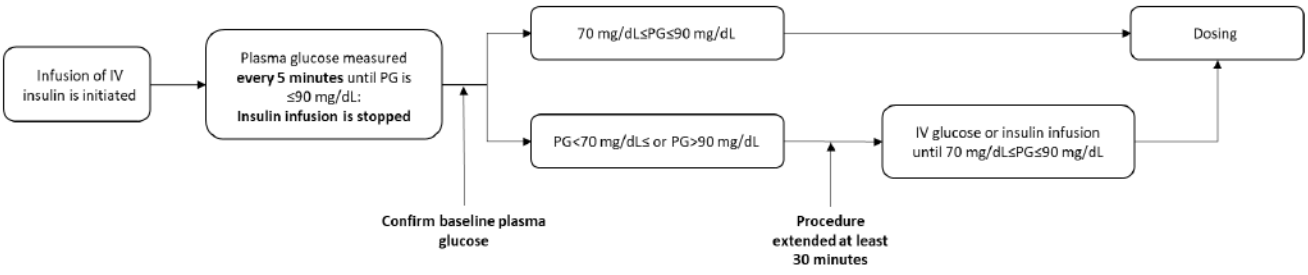
During the induction phase (during insulin infusion) and following the administration of dasiglucagon, glucose levels will be monitored by both CGM and by a glucose analyzer (see Section 6.1.1). Plasma glucose levels should preferably be checked by glucose analyzer approximately every 10 minutes while glucose levels are above 110 mg/dL (6.1 mmol/L), and preferably approximately every 5 minutes once glucose levels are at or below 110 mg/dL (6.1 mmol/L). Plasma glucose should be confirmed by the glucose analyzer prior to the administration of dasiglucagon. After dosing with dasiglucagon, once glucose levels reach ≥110 mg/dL (6.1 mg/dL), glucose levels should preferably be checked approximately every 30 minutes until 300 minutes. The monitoring frequency of plasma glucose via the glucose analyzer should be adjusted as needed based on the child's body weight to ensure that a maximum of approximately 1.5% is obtained during the dosing visit as specified in Section 6.5.3 (1.5% equals 1.13 to 1.20 mL/kg with the total blood volume estimated to be 75 to 80 mL/kg).[5]

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 (eg, symptoms) of a hypoglycemic episode.

A catheter (eg, an indwelling catheter) will be inserted into the child's arm for blood draw for PK/PD sampling. If possible, the same IV access as used for insulin infusion can be used for the PK/PD sampling to reduce the stress and discomfort for the child. Attention should be made to ensure blood samples will be collected from the circulating blood volume and not from a reservoir dead space created by the catheter. If direct venipunctures are considered more appropriate, especially for those children where the number of PK and PD samples are reduced (refer to Table 6-4), venipunctures can be used instead of indwelling catheters. The catheter will be placed in the morning prior to IMP administration. Blood samples for baseline assessment of plasma glucose and dasiglucagon PK will be collected approximately 5 minutes after the glucose concentration reaches ≥70 mg/dL and <90 mg/dL (3.9 to 5.0 mmol/L). These samples are the baseline samples and should be collected within 2 minutes before IMP administration. The baseline samples will be sent to the clinical laboratory and analyzed using a sensitive and validated assay for glucose measurements. The assay used for primary endpoint glucose measurements should fulfil the requirements of the US FDA Bioanalytical Method Validation Guidance for Industry Section 5 B and C for biomarkers.[6] The sampling procedure is described in Section 6.5.3. Before IMP administration, it must be confirmed by the glucose analyzer that the plasma glucose is still ≥70 mg/dL and <90 mg/dL.

If plasma glucose is < 70 mg/dL (3.9 mmol/L), IV glucose solution will be administered to raise plasma glucose to within the 70 to 90 mg/dL (3.9 to 5.0 mmol/L) target range. The run-in period will be adequately extended (at least 30 minutes) until the above target is achieved, the glucose infusion has been discontinued, and new baseline samples for plasma glucose will be collected. In this case, glucose should not be infused within 30 minutes before IMP 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).

Figure 6-1 Insulin-Induced Hypoglycemia and Dosing



Abbreviations: IV=intravenous; PG=plasma glucose

Notes: Patients will be admitted to the clinic with their parent(s)/legal guardian(s) in the night before or at early morning of the dosing day.

If insulin infusion is used; dosing should not be done until at least 5 minutes after infusion is stopped.

If glucose infusion is used dosing should not be done until at least 30 minutes after infusion is stopped.

Assessments and blood sampling will be performed ongoing from pre-procedure/pre-dose until 300 minutes post-dose according to protocol.

Patients will not be allowed to eat or drink (except water) from 4 hours before dosing until 35 minutes after dasiglucagon administration. Patients will only be allowed to eat and drink moderately (appropriate to their body size, with a maximum of 50 grams carbohydrates) starting 35 minutes after IMP administration to minimize discomfort in terms of potential nausea. Two hours after dosing, patients can eat and drink their usual meals. The amount and type of food and drink consumed will be recorded. Patients should remain in bed, to the extent possible, until completion of the test procedure 300 minutes after dosing (bathroom visits are allowed).

6.5.2 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 IMP (t=0), if their glucose levels become too low, patients may receive post-treatment IV glucose infusion to ameliorate hypoglycemia, as follows:

- The IV glucose infusion should be initiated if a patient experiences escalating symptoms of hypoglycemia (eg, start of moderate symptoms of hypoglycemia) at any time during the test procedure. Glucose infusion should then be initiated targeting a plasma glucose level >90 mg/dL.
- If plasma glucose is <70 mg/dL between t=8 and t=30 minutes, glucose infusion (eg, 1 to 2 mg/kg administered over 5 to 10 seconds) should be initiated to maintain plasma glucose between 70 mg/dL (inclusive) and 90 mg/dL. Pause glucose infusion if plasma glucose is >90 mg/dL.
- If plasma glucose is <90 mg/dL at t≥30 minutes, glucose infusion (eg, 2 to 3 mg/kg administered over 5 to 10 seconds) should be initiated. Pause glucose infusion when plasma glucose is >90 mg/dL.

6.5.3 Collection of Blood Samples

The total blood volume to be obtained from any individual child with a body weight of more than 10.0 kg will be about 22.5 mL (see Table 6-3 and Table 6-4 for details). For children with a body weight ≤ 10.0 kg, the frequency of PD (for efficacy) and PK samples will gradually be reduced as specified in Table 6-4 to minimize the blood volume obtained at a single visit (to approximately maximum 1.5% of total blood volume) and over a four-week period (to approximately maximum 3% of total blood volume). These values are within the recommended scope provided by guidelines and based on a child's total blood volume being 75 to 80 mL/kg.[5]

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

Table 6-3 Blood Volumes

Sample type	Visit 1 (mL)	Visit 2 (mL)	Visit 3 (mL)	
Biochemistry	2.00	-	2.00	
Hematology	1.20	-	1.20	
ADA	3.00	-	3.00	
PK*	-	5.00	-	
PD*	-	3.60	-	
Onsite-PG	-	1.50	-	
Sum for each visit	6.2	10.1	6.2	
Total				22.5

Abbreviations: ADA=anti-drug antibodies; Onsite-PG=onsite monitoring of plasma glucose; PD=pharmacodynamics; PK=pharmacokinetics

* Refer to Table 6-4 for details on which PD and PK samples to be collected based on body weight at Visit 1 and Visit 2.

Table 6-4 PD (for efficacy) and PK Sampling Based on Body Weight

Body weight at Visit 2	Visit 2	
	PD samples to draw	PK samples to draw
BW> 10.0 kg	pre-dose 15 min 30 min	pre-dose 10 min 20 min 30 min 40 min 60 min 90 min 140 min 220 min 300 min
10.0 ≥ BW > 9.0 kg	pre-dose 15 min 30 min	pre-dose 10 min 20 min 30 min 40 min 60 min 90 min 140 min 300 min
9.0 ≥ BW > 8.0 kg	pre-dose 30 min	pre-dose 10 min 20 min 30 min 40 min 60 min 90 min 140 min 300 min

Abbreviations: BW=body weight; PD=pharmacodynamics; PK=pharmacokinetics

6.6 Continued Access to Trial Intervention After Visit 2

After the dosing visit (Visit 2), patients should return to the standard of care that they received prior to the wash-out period. The treating physician/investigator will be responsible for supervising patients' diabetes management after the dosing visit (Visit 2). The parent(s)/legally authorized representative (LAR) will be encouraged to contact the investigator should they have any questions about the daily basal insulin treatment after the time of discharge. If there are any safety concerns at the time of discharge the in-house visit can be extended at the discretion of the investigator.

6.7 Treatment of Overdose

For this trial, any dose greater than 0.6 mg or 0.3 mg of dasiglucagon for the relevant body weight and age ranges will be considered an overdose.

Zealand Pharma A/S recommends symptomatic treatment in the event of an overdose.

In the event of an overdose, the investigator should:

- Contact the medical monitor immediately.
- Assess the patient to determine, in consultation with the medical monitor, whether trial intervention should be interrupted.
- Closely monitor the patient for any AE/SAE and laboratory abnormalities until dasiglucagon can no longer be detected systemically (at least 1 day).
- Document the quantity of the overdose.

6.8 Concomitant Therapy

Details of any concomitant medication must be recorded in the eCRF at trial entry (ie, at screening). Any changes in concomitant medication must be recorded in the eCRF at each visit as they occur.

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 to 7 days later. See Section 7 for possible reasons for patient discontinuation.

7 DISCONTINUATION OF TRIAL INTERVENTION AND PATIENT DISCONTINUATION/WITHDRAWAL

7.1 Discontinuation of Trial Intervention

This is a single dose intervention. For cases where the target plasma glucose cannot be achieved on the dosing day (Visit 2) while inducing hypoglycemia, see Section 5.5.

7.2 Patient Discontinuation/Withdrawal from the Trial

Participation in the trial is strictly voluntary. Patient's parent(s)/legal guardian(s) will be advised that they are free to withdraw their child from participation in this trial at any time, for any reason and without prejudice. If the patient's parent(s)/legal guardian(s) chooses 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 eCRF.

If withdrawal occurs following administration of the IMP, the patient will be asked to consider completing the PD sampling at 30 minutes and participate in the follow-up visit at trial Day 29. 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 IMP 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 a patient discontinues trial participation due to the ongoing severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) global pandemic, information should be captured on the eCRF so that this can be summarized in the clinical study report at the end of the trial, in line with the FDA Guidance on Conduct of Clinical Trials of Medical Products during COVID-19 Public Health Emergency (FDA 2020). The reason for discontinuation should be recorded on the eCRF as SARS-CoV-2/COVID-19, if applicable, and include as many details as possible. For example, specific reasons may include, but are not limited to:

- The patient exhibits symptoms consistent with COVID-19.
- The patient has a positive test result for SARS-CoV-2.
- The patient has neither symptoms nor a positive test but has chosen to discontinue study participation due to COVID-19 concerns.

7.3 Lost to Follow-Up

A patient will be considered lost to follow-up if he or she repeatedly fails to return for scheduled visits and is unable to be contacted by the trial site.

The following actions must be taken if a patient fails to return to the clinic for a required trial visit:

- The site must attempt to contact the patient's parent(s)/legal guardian(s) and reschedule the missed visit as soon as possible and ascertain whether or not the patient wishes to and/or should continue in the trial.
- Before a patient is deemed lost to follow-up, the investigator or designee must make every effort to regain contact with the patient's parent(s)/legal guardian(s) (where possible, telephone calls and, if necessary, a certified letter to the last known mailing address of the patient's parent(s)/legal guardian(s) or local equivalent methods). These contact attempts should be documented in the patient's medical record.
- Should the patient's parent(s)/legal guardian(s) continue to be unreachable, the patient will be considered to have withdrawn from the trial.

Discontinuation of specific sites or of the trial as a whole are handled as part of Appendix 1.

8 TRIAL ASSESSMENTS AND PROCEDURES

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

Informed consent will be obtained prior to any trial-related procedures; see Section 10.1.3.

- Trial procedures and their timing are summarized in the SoA. Protocol waivers or exemptions are not allowed.
- Immediate safety concerns should be discussed with the sponsor immediately upon occurrence or awareness to determine if the patient should continue or discontinue trial intervention.
- Adherence to the trial design requirements, including those specified in the SoA, is essential and required for trial conduct.
- All screening assessments must be completed and reviewed to confirm that potential patients meet all eligibility criteria. The investigator will maintain a screening log to record details of all patients screened and to confirm eligibility or record reasons for screening failure, as applicable.
- Procedures conducted as part of the patient's routine clinical management (eg, blood count) and obtained before signing of the ICF may be utilized for screening or baseline purposes provided the procedures met the protocol-specified criteria and were performed within the time frame defined in the SoA.

8.1 Assessments and Schedule of Measurements

8.1.1 Screening Visit

Patients will come to the trial site for screening assessments. Prior to any trial-related procedures, the informed consent procedure will be performed; the trial will be explained in detail to the patients' parent(s)/legal guardian(s) and consequently the ICF will be signed by legally responsible individual(s) per local regulations. Then, the screening procedures will be performed as described in the SoA.

Consented patients will be provided with a Patient Identification Card (ID card), stating that the patient is participating in the trial and whom to contact (site address, Investigator name and telephone number). The patients' parent(s)/legal guardian(s) should be instructed to return the ID card to the Investigator at the last visit or to destroy the card after the last 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 (including information on prior use of ZEGALOGUE® and other glucagon products [number of times used, date of last time used])
- Physical examination
- Vital signs
- 12-lead ECG
- AEs
- Biochemistry and hematology
- Antibodies against dasiglucagon

8.1.2 Dosing Visit

The dosing visit (Visit 2) will take place 4 to 7 weeks after Visit 1 in order to allow sufficient recovery between blood draws in order to accommodate pediatric volume restrictions. Patients will be admitted to the clinic with their parent(s)/legal guardian(s) in the night before or at early morning of the dosing day (Day 1). The patient's eligibility will be checked.

The following assessments will take place:

- Inclusion/exclusion criteria (prior to the start of the insulin-induced hypoglycemic procedure. Check of exclusion criteria at clinic admission on Visit 2 and changes between screening visit [Visit 1] and Visit 2)
- Body measurements
- Diabetes diagnosis and current diabetes treatment
- Concomitant medications (prior to the start of the insulin-induced hypoglycemic procedure, and during and after the insulin-induced hypoglycemic procedure)
- Exclusion criteria at clinic admission (prior to the start of the insulin-induced hypoglycemic procedure)
- Insulin-induced hypoglycemia
- Physical examination
- 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)
- 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, PK sampling as per Table 6-4. The actual time of blood sampling should not deviate from the nominal time by more than ± 1 minute. Pre-dose is defined as within 2 minutes prior to dosing.
- Plasma glucose (PD) sampling as per Table 6-4. The actual time of blood sampling should not deviate from the nominal time by more than ± 1 minute for the collection time points. Pre-dose is defined as within 2 minutes prior to dosing).
- If rescue treatment is required before 30 minutes after dosing, a blood sample for plasma glucose (PD sample) will be collected just before start of rescue treatment. No further plasma glucose (PD sample) samples are required after rescue treatment has been employed.
- Plasma glucose using glucose analyzer during induction of hypoglycemia and until 300 minutes after dosing. Plasma glucose levels must be checked by glucose analyzer approximately every 10 minutes while glucose levels are above 110 mg/dL (6.1 mmol/L), and approximately every 5 minutes once glucose levels are at or below 110 mg/dL (6.1 mmol/L). Plasma glucose should be confirmed by the glucose analyzer prior to the pre-dose PK and PD samples and prior to the administration of dasiglucagon. After dosing with dasiglucagon, once glucose levels reach ≥110 mg/dL (6.1 mg/dL), glucose levels should be checked approximately every 30 minutes until 300 minutes.
- Glucose levels using CGM during induction of hypoglycemia and until 300 minutes after dosing. Dexcom 6 will be fitted in the morning at Visit 2 and must remain fitted until 300 minutes after IMP administration.
- Administration of IMP (during hypoglycemic clamp procedure)

8.1.3 Follow-up Visit

A follow-up visit (Visit 3) is planned 4 weeks after the dosing day visit to assess:

- Concomitant medications
- Diabetes diagnosis and current diabetes treatment
- Physical examination
- Vital signs
- Local tolerability
- AEs
- Biochemistry and hematology
- Antibodies against dasiglucagon
- End-of-trial status

Patients with anti-dasiglucagon antibodies (treatment-induced or treatment-boosted [titer increase above 4-fold]) at Visit 3 will be invited for follow-up ADA sampling approximately 6 months after their follow-up visit (refer to Table 1-1 visit x). If no clinically relevant outcomes

related to anti-dasiglucagon antibodies are detected during this 6-month follow-up period or the ADA has returned to baseline, further follow-up sampling should be discontinued. If clinically relevant outcomes have been detected and the patient is still ADA-positive, a further sample will be collected after another 6 months.

8.2 Safety Assessments

8.2.1 Physical Examinations

Physical examination is performed at screening (Visit 1), dosing (Visit 2, prior to dosing) and end-of-trial (Visit 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, and lymph nodes.

8.2.2 Vital Signs

Vital signs will be measured at screening (Visit 1), dosing (Visit 2) and end-of-trial (Visit 3). Blood pressure, pulse and body temperature will be measured after 5 minutes in a seated position. See the SoA (Section 1.3) for the timing of vital sign measurements at the dosing visit.

8.2.3 Electrocardiograms

A 12-lead ECG assessment will be performed at screening (Visit 1). Details from the ECG assessments will be recorded, including heart rate, PR, QRS, and QT interval and QTc corrected by Fridericia´s formula interval (QTcF). In case the QTcF values are not readily available from the ECG machine, sites must perform the calculation based on the relevant parameters obtained from the device.

8.2.4 Local Tolerability

Local tolerability assessments at 30, 120, and 300 minutes after IMP administration will be performed to assess the following:

- Spontaneous pain
- Pain on palpation
- Itching
- Redness
- Edema
- Induration/infiltration
- Other

Injection site reactions and any findings will be reported as AEs (see Section 8.3).

8.2.5 Clinical Safety Laboratory Assessments

See Appendix 2 for the list of clinical laboratory tests to be performed and to the SoA (Section 1.3) for the timing and frequency.

8.3 AEs, SAEs, and Other Safety Reporting

The definitions of AEs and SAEs can be found in Appendix 3.

AEs will be reported by the patient (or, when appropriate, by a caregiver, parent, or the patient's LAR).

The investigator and any qualified designees are responsible for detecting, documenting, and recording events that meet the definition of an AE or SAE and remain responsible for following up all AEs.

The method of recording, evaluating, and assessing causality of AEs and SAEs and the procedures for completing and transmitting SAE reports are provided in Appendix 3.

8.3.1 Time Period and Frequency for Collecting AE and SAE Information

All AEs and SAEs will be collected from the first trial-related activity after signing of the ICF until the end of the post-treatment follow-up period (which may include contacts for follow-up of safety) at the time points specified in the SoA (Section 1.3).

All SAEs will be recorded and reported to the sponsor or designee immediately and under no circumstance should this exceed 24 hours, as indicated in Appendix 3. The investigator will provide any updated SAE data to the sponsor within 24 hours of it being available.

Investigators are not obligated to actively seek information on AEs or SAEs after conclusion of the trial participation. However, if the investigator learns of any SAE, including a death, at any time after a patient has been discharged from the trial, and he/she considers the event to be reasonably related to the trial intervention or trial participation, the investigator must promptly notify the sponsor.

8.3.2 Method of Detecting AEs and SAEs

Care will be taken not to introduce bias when detecting AEs and/or SAEs. Open-ended and non-leading verbal questioning of the patient and patient's parent(s)/legal guardian(s) is the preferred method to inquire about AE occurrences. In addition, patients will be observed for any signs or symptoms.

8.3.3 Follow-up of AEs and SAEs

After the initial AE/SAE report, the investigator is required to proactively follow each patient at subsequent visits/contacts. All SAEs, and AEs of special interest (as defined in Appendix 3), will be followed until resolution, stabilization, the event is otherwise explained, or the patient is lost

to follow-up (as defined in Section 7.3). Further information on follow-up procedures is provided in Appendix 3.

8.3.4 Regulatory Reporting Requirements for SAEs

Prompt notification by the investigator to the sponsor of an SAE is essential so that legal obligations and ethical responsibilities towards the safety of patients and the safety of a trial intervention under clinical investigation are met.

The sponsor has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a trial intervention under clinical investigation. The sponsor will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority, Institutional Review Boards (IRB)/Independent Ethics Committees (IEC), and investigators.

An investigator who receives an investigator safety report describing an SAE or other specific safety information (eg, summary or listing of SAEs) from the sponsor will review and then file it along with the IB and will notify the IRB/IEC, if appropriate according to local requirements.

Investigator safety reports must be prepared for suspected unexpected serious adverse reactions according to local regulatory requirements and sponsor policy and forwarded to investigators as necessary.

8.3.5 AEs of Special Interest

An AE of special interest (AESI) is an event that, in the evaluation of safety, has a special focus (eg, 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 Section 8.3.1 and Appendix 3).

8.3.6 Hypoglycemia

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

Since hypoglycemia will be induced during the dosing visit, in accordance with hypoglycemia clamp procedure described in Section 6.5.1, ONLY plasma glucose below 3.0 mmol/L (54 mg/dL) must be reported as an AE, at the dosing visit (Visit 2), or at instances that IV

glucose is given after IMP administration. The hypoglycemic event should be confirmed by glucose analyzer. If only the CGM device is used, the hypoglycemic event should be present for at least 15 minutes below 3.0 mmol/L for AE reporting.

8.3.7 Hypersensitivity

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.

8.3.8 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 × ULN and total bilirubin > 2 × ULN, where no alternative etiology exists
- Suspicion of transmission of infectious agents via the IMP
- Overdose of the IMP
- Suspected abuse or misuse of the IMP
- Medication error involving the IMP
- Inadvertent or accidental exposure to the IMP

8.3.9 Handling of Technical Complaints Regarding Dasiglucagon Pre-filled Syringes

A technical complaint is any communication that alleges product (IMP) deficiencies related to the identity, quality, durability, reliability, safety, effectiveness, or performance. The technical complaint may or may not be associated with an AE.

Recording and Follow-up of Technical Complaints

Technical complaints must be recorded on a technical complaint form in the eCRF. If the eCRF is not available, a paper form must be filled out, and the eCRF must be filled out when available.

Timelines for Reporting of Technical Complaints

The investigator must complete the technical complaint form in the eCRF within:

- 24 hours if related to a SAE
- 5 calendar days for all other technical complaints

Any AE/SAE associated with a technical complaint must be reported in accordance with Appendix 3. The relationship between the technical complaint and the AE/SAE must be assessed by the investigator.

Follow-up on Technical Complaints

The investigator is responsible for ensuring that new and/or updated information is recorded on the original technical complaint form.

Collection and shipment of technical complaint sample(s) (IMP)

The investigator must collect the technical complaint sample(s) and all associated parts and notify the monitor within 5 calendar days of obtaining the sample. The monitor must coordinate the shipment of the sample and associated parts as per instruction from the sponsor.

8.4 Pharmacokinetics

The exposure to dasiglucagon for assessment of PK will be assessed based on plasma concentration data from samples collected at the dosing visit (Visit 2):

- Area under the plasma concentration versus time curve (AUC) from 0 to 30 minutes post-dose ($AUC_{0\text{-}30min}$)
- AUC from 0 to 300 minutes post-dose ($AUC_{0\text{-}300min}$)
- AUC from 0 to the last time point with a measured concentration ($AUC_{0\text{-}t}$)
- AUC from 0 to infinity post-dose ($AUC_{0\text{-}inf}$)
- Maximum observed concentration (C_{max})
- Time to C_{max} (T_{max})
- Terminal elimination rate constant of plasma dasiglucagon (λz)
- Terminal plasma elimination half-life of dasiglucagon ($t_{½}$)
- Total body clearance of plasma dasiglucagon (Cl/f)
- Volume of distribution of plasma dasiglucagon (Vz/f)
- Mean residence time of plasma dasiglucagon (MRT)

Blood samples of approximately 5 mL will be collected for measurement of plasma concentrations of dasiglucagon (refer to Table 6-4). The sampling procedure is described in Section 6.5.3.

8.5 Pharmacodynamics

The plasma glucose profile for assessment of the primary and secondary clinical efficacy endpoints will be assessed based on plasma glucose concentration data from samples collected at the dosing visit (Visit 2). Blood samples of approximately 3.6 mL will be collected for measurement of the plasma glucose response (refer to Table 6-4). The samples will be sent to the clinical laboratory and analyzed using a sensitive and validated assay for glucose measurements.

The assay used for primary endpoint glucose measurements should fulfil the requirements of the US FDA Bioanalytical Method Validation Guidance for Industry Section 5 B and C for biomarkers.[6] The sampling procedure is described in Section 6.5.3.

Plasma glucose will be measured by glucose analyzer during induction of hypoglycemia at site. Plasma glucose should be confirmed by the glucose analyzer prior to the pre-dose PK and PD samples and prior to the administration of dasiglucagon (at Visit 2). Plasma glucose levels at baseline must be entered into the eCRF.

Furthermore, glucose levels will be measured by CGM. Investigator must ensure that CGM data are collected.

8.6 Immunogenicity Assessments

Assessment of antibodies against dasiglucagon is described in Appendix 2.

9 STATISTICAL CONSIDERATIONS

9.1 Statistical Hypotheses

No hypotheses are pre-specified, and no inferential testing will be carried out.

9.2 Sample Size Determination

Eight children will be treated in this trial. The number of children is not based on a formal sample size calculation, evaluating the power of the trial, however some considerations have been made with respect to the precision of the estimate of the primary endpoint. In a previous pediatric trial with dasiglucagon, the mean plasma glucose change from baseline at 30 minutes after IMP injection was 96 mg/dL with a standard deviation (SD) of 20 mg/dL in children aged 6 to 11 years. Assuming the variation is the same in children < 6 years of age, a 95% confidence interval will have a width of ± 25 mg/dL around the mean plasma glucose change, when 8 children are treated.

9.3 Analysis Sets

For the purposes of analysis, the following analysis sets are defined:

- Safety analysis set (SAF): All patients who were enrolled and received at least 1 dose of IMP
- Full analysis set (FAS): All patients of the SAF

9.4 Statistical Analyses

The statistical analysis plan will be finalized prior to database lock, and it will include technical and detailed description of the statistical analyses described in this section. This section is a summary of the planned statistical analyses of the most important endpoints including primary and key secondary endpoints.

9.4.1 General Considerations

All data collected in this trial and documented in the eCRFs will be presented in data listings. All endpoints will be summarized using descriptive statistics. Continuous data will be summarized in tables using number of patients (n), mean, median, SD, minimum and maximum by trial time point. In addition, 95% confidence intervals (based on a t distribution if not otherwise stated) will be calculated if appropriate and detailed in the table shells. For logarithm-transformed data (relevant for selected PK summaries), the geometric mean and coefficient of variation % will also be provided. Categorical data will be summarized using the count and percentage.

In case of termination of the trial, all data collected up to that time point will be included into the analysis.

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

9.4.2 Primary Endpoint

The primary efficacy endpoint, plasma glucose concentration change from baseline at 30 minutes after trial product injection or at the time of rescue by IV glucose if before 30 minutes, will be summarized using descriptive statistics and 95% confidence intervals.

9.4.3 Secondary Endpoints

9.4.3.1 Secondary Efficacy Endpoint

The secondary efficacy endpoint, plasma glucose concentration change from baseline at 15 minutes after IMP injection or at the time of rescue by IV glucose if before 15 minutes, will be summarized using descriptive statistics.

9.4.3.2 Pharmacokinetic Endpoints

The following PK parameters will be estimated by non-compartmental analysis (NCA) based on plasma dasiglucagon concentrations derived from 0 to 300 minutes and summarized with descriptive statistics:

- $AUC_{0\text{-}30min}$
- $AUC_{0\text{-}300min}$
- $AUC_{0\text{-}t}$
- AUC_{inf}
- C_{max}
- T_{max}
- λz
- $t_{½}$
- Cl/f
- Vz/f
- MRT

9.4.3.3 Safety and Tolerability 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.

AEs

AEs will be coded using the latest available version of the Medical Dictionary for Regulatory Activities. All AEs will be listed, however only AEs that are considered treatment emergent (AEs with an onset time at or after the initial dose of trial drug) will be tabulated. Such tables will include the counts and percentages of patients experiencing AEs by system organ class and preferred term. If a patient has more than 1 AE which codes to the same preferred term, the patient will be counted only once for that preferred term. The total number of events documented per system organ class and preferred term will also be displayed.

Other Safety Data

Administration and time to first IV glucose infusion, within 30 minutes after IMP administration (NB: 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.

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

Immunogenicity

Occurrence of ADA will be analyzed descriptively. ADA and result from associated characterization assays will be presented descriptively.

Continuous Glucose Monitoring (CGM)

At Visit 2, glucose levels will be monitored by CGM.

CGM data will be presented graphically as individual plots of glucose measurements over time from the time of stopping the participant's insulin pump to 300 minutes after dasiglucagon administration.

9.5 Interim Analysis

No interim analysis is planned for this trial.

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 Appendix 1: Regulatory, Ethical, and Trial Oversight Considerations

10.1.1 Regulatory and Ethical Considerations

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/IRB according to reporting timelines. If the trial is terminated early, the CA and the IEC/IRB will be notified within 15 days.

The IECs and/or IRBs meet the requirements of the International Council for Harmonisation (ICH) Harmonised Tripartite Guideline for Good Clinical Practice (GCP) E6 (R2) and local legislation. IRBs also meet the requirements of 21 Code of Federal Regulations (CFR) 56.

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

10.1.2 Financial Disclosure

Investigators and sub-investigators will provide the sponsor with sufficient, accurate financial information as requested to allow the sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities. Investigators are responsible for providing information on financial interests during the course of the trial and for 1 year after completion of the trial.

10.1.3 Informed Consent Process

Written informed consent will be obtained from the parent(s)/legal guardians 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 guardians 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:

- Their consent is voluntary
- 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, ie, 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 guardians 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.

10.1.4 Data Protection

Patients will be assigned a unique identifier by the sponsor. Any patient records or datasets that are transferred to the sponsor will contain the identifier only; patient names or any information which would make the patient identifiable will not be transferred.

The patient and the parent(s)/legal guardian(s) must be informed that the patient's medical records may be examined by Clinical Quality Assurance auditors or other authorized personnel appointed by the sponsor, including relevant partners, by appropriate IRB/IEC members, and by inspectors from regulatory authorities.

10.1.5 Committees Structure

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

A list with the names and addresses of the responsible institutions and persons is provided in Appendix 5 of this protocol.

10.1.5.1 Safety Data Review and Committee

Safety Committee

An internal Zealand Pharma A/S safety committee is constituted to perform ongoing blinded 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.

Trial Safety Group

In addition, new patients will only be dosed after safety assessment of the dosing visit for the preceding patient is completed and assessed by the Trial Safety Group (TSG). Thus, except for

the first patient being dosed in the trial, investigators need written permission (eg, via an email) from the TSG chair to dose a patient in the trial.

The TSG consists of the following personnel:

- Principal investigator or his deputy
- Medical responsible of the sponsor (chair)
- Clinical trial manager and/or clinical pharmacologist of the sponsor
- Safety responsible of the sponsor (pharmacovigilance [PV])
- Statistician of the sponsor (ad-hoc member)
- A representative from the partner of the sponsor (ad-hoc member)

The principal investigator for the site responsible for treatment of the patient being assessed may be invited to the TSG meeting.

The TSG will review the following non-verified data before deciding if the next patient can be dosed:

- Safety data (including SAEs, AEs, concomitant medication, local tolerability, vital signs, physical exam) and body weight data collected at Visit 1 and 2
- Safety data (standard safety 12-lead ECG, local tolerability, biochemistry, and hematology) and age collected at Visit 1
- Glucose data derived from the glucose analyzer collected at Visit 2
- Dose administered (0.3 mg or 0.6 mg)
- In case pharmacokinetic (PK) data collected at Visit 2 is available to the TSG for the preceding patient or other patients dosed earlier, these data will also be included in the review. Review of PK data is not a prerequisite for deciding to continue dosing a new patient; however, the TSG may decide to await the PK data before deciding on whether a new patient can be dosed.

The TSG may decide to pause the dosing of further patients and escalate any significant findings to the Chairperson of the Zealand Pharma A/S (ZP) Safety Committee.

Dosing of the next patient will be paused if a patient experiences an SAE judged as probably or possibly related to dasiglucagon. If this occurs, the TSG must notify the Chairperson of the ZP Safety Committee. The ZP Safety Committee will appoint an assessor (not otherwise involved in the trial) for evaluation of AE relationship to dasiglucagon. The conclusion from this assessment will be communicated to the TSG, and based on this assessment, the TSG will decide if dosing of the next patient can proceed. The TSG may introduce additional precautions to the trial before dosing of the next patient. The investigator must not initiate dosing of the next patient until permission to do so from the TSG has been obtained and the dose level (0.6 mg or 0.3 mg) for the next dosed patient has been confirmed by the sponsor.

10.1.6 Protocol Deviations

Continuous protocol deviation management is part of the monitoring activities and will be detailed in the Monitoring Plan. Before data are released for statistical analysis, a review of all data will take place to identify protocol deviations that may potentially affect the results. Spurious outliers will be assessed. In addition, protocol deviations that may potentially affect the results will be identified and it will be assessed 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 per protocol set.

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 IMP
- Significant deviation from time windows
- 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 full analysis set. In that case, the decision should be taken at the 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.

10.1.7 Protocol Changes

This trial protocol may be amended following the procedures specified by local laws and regulations. Substantial amendments to this trial protocol will only be implemented upon 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 IMP 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 event, and the measures taken.

10.1.8 Source Documents

Source documents provide evidence for the existence of the patient and substantiate the integrity of the data collected. Source documents are filed at the investigator's site and should be attributable, legible, contemporaneous, original, accurate, and complete.

Data entered in the eCRF that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The investigator may need to request previous medical records or transfer records, depending on the trial. Also, current medical records must be available.

For CGM, the definition of source data follows the Food and Drug Administration (FDA) Guidance on Electronic Source Data in Clinical Investigations (2013).[7]

The investigator must maintain adequate and accurate documentation (source data) that supports the information entered in the eCRF.

Trial monitors will perform ongoing source data verification to confirm that data entered into the eCRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of patients are being protected; and that the trial is being conducted in accordance with the currently approved protocol and any other trial agreements, ICH GCP, and all applicable regulatory requirements.

10.1.9 Electronic Case Report Forms

All the information collected during the trial will be recorded in the eCRFs, which are identified by patient number. A designated CRO 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.

10.1.9.1 Data Quality Control and Assurance

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 a designated CRO 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 (ICFs) for completeness and consistency.

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

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

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

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

The eCRFs will be used for all patients. The investigator's data will be accessible from the investigator's site 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 site to ensure that all patient discontinuations or changes in IMP or other medications entered in the patient's medical records are also made on the patient's eCRF.

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

10.1.10 Dissemination of Clinical Trial Data

Dissemination of clinical trial data and data management will be performed according to the designated CRO's standard operating procedures.

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

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 IMP has been approved or the sponsor has discontinued its research with the IMP, or
- At least 2 years have elapsed since the formal discontinuation of clinical development of the IMP

However, these documents should be retained for a longer period (25 years after the end of trial if data will be a part of an EU submission) 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.

10.1.12 Trial and Site Start and Closure

First Act of Recruitment

The trial start date is the date on which the clinical trial will be open for recruitment of patients.

The first act of recruitment is the first site open and will be the trial start date.

Trial/Site 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 IMP 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

Any site can be closed, and the trial terminated for the following reasons:

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

However, even in case of site discontinuation the site 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 site.

The investigator may initiate trial site closure at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended termination.

If the trial is prematurely terminated or suspended, the sponsor shall promptly inform the investigators, the IECs/IRBs, the regulatory authorities, and any contract research organization(s) used in the trial of the reason for termination or suspension, as specified by the applicable regulatory requirements. The investigator shall promptly inform the patient's parent(s)/legal guardian(s) and should assure appropriate patient therapy and/or follow-up.

10.1.13 Publication Policy

The information obtained during the conduct of this trial is considered confidential and will be used by or on behalf of Zealand Pharma A/S for regulatory purposes as well as for the general development of the IMP. 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 IMP, 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 IMP 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 contract research organization (CRO) involved in the trial.

The results of this trial will be patient 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.

10.2 Appendix 2: Clinical Laboratory Tests

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

- Clinical chemistry (measured in serum): sodium, potassium, calcium, creatinine, total bilirubin, AST, ALT. If additional clinical chemistry parameters are analyzed and reported as part of a standard chemistry panel, these data will also be evaluated.
- Hematology (measured in whole blood): hemoglobin, platelet count (thrombocytes), total white blood cell count (leucocytes). If additional hematology parameters are analyzed and reported as part of a standard hematology panel, these data will also be evaluated.
- Pharmacokinetic (PK) samples (measured in plasma): PK assessments from samples collected during Visit 2 will be analyzed by York Bioanalytical Solutions (York, United Kingdom).
- Immunogenicity: Serum samples for assessment of antibodies against dasiglucagon will be measured at screening Visit 1 and at follow-up Visit 3 by the special laboratory, York Bioanalytical Solutions (York, United Kingdom). The cell-based neutralizing antibody analyses will be performed by a special laboratory, BioAgilytix, Durham, North Carolina, USA. A description of the sample handling and sample processing, storage, and shipment at the site will be included in the laboratory manual. Immunogenicity samples are planned to be analyzed after the last patient's Visit 3.
- The clinical anti-drug antibody (ADA) assays 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 4-fold) ADA-positive patients will be assessed 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 as long as outcomes related to ADAs are detected or until the ADA level has returned to baseline.

Additional tests may be performed at any time during the trial as determined necessary by the investigator or required by local regulations. Considerations regarding the additional blood volume required for the tests should be made prior to performing additional tests, taking the child's body weight and estimated total blood volume (total estimated blood volume is 75 to 80 mL/kg) into account (refer to Section 6.5.3).

10.3 Appendix 3: Adverse Events and Serious Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting

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

10.3.1 Definition of Adverse Event

An adverse event (AE) is any untoward medical occurrence in a clinical trial patient, temporally associated with the use of trial intervention, whether or not considered related to the trial intervention. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the trial intervention.

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

Events Meeting the AE Definition

- A clinically significant worsening of a concomitant illness
- A clinical laboratory abnormality that is clinically significant, ie, 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 8.3.6)
- Injection site reactions
- New conditions detected or diagnosed after trial intervention administration even though it may have been present before the start of the trial
- Signs, symptoms, or the clinical sequelae of a suspected intervention- intervention interaction
- Lack of efficacy will not be reported as an AE or SAE. Such instances will be captured in the efficacy assessments. However, the signs, symptoms, and/or clinical sequelae resulting from lack of efficacy will be reported as AE or SAE if they fulfill the definition of an AE or SAE

Events NOT Meeting the AE Definition

- 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).

- Any clinically significant abnormal laboratory findings or other abnormal safety assessments which are associated with the underlying disease, unless judged by the investigator to be more severe than expected for the patient's condition.
- Situations in which an untoward medical occurrence did not occur (social and/or convenience admission to a hospital).
- Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the trial that do not worsen.

10.3.2 Definition of SAE

An SAE is defined as any SAE that, at any dose:

- Results in death
- Is life threatening
    - The term "life threatening" in the definition of "serious" refers to an event in which the patient was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.
- Requires inpatient hospitalization or prolongation of existing hospitalization
    - In general, hospitalization signifies that the patient has been admitted (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious.
    - Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE.
- Results in persistent or significant disability/incapacity
    - The term disability means a substantial disruption of a person's ability to conduct normal life functions.
    - This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (eg, sprained ankle) which may interfere with or prevent everyday life functions but do not constitute a substantial disruption.
- Is a congenital anomaly/birth defect

- Is medically important. Medical or scientific judgment should be exercised by the investigator in deciding whether SAE reporting is appropriate in other situations such as significant medical events that may jeopardize the patient or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These events should usually be considered serious.
    - Examples of such events include invasive or malignant cancers, intensive treatment for allergic bronchospasm, blood dyscrasias, convulsions or development of intervention dependency or intervention abuse.

Assessment of Intensity

The maximum intensity (severity) of all AEs must be assessed by the investigator and documented from the first reporting of the AE.

- 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.

Assessment of Causality

Causality relationship to IMP

- 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 IMP

Outcome of an AE

- 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's parent(s)/legal guardian(s) signed the informed consent.
- Recovering/resolving: The condition is improving, and the patient is expected to recover from the event.
- 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 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 IB.

10.3.3 Collection, Recording, and Reporting of AEs

All AEs, whether serious or non-serious, will be collected from the first trial-related activity after a signed and dated ICF is obtained until the end of the post-treatment follow-up period (which may include contacts for follow-up of safety). In addition, 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 site (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 IMP, 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 IMP. 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 IMP
- Measures taken due to AE
- Date and time of resolution and final outcome

10.3.4 Follow-up of AEs

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, it should be reported on an SAE form following the SAE reporting timelines.

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.
- 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.

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 treatment-emergent AE must be reported as follow-up with re-assessment of severity and/or seriousness of the event. For AEs with onset before IMP administration, any worsening of severity/seriousness after IMP

administration should be recorded as a separate AE, with the onset date of the event corresponding to the date of the severity/seriousness upgrade.

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.

10.4 Appendix 5: Abbreviations and Definitions

Abbreviation	Definition
ADA	anti-drug antibodies
AE	adverse event
AESI	adverse event of special interest
ALT	alanine aminotransferase
AST	aspartate aminotransferase
AUC	area under the plasma concentration versus time curve
CA	competent authority(ies)
CFR	Code of Federal Regulations
CGM	continuous glucose monitoring
Cl/F	apparent clearance (corrected for bioavailability)
C_{max}	maximum observed concentration
CRO	contract research organization
ECG	electrocardiogram
eCRF	electronic case report form
EDC	electronic data capture
FAS	full analysis set
FDA	Food and Drug Administration
GCP	Good Clinical Practice
IB	Investigator's Brochure
ICF	informed consent form
ICH	International Council for Harmonisation
IEC	independent ethics committee
IMP	investigational medicinal product
IRB	institutional review board
IV	intravenous(ly)
MRT	mean residence time
PD	pharmacodynamic(s)
PK	pharmacokinetic(s)
PT	preferred term

Abbreviation	Definition
SAE	serious adverse event
SAF	safety analysis set
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
SC	subcutaneous(ly)
SD	standard deviation
SoA	Schedule of Assessments
T1D	type 1 diabetes
ULN	upper limit of normal
US/USA	United States of America
Vz/f	volume of distribution of plasma dasiglucagon

Pharmacokinetic and Pharmacodynamic Abbreviations

Abbreviation	Definition
λ_z	terminal rate constant
$AUC_{0\text{-}15min}$	area under the plasma concentration versus time curve from administration to 15 minutes
$AUC_{0\text{-}30min}$	area under the plasma concentration versus time curve from administration to 30 minutes
AUC_{inf}	area under the plasma concentration versus time curve from administration to infinity post-dose
C_{max}	maximum plasma concentration
$T_{½}$	plasma concentration half-life
T_{max}	time to reach maximum plasma concentration

10.5 Appendix 6: List of Names and Addresses

Sponsor	Zealand Pharma A/S CVR No. DK 2004 5078 Sydmarken 11 DK-2860 Søborg, Denmark
Contract Research Organization (CRO)	Synteract Deutschland GmbH Stefan-George-Ring 6 81929 Munich, Germany
Safety CRO	PharmaLex A/S Agern Allé 24, DK-2970 Hørsholm, Denmark
Trial monitor	Synteract Deutschland GmbH Stefan-George-Ring 6 81929 Munich, Germany
Biostatistician	[redacted] [redacted] Synteract Deutschland GmbH Stefan-George-Ring 6 81929 Munich, Germany
Sponsor representative	[redacted] [redacted] [redacted] [redacted] Zealand Pharma A/S CVR No. DK 2004 5078 Sydmarken 11 DK-2860 Søborg, Denmark
Sponsor's medical expert	[redacted] MD [redacted] [redacted] Zealand Pharma A/S CVR No. DK 2004 5078 Sydmarken 11 DK-2860 Søborg, Denmark
Sponsor representative for pharmacovigilance	[redacted] PharmaLex A/S Agern Allé 24, DK-2970 Hørsholm, Denmark
Clinical laboratory	PPD Central Lab US Highland Heights, KY 2 Tesseneer Road Highland Heights, Kentucky 41076- 9167 United States +1 859 781 8877

Continuous glucose monitoring (GCM) vendor	IQVIA IQVIA Ltd. BNP Paribas, London Branch 10 Harewood Avenue London NW1 6AA [redacted]
Analytical laboratory (ZP4207 PK, ZP4207 anti-drug antibodies)	York Bioanalytical Solutions (YBS) Cedar House Northminster Business Park Northfield Lane York, YO26 6QR United Kingdom
(ZP4207 cell-based neutralizing antibodies)	BioAgilytix Labs 2300 Englert Drive Durham, NC 27713 USA

10.6 Appendix 7: Summary of Changes from Version 5 to Version 6

SUMMARY OF CHANGES FOR A PROTOCOL AMENDMENT

PROTOCOL AMENDMENT 6

Protocol Title:	A Phase 3, single-administration, open-label trial to assess the efficacy, safety, pharmacokinetics, and pharmacodynamics of dasiglucagon when administered as a rescue therapy for severe hypoglycemia in pediatric patients below 6 years of age with Type 1 Diabetes (T1D)
Protocol Number:	ZP4207-21052
Clinical Phase:	Phase 3
Protocol Version and Date:	Protocol Version 5.0, 01 Nov 2023
Original Protocol **Date Amended**	Version 1, 10 Mar 2022 Version 2.0, 14 Oct 2022 Version 3.0, 12 Dec 2022 Version 4.0, 09 Mar 2023 Version 5.0, 01 Nov 2023 Version 6.0, 09 Jul 2024
US Investigational New Drug Number:	127866
Sponsor:	Zealand Pharma A/S CVR No. DK 2004 5078 Sydmarken 11 DK-2860 Søborg Denmark

RATIONALE FOR AMENDMENT

The rationale for this amendment is to allow for more flexibility in recruiting children eligible for 0.3 mg dose by removing upper limit for body weight of 15 kg.

Changes to the protocol are summarized below.

Section number and heading(s)	Old text	New text	Justification for change
Sponsor Signatory 10.5 Appendix 6		Updated	Updated with a new signatory due to company team changes.
1.1 Synopsis	**Rationale:** Dasiglucagon is indicated for treatment of severe hypoglycemia in pediatric and adult patients with diabetes aged 6 years and above. As this condition also affects children below 6 years of age and there is a therapeutic need for them to get access to safe and efficacious emergency treatment, the present trial aims to assess the efficacy and safety of a single subcutaneous (SC) injection of dasiglucagon in children below 6 years of age with T1D. A total of 8 children will be included; 4 children receiving a dose of 0.3 mg and 4 children receiving a dose of 0.6 mg. Of the 4 children receiving 0.3 mg, 2 children should preferably be below 2 years at screening. The children aged 2 years or above receiving 0.3 mg should weigh below 15 kg at screening and should preferably be below 4 years at screening. For children receiving the 0.6 mg dose, all must be above 2 years at screening. The trial population will include children treated with SC insulin infusion via insulin pump or multiple daily injections in order to include children below 1 year of age. Overall, the eligibility criteria allow for enrollment of a trial population resembling the target population of children below 6 years of age with T1D.	**Rationale:** Dasiglucagon is indicated for treatment of severe hypoglycemia in pediatric and adult patients with diabetes aged 6 years and above. As this condition also affects children below 6 years of age and there is a therapeutic need for them to get access to safe and efficacious emergency treatment, the present trial aims to assess the efficacy and safety of a single subcutaneous (SC) injection of dasiglucagon in children below 6 years of age with T1D. A total of 8 children will be included; 4 children receiving a dose of 0.3 mg and 4 children receiving a dose of 0.6 mg. Of the 4 children receiving 0.3 mg, 2 children should preferably be below 2 years at screening. The **remaining 2** children aged 2 years or above receiving 0.3 mg should ~~weigh below 15 kg at screening and should~~ preferably be below 4 years at screening. For children receiving the 0.6 mg dose, all must be above 2 years at screening. The trial population will include children treated with SC insulin infusion via insulin pump or multiple daily injections in order to include children below 1 year of age. Overall, the eligibility criteria allow for enrollment of a trial population resembling the target population of children below 6 years of age with T1D.	The rationale for this amendment is to allow for more flexibility in recruiting children eligible for 0.3 mg dose by removing upper limit for body weight of 15 kg.

1.1 Synopsis 4.1 Overall Design	**Overall Design:** This trial will use a single-administration, open-label trial design to assess the ability of a single SC injection of dasiglucagon to increase plasma glucose in pediatric children with T1D with hypoglycemia. A total of 8 children will be included; 4 children receiving a dose of 0.3 mg and 4 children receiving a dose of 0.6 mg. Of the 4 children receiving 0.3 mg, 2 children should preferably be below 2 years at screening. The children aged 2 years or above receiving 0.3 mg should weigh below 15 kg at screening and should preferably be below 4 years at screening. For children receiving the 0.6 mg dose, all must be above 2 years at screening. Before an eligible child is dosed, the dose level (0.6 mg or 0.3 mg) must be confirmed by the sponsor. Each child will be dosed after the safety assessment of the preceding child has been completed and assessed by the Trial Safety Group.	Overall Design: This trial will use a single-administration, open-label trial design to assess the ability of a single SC injection of dasiglucagon to increase plasma glucose in pediatric children with T1D with hypoglycemia. A total of 8 children will be included; 4 children receiving a dose of 0.3 mg and 4 children receiving a dose of 0.6 mg. Of the 4 children receiving 0.3 mg, 2 children should preferably be below 2 years at screening. The **remaining 2** children aged 2 years or above receiving 0.3 mg should ~~weigh below 15 kg at screening and should~~ preferably be below 4 years at screening. For children receiving the 0.6 mg dose, all must be above 2 years at screening. Before an eligible child is dosed, the dose level (0.6 mg or 0.3 mg) must be confirmed by the sponsor. Each child will be dosed after the safety assessment of the preceding child has been completed and assessed by the Trial Safety Group.	
1.1 Synopsis	Number of Patients: At least 8 children below 6 years (at the screening visit) with T1D will be dosed in the trial. Two of these children should preferably be below 2 years of age at the screening visit and successfully complete Visit 2. Furthermore, 2 children should weigh below 15 kg at screening and should preferably be below 4 years.	Number of Patients: At least 8 children below 6 years (at the screening visit) with T1D will be dosed in the trial. Two of these children should preferably be below 2 years of age at the screening visit and successfully complete Visit 2. Furthermore, the remaining 2 children ~~should weigh below 15 kg at screening and should~~ preferably be below 4 years.	

1.1 Synopsis	**Intervention Groups and Duration:** All 8 children will be treated with dasiglucagon via a single SC injection into the buttocks at the dosing visit (Visit 2). Four children in the age range ≥ 2 and < 6 years: • will receive a single SC injection of 0.6 mg/0.6 mL of dasiglucagon by a prefilled syringe. Four children 2 of which should preferably be below 2 years, and the children aged ≥ 2 years and preferably < 4 years, with a body weigh below 15 kg • will receive a single SC injection of 0.3 mg/0.3 mL dasiglucagon by a syringe	**Intervention Groups and Duration:** All 8 children will be treated with dasiglucagon via a single SC injection into the buttocks at the dosing visit (Visit 2). Four children in the age range ≥ 2 and < 6 years: • will receive a single SC injection of 0.6 mg/0.6 mL of dasiglucagon by a prefilled syringe. Four children 2 of which should preferably be below 2 years, and the children aged ≥ 2 years and preferably < 4 years~~, with a body weigh below 15 kg~~ • will receive a single SC injection of 0.3 mg/0.3 mL dasiglucagon by a syringe	
Justification for Dose Pharmakokitenetics 4.3.1	Children weighing < 15 kg and preferably < 4 years: justification for 0.3 mg dose The final Pop PK model (Study 19-077) showed a mean Cmax and AUC that was 1.4 and 1.1 times higher in 0- to 1-year-olds for a dose of 0.3 mg compared to the exposure in adults with 0.6 mg, respectively. The ratio was 0.9 and 0.7 for Cmax and AUC in 3- to 4-year-olds for a dose of 0.3 mg compared to the exposure in adults with 0.6 mg, respectively. The alternative model indicated these values for Cmax and AUC to be 4.0 and 3.1 times higher in 0- to 1-year-olds and 2.0 and 1.7 times higher in 3- to 4-year-olds compared to adults. The span of PK predictions based	Children ~~weighing < 15 kg and~~ preferably < 4 years: justification for 0.3 mg dose The final Pop PK model (Study 19-077) showed a mean Cmax and AUC that was 1.4 and 1.1 times higher in 0- to 1-year-olds for a dose of 0.3 mg compared to the exposure in adults with 0.6 mg, respectively. The ratio was 0.9 and 0.7 for Cmax and AUC in 3- to 4-year-olds for a dose of 0.3 mg compared to the exposure in adults with 0.6 mg, respectively. The alternative model indicated these values for Cmax and AUC to be 4.0 and 3.1 times higher in 0- to 1-year-olds and 2.0 and 1.7 times higher in 3- to 4-year-olds compared to adults. The span of PK predictions based on	The rationale for this amendment is to allow for more flexibility in recruiting children eligible for 0.3 mg dose by removing upper limit for body weight of 15 kg.

	on the final and alternative PK models can be seen as a possible realistic range.	the final and alternative PK models can be seen as a possible realistic range.	
Justification for Dose Pharmacokinetics 4.3.2	Children weighing < 15 kg and preferably < 4 years: justification for 0.3 mg dose Since the predicted Cmax from both PK models (0.3 mg dose: final model: 926 to 3,660 pmol/L in children aged 0 to 1 years and alternative model: 2,600 to 10,500 pmol/L in children aged 0 to 1 years) is substantially lower (at least 2.9 times lower) than the observed concentration range, the use of 0.3 mg dasiglucagon is considered to be safe in children weighing below 15 kg (preferably below 4 years) with regards to the risk of QT prolongation.	Children ~~weighing < 15 kg and~~ preferably < 4 years: justification for 0.3 mg dose Since the predicted Cmax from both PK models (0.3 mg dose: final model: 926 to 3,660 pmol/L in children aged 0 to 1 years and alternative model: 2,600 to 10,500 pmol/L in children aged 0 to 1 years) is substantially lower (at least 2.9 times lower) than the observed concentration range, the use of 0.3 mg dasiglucagon is considered to be safe ~~in children weighing below 15 kg (preferably below 4 years)~~ with regards to the risk of QT prolongation.	The rationale for this amendment is to allow for more flexibility in recruiting children eligible for 0.3 mg dose by removing upper limit for body weight of 15 kg.

Notes: ~~Strikethrough~~ indicates deleted text, and **bold** idicates new text.

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