PROTOCOL TITLE: A Randomized, Double-Blind, Parallel-Group Trial Evaluating the Efficacy and Safety of Dasiglucagon for the Treatment of Children with Congenital Hyperinsulinism

PROTOCOL DATE: Original Protocol Version 1.0, 14-Dec-2017

NCT Number: NCT04172441

Document version	Date	Comments
Version 14.0 – Germany only	04-Jun-2021	Local protocol in Germany combining protocol 12.0 and 13.0.
Version 13.0 – All countries except Germany	04-Jun-2021	Updates to the secondary endpoints. Statistical section was updated with the updated endpoints. The safety section was clarified further.
Version 12.0 – Germany only	05-Nov-2020	Update requested by Germany competent authority to outline that only Dexcom G6 continuous glucose monitoring device was to be used in Germany.
Version 11.0 – Germany only	27-Aug-2020	Local protocol in Germany combining protocol 9.0 and 10.0 with additional to updating all device-specific sections related to Dexcom G4 to Dexcom G6.
Version 10.0 – All countries except Germany	08-Jul-2020	Dexcom G4 was changed to Dexcom G6 as the G4 was being phased out. Statistical section was updated focusing on the key secondary analysis description; the endpoint was rewritten to match the description in the endpoint section. Pharmacokinetics/drug exposure section was updated: Visit 5 corrected to Day 5.
Version 9.0 – All countries except Germany	29-Apr-2020	Local protocol in Germany combining protocol versions 6.1 and 8.0.
Version 6.1 – Germany only	05-Mar-2020	Device adapted protocol to fulfill the requirements from Germany competent authority in their query letters to protocol version 6.0. Protocol version includes protocol version 6.0 and mandated text on analysis of device related safety events.
Version 8.0 – Germany only	11-Oct-2019	Local protocol in Germany combining protocol versions 6.0 and 7.0.

Document version	Date	Comments
Version 7.0 – All countries except Germany	19-Sep-2019	First patient enrolled under this version.
Version 6.0 – Germany only	05-Jul-2019	Device adapted protocol to fulfill the requirements of the German competent authority. New section added to address the devices used in the trial and additional device related safety reporting added.
Version 5.0 – Global	03-Jun-2019	
Version 4.0 – Global	15-Apr-2019	
Version 3.0 – France, Germany, Israel, United Kingdom	25-Jul-2018	
Version 2.0 – Global	20-Jun-2018	
Version 1.0 – Global	14-Dec-2017	

PROTOCOL

PRODUCT NAME/NUMBER:	Dasiglucagon
PROTOCOL NUMBER:	ZP4207-17103
IND NUMBER:	135869
EUDRACT NUMBER:	2017-004545-24
DEVELOPMENT PHASE:	Phase 2/3
PROTOCOL TITLE:	A Randomized Trial in 2 Parts: Double-Blind, Placebo-Controlled, Crossover Part 1 and Open-label Part 2, Evaluating the Efficacy and Safety of Dasiglucagon for the Treatment of Children with Congenital Hyperinsulinism
PROTOCOL DATE:	Original Protocol Version 1.0, 14-Dec-2017 Final Version 14.0, 04-Jun-2021 (Germany)
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1. REVISION HISTORY

PROTOCOL TITLE:	A Randomized Trial in 2 Parts: Double-Blind, Placebo-Controlled, Crossover Part 1 and Open-label Part 2, Evaluating the Efficacy and Safety of Dasiglucagon for the Treatment of Children with Congenital Hyperinsulinism
PROTOCOL DATE:	Original Protocol Version 1.0, 14-Dec-2017
	This updated protocol version 14.0 is applicable for Germany only, and includes:
AMENDMENT No. 1	Final Version 2.0, 20-Jun-2018 (All Countries)
AMENDMENT No. 2	Final Version 3.0, 25-Jul-2018 (France, Germany, Israel, United Kingdom)
AMENDMENT No. 3	Final Version 4.0, 15-Apr-2019 (All Countries)
AMENDMENT No. 4	Final Version 5.0, 03-Jun-2019 (All Countries)
AMENDMENT No. 5	Final Version 6.0, 05-Jul-2019 (Germany)
AMENDMENT No. 5.1	Final Version 6.1, 05-Mar-2020 (Germany)
AMENDMENT No. 6	Final Version 7.0, 19-Sep-2019 (All countries except Germany)
AMENDMENT No. 7	Final Version 8.0, 11-Oct-2019 (Germany)
AMENDMENT No. 8	Final Version 9.0, 29-Apr-2020 (Germany)
AMENDMENT No. 9	Final Version 10.0, 08-Jul-2020 (All countries except Germany)
AMENDMENT No. 10	Final Version 11.0, 27-Aug-2020 (Germany)
AMENDMENT No. 11	Final Version 12.0, 05-Nov-2020 (Germany)
AMENDMENT No. 12	Final Version 13.0, 04-Jun-2021 (All countries except Germany)
AMENDMENT No. 13	Final Version 14.0, 04-Jun-2021 (Germany)

2. APPROVAL SIGNATURES

PROTOCOL NUMBER:	ZP4207-17103
PROTOCOL TITLE:	A Randomized Trial in 2 Parts: Double-Blind, Placebo-Controlled, Crossover Part 1 and Open-label Part 2, Evaluating the Efficacy and Safety of Dasiglucagon for the Treatment of Children with Congenital Hyperinsulinism

I, the undersigned, have read this protocol and confirm that to the best of my knowledge it accurately describes the planned conduct of the trial.

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Clinical Trial Manager
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Date

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Senior Manager Biostatistics
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Date

3. SYNOPSIS

PRODUCT NAME/NUMBER	Dasiglucagon
PROTOCOL NUMBER	ZP4207-17103
EUDRACT NUMBER	2017-004545-24
DEVELOPMENT PHASE	Phase 2/3
PROTOCOL TITLE	A Randomized Trial in 2 Parts: Double-Blind, Placebo-Controlled, Crossover Part 1 and Open-label Part 2, Evaluating the Efficacy and Safety of Dasiglucagon for the Treatment of Children with Congenital Hyperinsulinism
INDICATION	Congenital hyperinsulinism (CHI)
OBJECTIVES	Primary: To evaluate the efficacy of dasiglucagon in reducing glucose requirements in children with persistent CHI requiring continuous IV glucose administration to prevent/manage hypoglycemia. Secondary: To evaluate the safety and tolerability of dasiglucagon administered as a subcutaneous (SC) infusion in patients with CHI.
TRIAL DESIGN	This is a combined phase 2 and 3, randomized, multinational trial to evaluate the efficacy and safety of individually titrated dasiglucagon in children ≥7 days and <1 year of age who have been diagnosed with CHI, comprising 2 parts, a crossover (2 periods, 48 hours each), double-blind, placebo-controlled Part 1, and an open-label, single-arm Part 2 of 21 days. After screening, eligible patients will complete a minimum 24-hour run-in period to confirm the IV glucose randomization requirement (≥10 mg/kg/min). All non-nutritional carbohydrates and carbohydrate fortification of feeds are prohibited during this 24-hour run-in period. After the run-in period, patients will be randomly assigned in a double-blind fashion to receive dasiglucagon or placebo for 48 hours, after which they will be crossed over to the other trial treatment for an additional 48 hours. At the time of crossover, the trial drug will be initiated from the starting dose of 10 µg/hr, and IV glucose infusion rate (GIR) will be set to the rate obtained at the end of the run-in period and titrated accordingly. There will be no washout between the 2 periods in Part 1 to limit the length of time in which patients are reliant on IV GIR as their only means of preventing/treating hypoglycemia. This is deemed acceptable because: • The half-life ($t_{½}$) of dasiglucagon is short (~30 minutes for the 0.6 mg dose and potentially shorter for lower doses, ~22 minutes) relative to the 48-hour duration of each crossover period. Moreover, for the primary endpoint, only the last 12 hours of the 48-hour period will be used for endpoint assessment. • Assuming a positive effect of dasiglucagon, the absence of washout is more conservative with respect to key secondary endpoints since a potential carry over effect of dasiglucagon in Period 2 is attributed to placebo. During Part 1, the trial drug and IV GIR should be adjusted according to the protocol-specified algorithm. Non-nutritional carbohydrates and/or carbohydrate fortification of feeds during Part 1 is only allowed when the maximum tolerable volume and concentration of IV glucose for the patient is reached, and should be limited to the minimum needed to ensure the patient's safety. All feedings (administered as parenteral nutrition, by nasogastric [NG] tube, gastrostomy, or normal route), will be recorded during this period. Safety assessments will be performed daily after initiation of the trial drug (active or placebo).

	After Part 1, patients will continue in open-label Part 2 to receive dasiglucagon for 21 days. Additional CHI treatments can be introduced during Part 2 if needed, if up-titration of dasiglucagon is not possible because of undesirable side effects or if the maximum dose level (70 µg/hr) has been reached. Gradual transfer from IV glucose to oral and gastric carbohydrates should be initiated in Part 2, enabling weaning of IV glucose and hospital discharge. Patients will continue to be hospitalized until IV GIR is weaned off; however, as soon as local site criteria for discharge are met, patients can be discharged to continue the treatment period at home. Visits are planned at Day 11 and Day 18 in Part 2. These visits can be converted to telephone visits if appropriate, at the investigator's discretion. On Day 25, the End of Treatment Visit will take place, and based on the investigator's confirmation of continued positive benefit-risk balance, patients will be offered the opportunity to participate in the long-term extension trial (Trial ZP4207-17106) to continue dasiglucagon treatment. Patients who do not enter the long-term extension trial will have a Follow-up Visit 28 days after their last dose of trial drug.
PLANNED NUMBER OF PATIENTS	A total of 12 patients is planned to be randomized 1:1 to receive either placebo first and then dasiglucagon or vice versa.
TRIAL ENTRY CRITERIA	Eligible patients will be ≥7 days and <12 months of age at screening with a body weight of ≥2.0 kg (4.4 lbs.) and a diagnosis of CHI established based on the following: • Hyperinsulinemia (plasma insulin above the limit of detection of the assay documented during an event of hypoglycemia), and/or • Hypofattyacidemia (plasma free fatty acid <1.7 mmol/L), and/or • Hypoketonemia (beta-hydroxy-butyrate <1.8 mmol/L), and/or • Glycemic response (increase in plasma glucose [PG] of >30 mg/dL after 1 mg IV or intramuscular [IM] glucagon administration) Eligible patients will be dependent on continuous IV glucose to prevent hypoglycemia.
INVESTIGATIONAL PRODUCTS	Dasiglucagon injection 4 mg/mL in a 3 mL vial containing 1 mL for injection. Matching placebo for dasiglucagon injection in a 3 mL vial containing 1 mL for injection.
REFERENCE PRODUCT	None
TREATMENT REGIMENS	As long as the patient is receiving IV glucose, PG will be measured and reviewed hourly using a hand-held PG meter. The IV GIR will be titrated to achieve glycemia of at least 70 mg/dL (3.9 mmol/L) (minimum GIR will be established as the rate up-titrated after the patient drops at least once below 70 mg/dL, or 3.9 mmol/L). When the patient is no longer on IV GIR, the PG will be checked according to local practice, but at least 3 times daily. The PG will be measured using the same trial-supplied hand-held PG meter during the entire trial. Additionally, blinded continuous glucose monitoring (CGM) will be started 24 hours prior to randomization using either the Dexcom G4 or G6 (all countries except Germany), or Dexcom G6 system (Germany). CGM monitoring will continue until the end of Part 2. **Dosing of trial drug** Dosing of dasiglucagon will approximate continuous infusion by delivering small doses at frequent intervals via a trial-supplied infusion pump. The adjustment of trial drug dosing is closely linked to the PG level achieved, which in turn will determine the adjustments to the IV GIR.

	Part 1 (Crossover, double-blind, randomized, placebo controlled) The starting dose of trial drug is 10 µg/hr at t=0. Every 2 hours (t=2, 4, 6h, etc.), the dose will be increased by an additional 10 µg/hr until either: • The patient is totally weaned off IV glucose, or • PG during the last 2 hours was constantly above 120 mg/dL (6.7 mmol/L), or • IV GIR has not decreased despite 2 sequential dose increases (in this situation the dose of trial drug product should be maintained until the IV GIR can be further decreased or until crossover or the end of the treatment period), or • The maximum dose of 70 µg/hr is reached, or • Adverse events (AEs) emerge that are considered to be related to the trial drug (e.g., nausea/change in feeding patterns or increased vomiting) and that are limiting further dose escalation The 2-hour dose-adjustment interval will allow drug plasma levels to approach steady state before the dose is further increased. The cumulative dose will not exceed 1.26 mg over the first 24 hours and 1.68 mg for the subsequent 24-hour periods. At the time of crossover from the first 48-hour period to the second 48-hour period, the trial drug will again be titrated from the starting dose of 10 µg/hr, and IV GIR will be set to the rate obtained at the end of the run-in period and titrated accordingly. Part 2 (Open-label) After Part 1, all patients will continue in open-label Part 2 to receive dasiglucagon for an additional 21 days. Since treatment allocation during Part 1 remains blinded, all patients are required to initiate dasiglucagon dosing at 10 µg/hr in Part 2, while IV GIR in Part 2 should be started at the rate obtained at the end of the run-in period. During Part 2, the dasiglucagon dose, the amount and route of administration of carbohydrates (IV GIR, parenteral nutrition, gastric carbohydrate infusions, and carbohydrates from oral feeds) can be adjusted at the discretion of the investigator. Plasma glucose should be monitored at least hourly to adjust the IV GIR as appropriate and to avoid hypoglycemia. Additional concomitant medications, including somatostatin analogs and/or sirolimus/mechanistic target of rapamycin (mTOR) inhibitors, may be introduced at the investigator's discretion if the maximum dose level of dasiglucagon (70 µg/hr) has been reached or if further up-titration is not possible due to undesirable side effects. Adjustment of total carbohydrates administered (IV glucose, parenteral nutrition, gastric carbohydrate infusions, and carbohydrates from oral feeds) should continue throughout this period according to local practice to maintain PG levels in the range of 70 mg/dL to 120 mg/dL (3.9-6.7 mmol/L). The aim will be to obtain stable glucose levels with minimum rescue/hypoglycemia-prevention carbohydrates administered by invasive routes and to encourage/optimize oral feeds, and achieve weaning off IV glucose. Regardless of their discharge status, all patients will be offered the opportunity to participate in the long-term safety extension trial (ZP4207-17106), providing the investigator attests to the positive benefit-risk balance of continued dasiglucagon treatment.
PLANNED TRIAL SITES	Five to 7 sites in the United States, Europe, and Israel

ENDPOINTS	Primary endpoint *Part 1 (Day 1 to 4)* • Mean IV GIR in the last 12 hours of each treatment period during Part 1 (dasiglucagon or placebo administration) Key secondary endpoint *Part 1 (Day 1 to 4, for each 48-hour treatment period)* • Total amount (g) of carbohydrates administered (regardless of the route) per day.
STATISTICAL METHODS	Continuous endpoints will be summarized with number (n), mean, standard deviation (SD), median, minimum, and maximum. In addition, change from baseline values will be calculated at each time point and summarized descriptively; the exception to this is the time of PG within prespecified ranges, which will not have a baseline value. For categorical endpoints, descriptive summaries will include counts and percentages. All data will be presented in the data listings. Analysis Populations Four analysis populations have been defined for this trial: Safety analysis set: defined as all patients administered any randomized treatment. Full analysis set (FAS): defined as all patients in the Safety Set who have a valid baseline efficacy assessment. Patients will be analyzed according to randomized treatment (Part 1) or overall patients (otherwise). Per protocol (PP) set: defined as all patients in the FAS without any major protocol deviations. Patients will be excluded from the PP set if they do not receive both treatments and/or do not have efficacy data available to evaluate the primary endpoint for both treatments. Pharmacokinetic Set (PK): defined as all patients in the Safety Set who have at least 1 measurement with quantifiable plasma concentration of dasiglucagon. Efficacy Analyses The primary analyses of the primary and the key secondary endpoint will be based on the FAS. Supportive analyses of the primary endpoint will be based on the PP. *Primary Endpoint* *Part 1, Day 1 to 4* The mean IV GIR in the last 12 hours of each treatment period will be calculated as a weighted mean across the 12 hours, taking the actual time periods between the measurements into account (corresponding to calculating the area under the curve (AUC) and dividing it by the length of the time period, i.e., weighted mean IV GIR). The IV GIR endpoint is expressed as glucose in mg/kg/min, i.e., data reported from different concentrations of the glucose used for infusion will be transformed to this standardized unit prior to analysis. The primary analysis is defined by the estimand based on the treatment policy (de-facto) strategy, where the actual GIR measurement reported irrespective of adherence to treatment or use of subsequent therapy is used. The reduction in weighted mean IV GIR, as determined as the difference in weighted mean IV GIR between placebo and dasiglucagon, will be estimated. The weighted mean IV GIR will be analyzed using a mixed-model regression approach, with treatment and period as fixed effects and patient as a random effect. The 2-sided 95% confidence interval (CI) for the treatment difference will be calculated from the mixed regression model. Handling of missing data and sensitivity analyses are defined in the body of the protocol.

	Key Secondary Endpoints *Part 1, Day 1 to 4* The key secondary endpoint of total amount of gastric carbohydrates administered will be analyzed by using a mixed-model regression approach, with treatment and period as fixed effects and patient as a random effect. <u>Safety Analyses</u> Adverse events will be coded using the latest version of the Medical Dictionary for Regulatory Activities (MedDRA). Adverse events that begin after the first dose of trial drug will be defined as treatment emergent. The overall incidence of AEs will be displayed by system organ class, preferred term, and treatment. The incidence of AEs will also be presented by severity and by relationship to the trial drug. Vital signs, clinical laboratory measures (including hematology, biochemistry, and incidence of anti-drug antibodies [ADAs]), 12-lead electrocardiograms (ECGs), echocardiography, physical examinations, and local tolerability data will be summarized by treatment, where applicable. Out-of-range safety endpoints may be categorized as low or high, where applicable. Safety results will be summarized by treatment received within treatment period and part and by trial visit, if applicable. A formal statistical analysis plan (SAP) will be prepared to provide further details on the methods for statistical analysis. No interim analysis is planned.
SAMPLE SIZE DETERMINATION	A total of 12 patients will be randomized in this crossover trial on the basis of the following considerations: The GIR results from 40 infants treated with IV glucagon for 24 hours published in the JIMD Research Report "The Effect of Continuous Intravenous Glucagon on Glucose Requirements in Infants with Congenital Hyperinsulinism" (Hawkes et al 2019). "Overall there was a statistically significant reduction in the median (IQR) GIR during the 24h following initiation of continuous glucagon infusion compared to 24 h before initiation (18.5 (12.9, 22.8) to 11 (6.6, 17.5) mg/kg/min…" This trial is powered to detect an effect in GIR for dasiglucagon after 48 hours compared to placebo of at least this effect size. Based on the longer infusion and titration of dasiglucagon in this trial, this is considered a conservative approach, allowing for some uncertainty when translating the published data into a clinical trial setting. Using unpublished individual patient data from the above-referenced study, the difference in GIR between the 2 treatment groups is assumed to follow a normal distribution. Assuming the true mean difference is 7.5 mg/kg/min with a standard deviation of differences of 7.36, the trial will have 89% power using a one-sample t-test with 12 patients randomized to receive either placebo first and then dasiglucagon or vice versa.
TRIAL AND TREATMENT DURATION	The sequence and maximum duration of the trial periods will be as follows: 1. Screening Period: up to 28 days, including a minimum 24-hour run-in period 2. Part 1 (2x48-hour period: double-blind, randomized, placebo-controlled): 96 hours 3. Part 2 (Open-label Active Treatment Period): 21 days 4. Follow-up Period: Patients who do not enter the extension trial will have a Follow-up Visit 28 days after their last dose of trial drug The maximum trial duration for each patient is 81 days. The maximum treatment duration for each patient is 25 days.

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5. LIST OF ABBREVIATIONS

ADA	anti-drug antibody
ADE	adverse device effect
A&E	accident and emergency
AE	adverse event
AESI	adverse event of special interest
ANOVA	analysis of variance
$AOC_{glucose}$	area over the glucose curve
AUC	area under the curve
$AUC_{0\text{-}inf}$	area under the plasma concentration-time curve from time zero to infinity
BfArM	Bundesinstitut für Arzneimittel und Medizinprodukte
CGM	continuous glucose monitoring
CHI	congenital hyperinsulinism
CI	confidence interval
C_{max}	maximum observed concentration
CRA	clinical research associate
CRF	case report form
CRO	contract research organization
CTR	clinical trial report
DMC	independent data monitoring committee
ECG	electrocardiogram
eCRF	electronic case report form
EDC	electronic data capture
ER	emergency room
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GI	gastrointestinal
GIR	glucose infusion rate
GLMM	generalized linear mixed model
H	hypothesis
ICF	informed consent form

ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IEC	independent ethics committee
IM	intramuscular(ly)
IRB	institutional review board
ISF	investigator site file
IV	intravenous(ly)
MedDRA	Medical Dictionary for Regulatory Activities
mTOR	mechanistic target of rapamycin
NG	nasogastric
NME	necrolytic migratory erythema
PD	pharmacodynamic(s)
PG	plasma glucose
PK	pharmacokinetic(s)
PT	preferred term
RSI	Reference Safety Information
SADE	serious adverse device effect
SAE	serious adverse event
SAP	statistical analysis plan
SC	subcutaneous(ly)
SMPG	self-monitored plasma glucose
SoC	standard of care
SpO_2	peripheral capillary oxygen saturation
SUSAR	suspected unexpected serious adverse reaction
$t_{½}$	half-life
TMM	trial materials manual
USADE	unanticipated serious adverse device effect
Zealand	Zealand Pharma A/S

6. INTRODUCTION

6.1. Background and Rationale

Congenital hyperinsulinism (CHI) is a rare and challenging disorder in which β-cells in the pancreas secrete insulin irrespective of plasma glucose (PG) concentration, resulting in persistent and often severe hypoglycemia.[1] Congenital hyperinsulinism affects up to 1 in 50,000 newborns. It is typically diagnosed on the basis of signs and symptoms of hypoglycemia during the neonatal period or in infancy; however, the diagnosis may be made later in childhood. Mutations in several different β-cell genes have been described to cause CHI. Hypoglycemia that results from CHI is of particular concern because it is an important cause of brain injury in neonates, infants, and children with this disease, which leads to long-term neurological impairments.[1,2] Up to approximately 50% of children with CHI experience neurodevelopmental abnormalities caused by severe hypoglycemia that results from inadequate treatment and/or delays in diagnosis.[1,3] Severe brain damage is the consequence of severe hypoglycemia, which presents as coma and/or long-lasting epileptic seizures in neonates. Major intellectual disability is, therefore, most frequent in patients with neonatal initial onset, whereas hypoglycemia is usually less severe and brain damage less frequent in children with CHI diagnosed later in childhood.[1] Since symptoms and severity of hypoglycemia can vary and pose a diagnostic challenge in neonates, infants, and children with CHI, prompt recognition and treatment of hypoglycemia is critical to reduce the risk of long-term neurological consequences.

6.2. Current Treatment and Unmet Medical Need

Medical treatment for CHI is focused on chronic therapies to avoid hypoglycemia, as well as on rescue therapy during acute episodes of severe hypoglycemia. Available medical therapies (mainly diazoxide, octreotide, or glucagon alone or in combination with glucose infusion) are often inadequate and accompanied by inability to control PG, as reflected in a large proportion of patients requiring sub-total pancreatectomy.[4,5,6] With the exception of surgery for focal CHI, which is curative in the vast majority of patients, sub-total pancreatectomy for diffuse CHI has substantial inadequacies. A recent retrospective chart review[7] showed that 60% of patients who underwent near-total pancreatectomy had persistent hypoglycemia after surgery. Moreover, 96% had developed insulin-dependent diabetes within 11 years after surgery.

First-line medical treatment of CHI is diazoxide, which is the only EU- and US-approved drug for treatment of hyperinsulinemic hypoglycemia. Diazoxide acts to open K_{ATP} channels of the pancreatic β-cells, thereby inhibiting insulin secretion. Unfortunately, many patients with CHI are resistant to diazoxide because of mutations in the genes encoding the K_{ATP} channel of the pancreatic β-cells.[6] For those who respond to diazoxide treatment, the more common side effects comprise hypertrichosis, fluid retention, and gastrointestinal (GI) symptoms; however, side effects are usually not severe. In diazoxide nonresponders, second-line (and off-label) treatment is a somatostatin analog (octreotide or lanreotide [long acting]), which (among other effects) inhibits secretion of insulin and glucagon from the pancreas and suppresses GLP-1 secretion. Factors that limit their use comprise tachyphylaxis, as well as possible side effects, including necrotizing enterocolitis, gallstones, and hepatitis.[6]

The glycogenolytic effect of glucagon and its ability to increase PG levels has been confirmed in children with CHI or neonatal hypoglycemia.[8,9] Administration of marketed glucagon (intravenous [IV] or subcutaneous [SC] injection) is often used in the diagnostic phase of CHI to

stabilize patients before surgery or initiation of other medical treatments.[10] In a retrospective review of 55 neonates with resistant hypoglycemia, native glucagon IV infusion achieved a median rise in plasma glucose of 45 mg/dL (2.5 mmol/L) after an hour of administration, regardless of the underlying cause for the persistent neonatal hypoglycemia.[9] Furthermore, glucagon is administered as single SC doses to treat severe hypoglycemic episodes. While IV administration of glucagon to patients with CHI is used short-term in the hospital setting, e.g., before pancreatectomy,[2,10,11] long-term glucagon treatment is challenging since currently available glucagon products are unstable and form fibrils within hours after reconstitution.[12] Catheter obstruction and occlusion because of glucagon instability were observed daily to 2 to 3 times weekly in a retrospective review of 9 patients with CHI receiving continuous SC infusion of glucagon for weeks or months.[11] In another series of patients, 60% of the patients treated with short-term SC glucagon experienced catheter occlusion.[6] Instability of currently available glucagon products and the associated risk of dosing errors contributing to hypoglycemic events is a major barrier for using glucagon for long-term treatment of patients with CHI.

When successfully administered, long-term home treatment with subcutaneously infused glucagon over extended periods (years) has been beneficial, with a potentially good safety profile as compared to diazoxide and octreotide.[6,11,13]

6.3. Dasiglucagon for the Treatment of Congenital Hyperinsulinism

6.3.1. Dasiglucagon

Dasiglucagon is a peptide analog of human glucagon in development for the treatment and prevention of hypoglycemia in patients with diabetes mellitus and CHI via SC or intramuscular (IM) administration. Dasiglucagon is a stable analog of glucagon that has been specifically designed to overcome the issues with fibril formation and instability in solution observed with marketed glucagon products. Compared to native human glucagon, dasiglucagon also comprises 29 amino acids. As a result of chemical modifications (7 amino acid substitutions compared to human glucagon), the pronounced tendency of glucagon to form fibrils and aggregate has been effectively prevented in dasiglucagon. In addition, the chemical stability in aqueous media at physiological pH has been improved.

To support the use of dasiglucagon in the pump for this trial, compatibility/in-use studies have been performed with dasiglucagon 4 mg/mL in Roche Accu-Chek® Combo pump using the Accu-Chek® Spirit 3.15 mL cartridge system and the Accu-Chek® FlexLink infusion set. The studies support an in-use time for up to 6 days at 37ºC.

Dasiglucagon has been granted orphan drug designation by the European Commission on 20 June 2017 for the '*treatment of congenital hyperinsulinism*.' Furthermore, the FDA granted an orphan drug designation for the '*treatment of hypoglycemia in patients with congenital hyperinsulinism (CHI)*' on 10 August 2017.

6.3.2. Nonclinical Experience

The completed nonclinical pharmacology program has determined that dasiglucagon is a specific glucagon receptor agonist with comparable in vitro potency to glucagon, promoting a rapid onset of PG increase in both normoglycemic and insulin-induced hypoglycemic animals, similar to that of glucagon.

Results of the toxicity studies with dasiglucagon are comparable to what has been reported for glucagon. Those from chronic toxicity studies with dasiglucagon in rats and dogs are in line with the results of short-term toxicity studies, indicating that long-term treatment with dasiglucagon is safe and that the pharmacodynamic (PD) effects noted do not adversely affect organ function following chronic use.

6.3.3. Clinical Experience

Dasiglucagon is being developed to manage patients with CHI 1) as an initial short-term therapy to stabilize PG levels and reduce glucose infusion needs, and 2) as a long-term treatment to help maintain euglycemia. Dasiglucagon has not previously been studied in patients with CHI. Clinical experience with dasiglucagon comes from the following studies in healthy subjects and type 1 diabetics.

The safety, tolerability, pharmacokinetic (PK), and PD characteristics of dasiglucagon were investigated in 2 phase 1 trials, 3 phase 2 trials, and 2 phase 3 trials.

ZP4207-14013 was a phase 1 randomized, double-blind, 2-part, single-dose trial designed to evaluate the pharmacokinetics, PD, safety, and tolerability of dasiglucagon compared to GlucaGen®. Part 1 consisted of SC and IM administration of single ascending doses of dasiglucagon in 64 adult healthy volunteers. Part 2 consisted of 20 adult patients with type 1 diabetes using a crossover design with IM administration of 0.7 mg dasiglucagon. GlucaGen 1.0 mg was used as a comparator in both parts of the trial.

ZP4207-15007 was a phase 1 randomized, placebo-controlled, double-blind, multiple-dose trial designed to evaluate the PK, PD, safety, and tolerability of repeated doses of dasiglucagon SC in 24 healthy adult volunteers. The trial included 3 dose cohorts of 8 healthy volunteers each. In each cohort, patients were randomly assigned in a 3:1 ratio to receive 5 repeated daily doses of 0.1, 0.3, and 1.0 mg dasiglucagon (n=6) or placebo (n=2).

After the 2 phase 1 trials were completed, the composition of the dasiglucagon formulation was optimized with respect to its excipients (buffer, tonicity adjusting agent, and stabilizer). The PK and PD properties, safety, and tolerability of the optimized formulation were evaluated in the phase 2 trials.

ZP4207-15126 was a phase 2 randomized, double-blind, single-dose trial designed to evaluate the PK, PD, safety, and tolerability of a single SC dose of dasiglucagon in the optimized formulation (0.1, 0.3, 0.6, and 1.0 mg) or GlucaGen (0.5 and 1.0 mg) in 58 adult patients with type 1 diabetes.

ZP4207-16051 was a phase 2 open-label, randomized, crossover trial designed to evaluate the feasibility of using dasiglucagon 1 mg/mL SC in conjunction with insulin in a pump setting using an iLet algorithm as part of an automated dual hormone pump delivery system in 12 patients with type 1 diabetes. The primary objective was to evaluate the safety and tolerability of dasiglucagon compared to marketed glucagon (Lilly Glucagon™). The trial also aimed to demonstrate the functionality and accuracy of the iPhone-based bionic pancreas in a controlled,

clinical setting. Patients were randomly assigned to two 1-day treatment arms: dasiglucagon or Lilly Glucagon in the iPhone-based bionic pancreas.

ZP4207-16098 was a phase 2 randomized, crossover, double-blind trial designed to evaluate the safety, tolerability, PK, and PD of dasiglucagon in 23 patients with type 1 diabetes using a 4 mg/mL formulation of dasiglucagon (0.03 mg, 0.08 mg, 0.2 mg, and 0.6 mg) compared to an active comparator, Lilly Glucagon.

ZP4207-16136 was a phase 3 randomized, double-blind parallel-group safety trial designed to evaluate the immunogenicity of dasiglucagon compared to GlucaGen in 111 adult patients with type 1 diabetes.

ZP4207-16137 was a phase 3 randomized, double-blind, parallel-group trial designed to confirm the clinical efficacy and safety of a single dose of dasiglucagon (0.6 mg) for the rescue treatment of hypoglycemia compared to placebo and with reference to GlucaGen (1 mg) in 168 adult patients with type 1 diabetes.

Overall, no safety concerns were observed for dasiglucagon at the doses investigated in these clinical trials. The most frequently reported adverse event (AE) in each trial was nausea, which is a known side effect after administration of glucagon, and appeared at a similar frequency to marketed glucagon, which was used as active comparator in some of the trials. No local tolerability issues were reported. Injection site reactions were observed only sporadically after administration with dasiglucagon, placebo, or marketed glucagon in trials with SC or IM administration, and all events were mild and transient. No treatment-induced or treatment-boosted anti-drug antibodies (ADAs) were noted in any of the reported trials, except for trial ZP4207-16098, in which 1 patient tested positive for both anti-dasiglucagon and anti-glucagon antibodies. Due to the crossover nature of this trial, the induction of ADAs could not be associated with a specific treatment.

Dose proportionality for dasiglucagon PK using the 1 mg/mL formulation was confirmed in the clinical trials, with doses ranging between 0.1 and 1.0 mg, characterized by a fast absorption with a peak plasma concentration obtained after 35 minutes. The median time to maximum concentration was later for dasiglucagon compared to marketed glucagon (GlucaGen) (35 vs 20 minutes). In the phase 2 trial ZP4207-15126, doses of 0.3 mg dasiglucagon and 0.5 mg GlucaGen and also 0.6 mg dasiglucagon and 1.0 mg GlucaGen were similar with regard to maximum observed concentration (C_{max}). The total exposure in terms of area under the concentration-time curve (AUC) from time zero to infinity ($AUC_{0\text{-}inf}$) was consistently higher for dasiglucagon compared to GlucaGen. This was likely due to a higher bioavailability of dasiglucagon since nonclinical data consistently show that the bioavailability of dasiglucagon is higher compared to marketed glucagon formulations. For the 4 mg/mL formulation, dose-proportionality of dasiglucagon doses between 0.03 and 0.6 mg was indicated for $AUC_{0\text{-}240min}$ and $AUC_{0\text{-}inf}$ in trial ZP4207-16098. Pharmacokinetic parameters were all similar for dasiglucagon administration under both euglycemic and hypoglycemic conditions. An approximately 30 to 40% greater exposure in terms of $AUC_{0\text{-}inf}$ and C_{max} was observed with the 4 mg/mL formulation as compared with the 1 mg/mL formulation.

In the phase 2 trial ZP4207-15126, all patients achieved a PG level of at least 70 mg/dL (3.9 mmol/L) at all dose levels of dasiglucagon after insulin-induced hypoglycemia, as well as an increase in PG by at least 20 mg/dL (1.1 mmol/L) within 30 minutes postdose. The PD responses of 0.3 mg of dasiglucagon and 0.5 mg of marketed glucagon (GlucaGen) were similar in the first 30 minutes postdose, as were the PD responses of 0.6 mg of dasiglucagon and 1.0 mg of

GlucaGen. The PD response over the entire observation time (0-360 minutes) was significantly greater after dosing with dasiglucagon than with GlucaGen for all prespecified dose comparisons. This was likely an effect of the higher total drug exposure ($AUC_{0\text{-}inf}$) of dasiglucagon mentioned previously.

In the phase 3 trial ZP4207-16137, the primary endpoint was time to plasma glucose recovery after insulin-induced hypoglycemia, which was defined as first increase in plasma glucose of ≥20 mg/dL (1.1 mmol/L) from baseline. Superiority was shown for dasiglucagon relative to placebo, with a median time to plasma glucose recovery of 10 versus 40 minutes. The median time to recovery for GlucaGen was 12 minutes. All patients in the dasiglucagon and GlucaGen treatment arms recovered from insulin-induced hypoglycemia within 30 minutes. All patients but one receiving dasiglucagon and one receiving GlucaGen had recovered within 20 minutes (dasiglucagon: 99% and GlucaGen: 98%). At 15 minutes, 99% of patients receiving dasiglucagon and 95% of those receiving GlucaGen had recovered. At 10 minutes, 65% of patients receiving dasiglucagon and 49% of those receiving GlucaGen had recovered.

No major differences in PD responses were observed between dasiglucagon doses under euglycemic or hypoglycemic conditions in phase 2 trial ZP4207-16098. Under euglycemic conditions, all patients achieved an increase in PG of at least 20 mg/dL (1.1 mmol/L) within 30 minutes postdose with 0.08 mg dasiglucagon and above, while this target was reached only for 0.2 mg of marketed glucagon (Lilly Glucagon). Under hypoglycemic conditions, dasiglucagon met the target at doses of 0.2 mg and above. The PD response over the entire observation time of 240 minutes was significantly higher with dasiglucagon than with Lilly Glucagon at the same doses. In the bionic pancreas feasibility trial ZP4207-16051, results demonstrated comparable autonomous glycemic control with dasiglucagon compared to Lilly Glucagon. This was despite the stressed conditions of the trial in terms of fasted patients being on up to twice their basal insulin rate to stimulate glucagon use during the 8-hour test, and inclusion of a structured exercise period. With dasiglucagon, patients obtained a glucose value within target (70-180 mg/dL [3.9-10.0 mmol/L]) 70.9% of the time vs 65.6% with Lilly Glucagon.

6.3.4. Literature Data

In a retrospective review of 223 cases of diffuse or focal CHI, glucagon was reported to be used in 55% of patients with diffuse CHI and in 31% of patients with focal CHI.[10] In an observational trial of 55 newborns who received glucagon because of hypoglycemia after birth, applied doses were mainly in the range of 0.5 to 1.0 mg/day, and results indicated an increase in PG from a mean of 36.3 md/dL to a mean of 93.0 mg/dL, observed within 4 hours after the start of glucagon infusion.[9] The frequency of hypoglycemic episodes was significantly reduced, and no further episodes of severe hypoglycemia were observed.

In another retrospective chart review of 40 children with CHI who received continuous IV glucagon for prevention of hypoglycemia,[14] a median (inter quartile range) glucagon dose of 205 (178, 235) mcg/kg/day over a median duration of 5 (3, 9) days enabled a glucose infusion rate (GIR) reduction from 18.5 (12.9, 22.8) to 11 (6.6, 17.5) mg/kg/min per 24 h ($p < 0.001$), and reduced hypoglycemia frequency from 1.9 (1.3, 2.9) to 0.7 (0.3, 1.2) episodes per day.

The long-term use of glucagon in patients with CHI is limited by the instability of marketed glucagon after reconstitution. A literature review on the long-term medical treatment of CHI revealed that only 1% of 619 patients identified received glucagon as part of their medical management.[6] A retrospective review of 9 children with CHI who received continuous SC

infusion of glucagon for weeks or months showed that introduction of glucagon allowed the reduction or discontinuation of central glucose infusion in all patients.[11] Six of 9 patients were discharged with continued glucagon therapy that their parents were able to continue without further symptomatic hypoglycemia, convulsions, or unconsciousness. In 3 children, glucagon therapy was continued for 1 to 4 years, which led to stable euglycemia.

The data reported on marketed glucagon use in patients with CHI indicate that continuous SC infusion of a glucagon agonist could provide therapeutic benefit to patients by stabilizing PG levels and reducing the frequency of hypoglycemic episodes.[5,6,9,10,11]

6.3.5. Anticipated Medical Benefit of Dasiglucagon in the Treatment of CHI

With its physio-chemical stability in liquid formulation, dasiglucagon could provide significant added benefit in the treatment of CHI relative to currently marketed glucagon by enabling long-term reliable IV infusion. Long-term subcutaneous infusion of dasiglucagon through a pump may be an attractive alternative or addition to diazoxide and octreotide since it may reduce the dependency on intensive nutritional support, both IV and gastric (NG tube or gastrostomy), whilst maintaining euglycemia by harnessing physiological mechanisms for combating hypoglycemia. Furthermore, if long term euglycemia is achieved with medical therapy, pancreatectomy for the treatment of diffuse CHI could eventually be avoided. In one cohort of nonsurgically treated children, the mean clinical remission rate was 5 (1.5-12) years for diffuse CHI.[5] This suggests that a significant proportion of infants with CHI could avoid surgery if medical treatment allowed for the effective long-term control of hyperinsulinism.

The target population in this trial is children with CHI who are especially challenging to treat, and who need continuous IV glucose infusion to prevent or treat hypoglycemia, necessitating ongoing hospitalization. These children have limited options in terms of other treatments, and represent very high unmet medical need group within already ultra-rare CHI population.

6.3.6. Anticipated Risks of Dasiglucagon in the Treatment of CHI

So far, there is no previous clinical experience with dasiglucagon SC infusion for the treatment of CHI. The following anticipated risks are derived from other clinical trials with dasiglucagon conducted in other indications, from marketed glucagon labelling information, and from native glucagon's physiological effects.

In clinical trials with dasiglucagon conducted up to now, the most frequent treatment-related AEs were nausea and vomiting, followed by headache.

Glucagon exerts positive inotropic and chronotropic effects and may, therefore, cause tachycardia and hypertension. Transient increases in heart rate and hypertension have been observed in clinical trials with dasiglucagon. Additionally, several episodes of hypotension and bradycardia have been noted in the clinical program, often in association with nausea and/or vomiting.

Accidental overdose may occur due to inappropriate handling of the infusion pump or due to pump malfunction. Overdose may result in nausea, vomiting, inhibition of GI tract motility, short-term increase in heart rate or blood pressure, and/or hypokalemia. Symptomatic care for nausea and vomiting, as well as monitoring of heart rate, blood pressure, and hypokalemia is advised.

Injection site reactions are seen with many injectable peptides. Injection site reactions have been reported across trials, with similar frequency in all treatment groups (dasiglucagon, marketed

glucagons, and placebo), irrespective of dose. The injection site reactions were generally mild and transient.

Overall, the clinical trial data indicate that dasiglucagon has a low risk for induction of ADAs. Out of more than 350 doses of dasiglucagon administered to more than 200 patients, only 1 low-titer transient ADA incident has been reported to date, in a patient following administration of 11 SC doses of dasiglucagon or Lilly Glucagon ranging from 0.03 to 0.6 mg, with low binding and low *in vitro* neutralizing capability. No apparent clinical effects on PK, PD, or AEs were noted. Due to the crossover trial design, the ADA induction could not be ascribed to a specific treatment. Based on the current clinical experience and the product characteristics, the risk of dasiglucagon inducing an ADA response is considered low.

Administration of glucagon or dasiglucagon may be associated with a risk of allergic reactions similar to those observed for other therapeutic peptides or proteins.

Data on the risks of chronic administration of dasiglucagon are not available. From sporadic reports of extended SC/IV infusion of marketed glucagon and in glucagonoma patients,[15] sustained exposure to high levels of glucagon may lead to development of the skin condition necrolytic migratory erythema (NME), a highly specific migrating, erythematous rash with predilection for perioral, perianal, and lower leg distribution.[11]

For further information on risks, please refer to the current version of the investigator's brochure (IB).

6.3.7. Summary of Potential Benefits and Risks

As with all treatment interventions, the anticipated benefits to trial patients should be balanced against the potential risks. The accumulated experience from nonclinical studies and clinical trials with dasiglucagon supports that dasiglucagon is a specific glucagon receptor agonist and is well tolerated. Glucagon and its analogs belong to a well-known drug class with known mode of action. The clinical investigators involved in the trial will all have had experience with use of glucagon in patients with CHI.

The investigator will inform the patients/parent(s)/guardian of the potential risks of dasiglucagon treatment and other trial-related procedures before they enter the trial. The investigator must become familiar with all sections of the dasiglucagon IB before the start of the trial.

In this trial population, the major and clinically relevant benefit is the expected reduction and eventually elimination of need for IV glucose, together with the reduction in gastric nutritional interventions while avoiding hypoglycemia. The reduced volume of nutritional interventions should limit the risk of volume overload. Furthermore, by removing the invasive methods of nutrition, the children should better retain or re-establish orality – the intake of nutrients by mouth.

In summary, with its marked improvements in stability in solution and solubility in aqueous media compared to currently marketed glucagon products, dasiglucagon is expected to have significant clinical benefits in the treatment of CHI and to substantially reduce the disease burden in these patients. This includes discharge from the hospital by enabling convenient and reliable long-term treatment via a pump device in a home setting, together with the potential to delay and ultimately avoid pancreatectomy and its related exo/endocrine complications, particularly the development of insulin-dependent diabetes.

Overall, the benefit to risk ratio for patients entering the ZP4207-17103 trial is considered acceptable.

7. OBJECTIVES AND ENDPOINTS

7.1. Objectives

7.1.1. Primary Objective

To evaluate the efficacy of dasiglucagon in reducing glucose requirements in children with persistent CHI requiring continuous IV glucose administration to prevent/manage hypoglycemia.

7.1.2. Secondary Objectives

To evaluate the safety and tolerability of dasiglucagon administered as an SC infusion in patients with CHI.

7.2. Endpoints

7.2.1. Primary Endpoint

Part 1 (Day 1 to 4)

- Mean IV GIR in the last 12 hours of each treatment period during Part 1 (dasiglucagon or placebo administration)

7.2.2. Key Secondary Efficacy Endpoint

Part 1 (Day 1 to 4, for each 48-hour treatment period)

1. Total amount (g) of carbohydrates administered (regardless of the route) per day.

7.2.3. Secondary Efficacy Endpoints

Part 1 (Day 1 to 4, for each 48-hour treatment period)

- Mean IV GIR for each 48-hour treatment period during Part 1 (dasiglucagon or placebo administration)
- Mean IV GIR below 10 mg/kg/min in the last 12 hours of each treatment period during Part 1 (yes/no) (dasiglucagon or placebo administration)

Part 2 (Day 5 to 25, assessed from the start of treatment in part 2)

- Time to complete weaning off IV GIR.
- Hypoglycemia event rate, defined as number of hypoglycemic events (PG <70 mg/dL or 3.9 mmol/L), as detected by SMPG.
- Clinically significant hypoglycemia event rate, defined as number of events <54 mg/dL (3.0 mmol/L), as detected by SMPG.
- Time to actual hospital discharge.
- Time to pancreatic surgery (sub-total or total pancreatectomy).
- Total amount (g) of carbohydrates administered (regardless of the route) per day, together with amounts (g) of carbohydrates administered per day:
  - via IV glucose infusion, or bolus (not as part of total parental nutrition),
  - as part of total parenteral nutrition (if applicable),

  - via oral route, and
  - via NG tube or gastrostomy.
- CGM percent time in range 70-180 mg/dL (3.9-10.0 mmol/L).
- CGM percent time in hypoglycemia (<70 mg/dL or 3.9 mmol/L).
- CGM percent time in clinically significant hypoglycemia (<54 mg/dL or 3.0 mmol/L).
- Rate of hypoglycemia episodes, defined as number of episodes <70 mg/dL (3.9 mmol/L) for 15 min or more, as measured by CGM.
- Rate of clinically significant hypoglycemia episodes, defined as number of episodes <54 mg/dL (3.0 mmol/L) for 15 min or more, as measured by CGM.
- Extent of hypoglycemia (area over the glucose curve [$AOC_{glucose}$] below 70 mg/dL [3.9 mmol/L]) as measured by CGM.
- Extent of clinically significant hypoglycemia ($AOC_{glucose}$ below 54 mg/dL [3.0 mmol/L]) as measured by CGM.
- CGM percent time in hyperglycemia (>180 mg/dL or 10.0 mmol/L).

7.2.4. Safety Endpoints

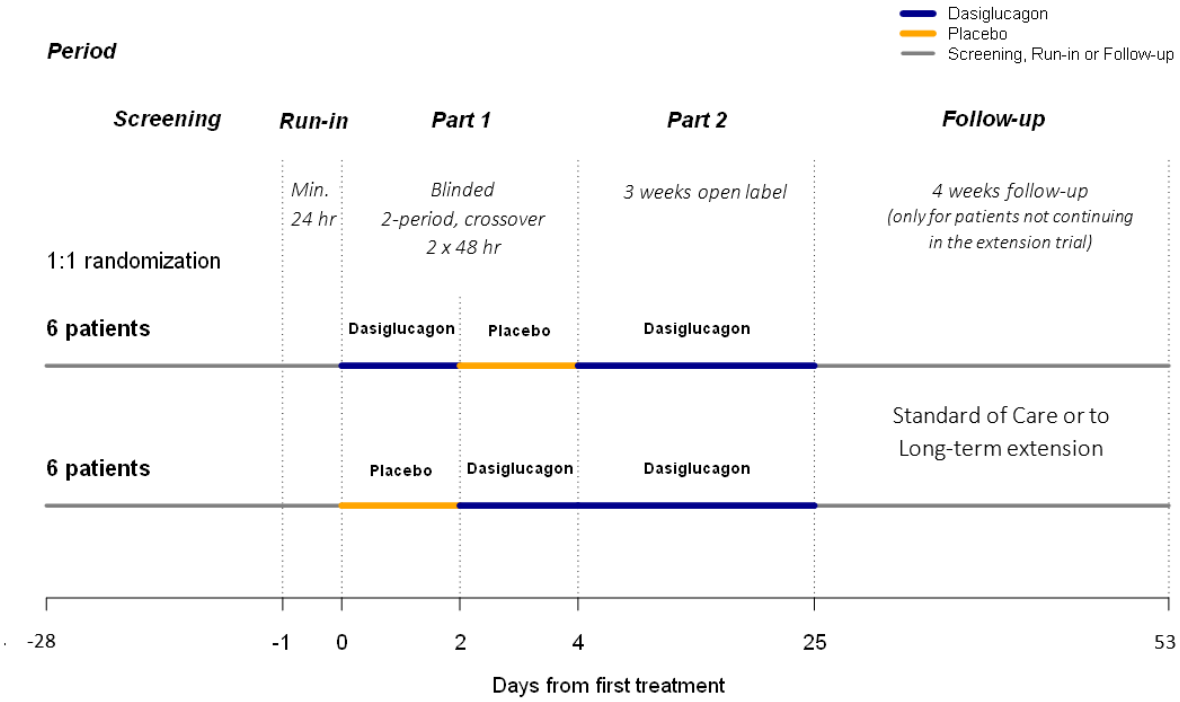
By Part 1 or 2 of the trial:

- Adverse events
- Changes in clinical evaluations:
  - Vital signs
  - Physical examination
  - 12-lead ECG
  - Echocardiogram
- Changes in clinical laboratory assessments:
  - Hematology
  - Biochemistry
  - ADA

8. TRIAL DESIGN

8.1. Overall Trial Design and Plan

This is a combined phase 2 and 3, randomized, multinational trial to evaluate the efficacy and safety of individually titrated dasiglucagon in children ≥7 days and <1 year of age who have been diagnosed with CHI, comprising 2 parts, a crossover (2 periods, 48 hours each), double-blind, placebo-controlled Part 1, and an open-label, single-arm Part 2 of 21 days (Figure 1).



Abbreviations: Min = minimum

Figure 1 Design of Trial ZP4207-17103

After screening, eligible patients will complete a minimum 24-hour run-in period to confirm the IV glucose randomization requirement (≥10 mg/kg/min). All non-nutritional carbohydrates and carbohydrate fortification of feeds are prohibited during this 24-hour run-in period. After the run-in period, patients will be randomly assigned in a double-blind fashion to receive dasiglucagon or placebo for 48 hours, after which they will be crossed over to the other trial treatment for an additional 48 hours. At the time of crossover, the trial drug will be initiated from the starting dose of 10 µg/hr, and IV GIR will be set to the rate obtained at the end of the run-in period and titrated accordingly.

There will be no washout between the 2 periods in Part 1 to limit the length of time in which patients are reliant on IV GIR as their only means of preventing/treating hypoglycemia. This is deemed acceptable because:

- The half-life ($t_{½}$) of dasiglucagon is short (~30 minutes, for the 0.6 mg dose, and potentially shorter for lower doses, ~22 minutes) relative to the 48-hour duration of each crossover

period. Moreover, for the primary endpoint, only the last 12 hours of the 48-hour period will be used for endpoint assessment.

- Assuming a positive effect of dasiglucagon, the absence of washout is conservative with respect to key secondary endpoints since a potential carry over effect of dasiglucagon in Period 2 is attributed to placebo.

During Part 1, the trial drug and IV GIR should be adjusted according to the protocol-specified algorithm (see Section 10.2). Non-nutritional carbohydrates and/or carbohydrate fortification of feeds during Part 1 is only allowed if the maximum tolerable volume and concentration of IV glucose for the patient is reached, and should be limited to the minimum needed to ensure the patient's safety (Section 9.5). All feedings (administered as parenteral nutrition, by NG tube, gastrostomy, or normal route), will be recorded during this period. Safety assessments will be performed daily after initiation of the trial drug (active or placebo).

After Part 1, patients will continue in open-label Part 2 to receive dasiglucagon for 21 days. Additional CHI treatments can be introduced during Part 2 if needed, if up-titration of dasiglucagon is not possible due to undesirable side effects or if the maximum dose level (70 µg/hr) has been reached. Gradual transfer from IV glucose to oral and gastric carbohydrates should be initiated in Part 2, enabling weaning of IV glucose and hospital discharge. Patients will continue to be hospitalized until IV GIR is weaned off; however, as soon as local site criteria for discharge are met, patients can be discharged to continue the treatment period at home. Visits are planned on Day 11 and Day 18 in Part 2. These visits can be converted to telephone visits if appropriate, at the investigator's discretion. On Day 25, the End of Treatment Visit will take place, and based on the investigator's confirmation of continued positive benefit-risk balance, patients will be offered the opportunity to enter the long-term extension trial (Trial ZP4207-17106) to continue dasiglucagon treatment. Patients who do not enter the long-term extension trial will have a Follow-up Visit 28 days after their last dose of trial drug.

8.2. Discussion of Trial Design

The initial 2x48-hour treatment period (Part 1) is double-blind and placebo controlled to avoid bias in the assessment of the effect of dasiglucagon on GIR. A crossover design has been chosen to limit the influence of inherent variability of the population, which allows for recruitment of fewer patients compared to a parallel-group design, reflecting the ultra-rare status of the disease.

All patients will receive dasiglucagon in open-label Part 2. According to International Council for Harmonisation (ICH) guidelines, for the conduct of clinical trials in rare diseases, where the anticipated likelihood of considerable benefit is high, such design has some potential efficiencies because all patients will be exposed to test drug, which is of particular importance in rare diseases.[16]

8.3. Trial Sites

The trial will take place at 5 to 7 sites in the US and Europe that are experienced in the treatment of CHI. A total of 12 patients with CHI is planned to be randomized and exposed to the trial product. Patients who withdraw prematurely will not be replaced.

8.4. Point of Contact

A point of contact will be identified to provide information to each patient's parent(s)/guardian about where to obtain information on the trial, the patient's rights, and whom to contact in case of trial-related injury. This information will be provided in the patient information and informed consent form (ICF).

9. PATIENT POPULATION

9.1. Selection of Trial Population

A screening log of potential trial candidates must be maintained at each trial site.

9.2. Trial Entry Criteria

9.2.1. Inclusion Criteria

A patient will be eligible for trial participation if he or she meets all of the following criteria:

1. CHI diagnosis established based on the following:
   a. Hyperinsulinemia: plasma insulin above the limit of detection of the assay documented during an event of hypoglycemia, and/or
   b. Hypofattyacidemia: plasma free fatty acid <1.7 mmol/L, and/or
   c. Hypoketonemia: Beta-hydroxybutyrate <1.8 mmol/L, and/or
   d. Glycemic response: an increase in PG of >30 mg/dL (1.7 mmol/L) after 1 mg IV or IM glucagon administration
2. Male or female, age ≥7 days and <12 months at screening
3. Body weight of ≥2.0 kg (4.4 lbs.)
4. Continuous IV glucose requirement to prevent hypoglycemia
5. One or both parents* or guardians of the patient must provide signed informed consent before any trial related activity is performed. (*according to local regulations)

9.2.2. Exclusion Criteria

A patient will be excluded from the trial if he or she meets any of the following criteria:

1. Is suspected of having a transient form of CHI (e.g., transient hyperinsulinism due to maternal diabetes or perinatal stress)
2. Was born preterm below 34 weeks of gestational age
3. Presence of hypertension or hypotension, including circulatory instability requiring supportive medication or presence of pheochromocytoma
4. Known or suspected presence of severe brain damage
5. Evidence of metabolic, endocrine, or syndromic causes of hypoglycemia not due to hyperinsulinism
6. Use of systemic corticosteroids, e.g., hydrocortisone >20 mg/m^2 body surface area or equivalent within 5 days before screening

7. Prior use of lanreotide, sirolimus (mechanistic target of rapamycin [mTOR inhibitors]), anti-inflammatory biological agents, or other immune-modulating agents. Prior use of octreotide is allowed after a minimum of 48-hour washout before randomization.
8. Aspartate aminotransferase (AST) or alanine aminotransferase (ALT) >2.5 X the upper limit of normal (ULN), or estimated glomerular filtration rate (eGFR) <30 mL/min/1.73m^2 adjusted by a pediatric formula (e.g., Schwartz formula)
9. Any clinically significant abnormality identified on echocardiogram that in the opinion of the investigator would affect the subject's ability to participate in the trial
10. Known history of laboratory test results obtained before screening that show presence of HIV, hepatitis B surface antigen, hepatitis C antibody, or hepatitis A immunoglobulin M
11. Any recognized clotting or bleeding disorder
12. Previous administration of dasiglucagon (previously referred to as ZP4207)
13. Known or suspected allergy to the trial drug or related products
14. Previous participation (randomization) in the clinical program (Trials ZP4207-17103 or -17109)
15. Has participated in an interventional clinical trial (investigational or marketed product) within 30 days of screening, or plans to participate in another clinical trial, except for 18F-Dopa PET CT/MRI investigation (where performed as a part of a research trial), which is allowed for diagnosis of focal CHI.
16. The use of prescription or non-prescription medications known to cause QT prolongation.

9.2.3. Randomization Exclusion Criteria

A screened patient will not be randomized if:

1. Mean IV glucose requirement is <10 mg/kg/min to maintain glycemia above 70 mg/dL (3.9 mmol/L) during the previous 24 hours prior to randomization
2. Use of glucagon within 24 hours before randomization.
3. Use of additional enteral glucose within 24 hours before randomization. Patients should be transitioned to IV glucose infusions only at least 24 hours before randomization.
4. Is not sufficiently clinically stable on IV GIR only (± diazoxide, as applicable), in the opinion of the investigator, to undergo the placebo-controlled randomized Part 1 of the trial (2x48 hours).

The Randomization Visit may be rescheduled once if Randomization Exclusion Criterion 2 is met.

9.3. Premature Patient Withdrawal

Randomization day exclusion criteria are described in Section 9.2.3.

Patients' parent(s)/guardian 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. Every reasonable attempt should be made by the investigator to keep patients in the trial to obtain data for the primary endpoint in Part 1 and for safety follow-up even if trial treatment has been discontinued. However, patients must be withdrawn from the trial if their parent(s)/guardian withdraw consent to participate.

Investigators must attempt to contact patients' parent(s)/guardian who fail to attend scheduled visits by telephone or other means to exclude the possibility of an AE being the cause of withdrawal. Attempts to contact the patient must be documented. At least 3 phone calls and 3 written attempts to contact the patient will be made prior to considering them lost to follow-up. Should an AE be the cause of withdrawal, it must be documented, reported, and followed up as described in Section 12.3.

If a patient/parent(s)/guardian withdraws consent, the reason for withdrawal and the date of withdrawal will be recorded on the appropriate page of the electronic case report form (eCRF). Whenever possible and reasonable, the evaluations that were to be conducted at the completion of the trial should be performed at the time of premature withdrawal.

9.4. Treatment Discontinuation

To prevent missing data, patients should, to the extent possible, be kept in the trial; therefore, treatment discontinuation is often the preferred option in case of, e.g., substantial noncompliance with trial procedures or initiation of prohibited treatment that interferes with the efficacy and safety evaluation. If it is an investigator's decision to discontinue the patient's treatment, the investigator should, whenever possible, discuss the potential discontinuation of the treatment with the medical monitor. If the patient is discontinued from trial treatment by the investigator or by parent/guardian's decision, the reason for treatment discontinuation and the date of treatment discontinuation will be recorded on the appropriate page of the eCRF. The patient should be asked to continue in the trial by following the planned visit schedule and to have trial assessments performed according to the Schedule of Events. This is especially important during Part 1 of the trial where the primary endpoint is evaluated. As a minimum, the patient will be asked to attend the Follow-up Visit 28 days (± 3 days) after discontinuation of trial treatment.

9.5. Rescue Criteria

During Part 1, the IV glucose concentration and rate of infusion can be increased to avoid hypoglycemia. When the maximum (or maximum tolerable) dose of trial drug has been reached and the necessary amount of GIR to prevent hypoglycemia is likely to cause (in the investigator's opinion) a moderate or severe AE (e.g., volume overload), oral/gastric non-nutritional carbohydrates can be introduced to ensure the patient's safety whilst maintaining the maximum tolerable concentration and infusion rate of IV glucose.

During Part 2, patients are allowed to initiate and titrate oral/gastric non-nutritional carbohydrates, and initiate any CHI treatment.

9.6. Patient Replacement Criteria

Patients who withdraw from the trial prematurely will not be replaced. If a substantial number of patients withdraw prematurely, the sponsor will evaluate the need for developing replacement criteria.

Enrolled patients (defined as randomized) who are subsequently withdrawn from the trial may not re-enter. The patient number for a withdrawn patient will not be reassigned to another patient.

10. TREATMENTS

10.1. Identification of Investigational Product

Dasiglucagon Injection, 4 mg/mL will be supplied by the sponsor in a 3 mL vial containing 1 mL of drug product.

Matching placebo for dasiglucagon injection will be supplied by the sponsor in a 3 mL vial containing 1 mL of drug product.

Dasiglucagon and matching placebo will be provided in the form of solution for injection for subcutaneous administration through an infusion pump.

The trial drug product, as applicable, must be transferred from the vial to the Accu-Chek Spirit Cartridge. The amount of drug product dosed via the pump will vary among patients.

Cartridges and infusion sets should be filled and replaced as indicated in the instructions for use.

10.1.1. Packaging and Labeling

Trial drug products will be packaged and labeled by the sponsor.

Part 1 dispensing unit configuration: 3 vials containing dasiglucagon, 4 mg/mL or placebo for dasiglucagon, packed in an outer carton. The vials and carton will be packaged and labeled in local language without revealing the treatment.

Part 2 dispensing unit configuration: 3 vials containing dasiglucagon, 4 mg/mL, packed in an outer carton. The vials and carton will be packaged and labeled in local language, revealing the treatment.

The storage conditions for trial drug products will be described on the trial drug product label. The labels will supply no information about patients. Each dispensing unit (3 vials) will have a unique Dispensing Unit Number for drug allocation, drug accountability, and traceability purposes.

Labelling will be performed according to Annex 13 of the Good Manufacturing Practice guidelines of the European Commission, ICH Good Clinical Practice (GCP) guidelines, local laws, and regulations.

10.2. Treatments Administered

As long as the patient is receiving IV glucose, the PG will be measured and reviewed hourly using a hand-held PG meter. The IV GIR will be titrated to achieve glycemia of at least 70 mg/dL (3.9 mmol/L) (a minimum GIR will be established as the rate up-titrated after the patient drops at least once below 70 mg/dL [3.9 mmol/L]). When the patient is no longer on IV GIR, the PG will be checked according to local practice, but at least 3 times daily. The PG will be measured using the same trial-supplied hand-held PG meter during the entire trial.

Additionally, blinded CGM will be started 24 hours prior to randomization and continued until the end of Part 2.

Dosing of dasiglucagon will approximate continuous infusion by delivering small doses at frequent intervals via the infusion pump. The adjustment of trial drug dosing is closely linked to the PG level achieved, which in turn will govern the IV GIR. In Part 1 the algorithm in Table 1 should be used.

The pump administers 0.000025 mL/dose ~ 0.1 µg/dose (4 mg/mL formulation):

- 10 µg/hr ~ 0.5 µg every 3 min
- 20 µg/hr ~ 1 µg every 3 min
- 30 µg/hr ~ 1.5 µg every 3 min
- 40 µg/hr ~ 2µg every 3 min
- 50 µg/hr ~ 2.5µg every 3 min
- 60 µg/hr ~ 3µg every 3 min
- 70 µg/hr ~ 3.5µg every 3 min

Table 1 Algorithm to Maintain Plasma Glucose

Plasma Glucose (mg/dL)	Plasma Glucose (mmol/L)	Action
<50	<2.8	Give 200 mg/kg of dextrose as a bolus and increase IV GIR by 2 mg/kg/min
50-59	2.8-3.3	Increase IV GIR by 2 mg/kg/min
60-69	3.3-3.9	Increase IV GIR by 1 mg/kg/min
70-80	3.9-4.4	No change
81-90	4.4-5.0	Reduce IV GIR by 0.5 mg/kg/min
91-100	5.0-5.5	Reduce IV GIR by 1 mg/kg/min
101-120	5.5-6.7	Reduce IV GIR by 1.5 mg/kg/min
>120	>6.7	Reduce IV GIR by 2 mg/kg/min

Abbreviations: GIR = glucose infusion rate; IV = intravenous

The IV GIR will be reviewed, evaluated, and adjusted (if indicated) every hour according to the above PG-driven algorithm to maintain a PG of >70 mg/dL (>3.9 mmol/L). Rechecks of PG are allowed.

Part 1 (Crossover, double-blind, randomized, placebo-controlled)

The starting dose of trial drug is 10 µg/hr at t=0. Every 2 hours (t=2, 4, 6, etc.), the dose will be increased by an additional 10 µg/hr until either:

- The patient is totally weaned off IV glucose, or
- Plasma glucose during the last 2 hours was constantly above 120 mg/dL (6.7 mmol/L), or
- IV GIR has not decreased despite 2 sequential dose increments of trial drug (in this situation, the dose of trial drug should be maintained until the IV GIR can be further decreased or until crossover or the end of Part 1), or
- The maximum dose of 70 µg/hr is reached, or
- AEs emerge that are considered to be related to dasiglucagon (e.g., nausea/change in feeding pattern or increased vomiting) that are limiting further dose escalation

The 2-hour dose-adjustment interval will allow drug plasma levels to approach steady-state before the dose is further increased. The cumulative dose will not exceed 1.26 mg over the first 24 hours and 1.68 mg for each of the subsequent 24-hour periods.

At the time of crossover from the first 48-hour period to the second 48-hour period, the trial drug will again be titrated from the starting dose of 10 µg/hr, and IV GIR will be set to the rate obtained at the end of the run-in period and titrated accordingly.

Part 2 (Open label)

After Part 1, patients will continue in open-label Part 2 to receive dasiglucagon for a further 21 days. Since treatment allocation during Part 1 remains blinded, all patients are required to initiate dasiglucagon dosing at 10 µg/hr in Part 2, while IV GIR should be started at the rate obtained at the end of the run-in period.

During Part 2, the dasiglucagon dose, the amount and route of administration of carbohydrates (IV GIR, parenteral nutrition, gastric carbohydrate infusions, and carbohydrates from oral feeds) can be adjusted at the discretion of the investigator. The PG should be monitored at least hourly to adjust the IV GIR as appropriate and to avoid hypoglycemia. Additional concomitant medications, including somatostatin analogs and/or sirolimus/mTOR inhibitors, may be introduced at the investigator's discretion if the maximum dose level of dasiglucagon (70 µg/hr) has been reached or if further up-titration is not possible due to undesirable side effects.

Adjustment of total carbohydrate administered (IV glucose, parenteral nutrition, carbohydrate infusions, and carbohydrates from oral feeds) should continue throughout this period according to local practice to maintain PG levels in the range of 70 mg/dL to 120 mg/dL (3.9-6.7 mmol/L).

The aim will be to obtain stable glucose levels with minimum rescue/hypoglycemia-preventative carbohydrates administered by invasive routes, and to encourage/optimize oral feeds, and achieve weaning off IV glucose. Regardless of their discharge status, all patients will be offered the opportunity to participate in the long-term safety extension trial (ZP4207-17106), providing the investigator attests to the positive benefit-risk balance of continued dasiglucagon treatment.

10.3. Trial Supplies

The device and ancillaries listed in the following table will be supplied by the sponsor throughout the trial. Trained trial personnel will train parent(s)/guardian on the use of the devices.

Instructions for the use of all these supplies will be provided in separate manuals.

Item	Name	Manufacturer
Pump	Accu-Chek Spirit Combo	Hoffman-La Roche AG, Basel, Switzerland
Cartridge	Accu-Chek Spirit 3.15 mL Cartridge system	Hoffman-La Roche AG, Basel, Switzerland
Infusion sets	Accu-Chek FlexLink Infusion set (Accu-Check UltraFlex Infusion set in US) and Accu-Chek Rapid-D Link Infusion set	Hoffman-La Roche AG, Basel, Switzerland
Infusion set inserter	Accu-Chek LinkAssist Insertion device (can be used with FlexLink & UltraFlex)	Hoffman-La Roche AG, Basel, Switzerland
SMPG	StatStrip Xpress2	Nova Biomedical, Waltham, MA, USA
CGM	Dexcom G4 (not to be used in Germany) Dexcom G6	Dexcom Inc., San Diego, CA, USA

The infusion pump system is CE-marked for the management of diabetes mellitus in persons requiring insulin, as prescribed by a physician. In this trial, the pump system is used outside of the CE-marked intended use since the pump system will be delivering dasiglucagon to patients with CHI. The PG meter is used as intended according to the CE mark, except for the use by a lay person in a home care setting. The CGM devices are used as intended according to the CE mark, except for the age group and the disease.

The pump, the SMPG, and the CGM will be labeled for use in an investigational trial. For more information on the devices please refer to Section 20.

10.4. Dispensing and Storage

The trial drug products supplied by the sponsor is to be dispensed exclusively to patients randomized in this clinical trial according to the instructions of this protocol and the pharmacy manual/trial materials manual (TMM). The investigator is responsible for dispensing the trial drug product according to the dosage scheme. Trial drug products will be allocated using an interactive automated web response system (IWRS), according to Section 10.5.

Dasiglucagon and placebo for dasiglucagon injection must be stored at 2–8°C in a refrigerator.

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

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

The investigator must contact the clinical research associate (CRA) in case of temperature deviations outside the acceptable range.

Please see the pharmacy manual/TMM for additional information on handling of the trial drug.

The investigator must maintain adequate records showing the receipt, dispensing, return, or other disposition of the trial drug, including the date, quantity, batch or code number, and identification of patients (patient number) who received the trial drug. The investigator will not supply the trial drug to any person except subinvestigators, designated trial personnel, and patients in this trial. The trial drug may not be relabeled or reassigned for use by other patients. If any of the trial drug is not dispensed, is lost, stolen, spilled, unusable, or is received in a damaged container, this information must be documented and reported to the sponsor and the appropriate regulatory agencies as required.

10.5. Method of Assigning Patients to Treatment Groups

For Part 1, patients will be randomly assigned 1:1 in a double-blind fashion to 1 of 2 sequences:

- Dasiglucagon 4 mg/mL for 48 hours, followed by placebo for dasiglucagon for 48 hours, or
- Placebo for dasiglucagon for 48 hours, followed by dasiglucagon 4 mg/mL for 48 hours.

Randomization will be performed using a block randomization scheme stratified by region (US/non-US). The randomization scheme will be generated prior to the initiation of the trial by

an independent statistician/programmer who will not be a member of the trial team; investigators will not be aware of the block size of the randomization scheme.

Patients will be randomly assigned to a trial treatment sequence using an IWRS that has been validated for the intended use under the International Society of Pharmaceutical Engineers Good automated manufacturing practice guidelines, 21CFR Part 11 (FDA regulation for Electronic Records and Electronic Signatures), and the ICH Guidance E6 for Industry on Good Clinical Practice.

After completing Part 1, patients will continue in Part 2 to receive active treatment for a maximum of 21 days.

10.6. Blinding and Unblinding Treatment Assignment

Trial personnel will endeavor to safeguard the integrity of the trial blind to minimize bias in the conduct of the trial. Treatment unblinding is discouraged if knowledge of the treatment assignment will not materially change the planned management of a medical emergency. Unblinding will be permitted in a medical emergency that requires immediate knowledge of the patient's treatment assignment.

For emergency unblinding, the investigator will use the measures provided through the IWRS. Unblinding should be discussed in advance with the medical monitor if possible. If the investigator is not able to discuss treatment unblinding in advance, then he or she must notify the medical monitor as soon as possible about the unblinding incident without revealing the patient's treatment assignment.

The investigator or designee must record the date and reason for unblinding on the appropriate eCRF page for that patient.

10.7. Selection of Doses in the Trial

Both the starting dose and the maximum allowed doses are based on experience with marketed glucagon products in this patient population.[11]

At present, no pediatric PK data are available for dasiglucagon. However, an approximate estimation of expected drug concentration in a 3 kg patient has been made by extrapolation of a previously made PK model for pediatric patients with weights between 25 and 45 kg.[17]

In this trial, dasiglucagon will be titrated to meet the needs of the individual patient on the basis of PD effects in regards to efficacy and tolerability, and it is expected that the majority of patients will be adequately treated with a dasiglucagon infusion rate of approximately 20 to 40 µg/hr. The up-titration will stop when IV glucose is completely weaned, if undesirable side effects arise, or when no additional PD effects are observed as the dasiglucagon infusion rate is increased.

Based on the current PK/PD model, the predicted plasma concentration is expected to result in a medium PD response at the lowest dose level and be approaching maximum effect at the highest dose level. The PD response of patients will be monitored closely in this trial, and the appropriateness of the applied titration algorithm will be evaluated.

Although the PK of dasiglucagon has been shown to be dose-proportional in adults and in all toxicologically tested species over a wide range of doses, limited PK sampling at each dose level is planned based on the limited available blood volume in this population.

10.8. Selection of Timing of Dose for Each Patient

Dosing details are provided in Section 10.2.

10.9. Dose Adjustment Criteria

Dose adjustment criteria are presented in Section 10.2.

10.10. Treatment Compliance

Compliance data will be collected. Infusion details will be recorded in the patient's eCRF and drug accountability will be performed as detailed in the pharmacy manual/TMM.

10.11. Permitted and Prohibited Therapies

All concomitant medications used (including over-the-counter medications and herbal supplements) will be recorded in the source document and on the appropriate eCRF.

Caution is advised when beta-blockers, indomethacin, anticholinergic drugs, and warfarin are prescribed due to reports of interaction with marketed glucagon products.

10.11.1. Permitted Therapies

Regarding diazoxide, patients entering the trial either:

- Have tried diazoxide, but discontinued due to lack of efficacy. Minimum of 6 days' washout is required prior to randomization.
- Have tried diazoxide, but discontinued due to adverse events, or family choice, even if efficacy was established. Minimum of 6 days' washout is required prior to randomization.
- Have initiated diazoxide prior to screening, and continue on the treatment. Diazoxide dose should remain stable during Part 1 of the trial. Diazoxide should not be initiated during screening.
- Have never tried diazoxide. Diazoxide should not be initiated during screening or Part 1 of the trial.

Prior use of octreotide is allowed after a minimum 48-hour washout prior to randomization. Washout is required to eliminate the effect of octreotide during Part 1, where adjustment of GIR has to follow the protocol-defined PG-driven algorithm (Table 1) and the primary endpoint on mean GIR is assessed.

Somatostatin analogs and/or diazoxide can be introduced during Part 2 if needed, at the investigator's discretion, when the maximal limit of dasiglucagon (70 µg/hr) has been reached or if further up-titration is not possible due to undesirable side effects.

Addition of other CHI-specific treatments (e.g., sirolimus/mTOR inhibitors) during Part 2 need to be discussed with the medical monitor.

10.11.2. Prohibited Therapies

The following therapies are prohibited:

During the entire trial:

- Systemic corticosteroids, e.g., hydrocortisone >20 mg/m^2 body surface area or equivalent within 5 days before screening.
- Anti-inflammatory biological agents or other immune-modulating agents.

- Other investigational agents.
- Marketed glucagon products within the last 24 hours prior to randomization and throughout the trial unless necessary for rescue therapy in case of severe hypoglycemia, as per local standard of care.
- Prescription or non-prescription medications known to cause QT prolongation.

During Screening and Part 1:

- Somatostatin analogs and sirolimus (mTOR inhibitors). No new CHI treatment is to be initiated during screening and Part 1. Octreotide is allowed in the screening period if discontinued at least 48 hours before randomization.

During Part 2 and follow-up:

- Initiation of somatostatin analogs, diazoxide and sirolimus (mTOR inhibitors) unless the maximum dose level of dasiglucagon (70 µg/hr) has been reached or further dasiglucagon up-titration is not possible due to undesirable side effects.

10.12. Discharge of Patients for Home Treatment

Patients can be discharged during Part 2 to complete the treatment at home as soon as IV GIR has been weaned off and local site criteria for discharge are met. Discharge for home treatment is contingent on the parent(s)/guardian being capable of taking care of the administration of investigative product via pump at home, can use SMPG and CGM device, and are able to attend planned assessment visits at the hospital.

Before discharge of patients from hospital care, the investigator will ensure:

- Appropriate training of patient's parent(s)/guardian in the use of dasiglucagon in the Accu-Chek Spirit Combo pump based on the training material provided. The first pump infusion set change should be performed at the hospital
- Parent(s)/guardian are trained appropriately on the handling of CGM device
- Parent(s)/guardian are trained appropriately on how to perform SMPG measurements and how to complete the diary. They will check their child's SMPG at least 3 times daily (preferably before main meals) and in case of suspected hypoglycemia
- Parent(s)/guardian are instructed not to change the dose of trial drug without prior consultation with the investigator
- Parent(s)/guardian are instructed how to recognize and handle signs of hypoglycemia
- Parent(s)/guardian are instructed to call the investigator/site staff in case of questions

Patients who are discharged from hospital before Day 25 will be contacted by the investigator by telephone the day after discharge. The investigator will ask the parent(s)/guardian if they have any questions about the trial procedures and if their child has experienced any AEs.

10.13. Treatment After End of Trial

On Day 25, the End of Treatment Visit will take place. Based on the investigator's confirmation of continued positive benefit-risk balance, patients will be offered the opportunity to enter the long-term extension trial (Trial ZP4207-17106) to continue dasiglucagon treatment. Patients who do not enter the long-term extension trial will have a Follow-up Visit 28 days after their last dose of trial drug and return to standard of care as per investigator discretion. No further treatment with dasiglucagon will be offered.

11. TRIAL PROCEDURES

Patients' parent(s)/guardian (according to local law) must provide written informed consent before any trial-related procedures are initiated, including the cessation of prohibited concomitant therapy.

For the timing of assessments and procedures throughout the trial, refer to the Schedule of Events (Section 18.1). Throughout the trial, trial personnel should make every reasonable effort to follow the timing of assessments and procedures in Section 18.1 for each patient. If a patient misses a trial visit for any reason, it should be rescheduled as soon as possible.

11.1. Trial Duration

The sequence and maximum duration of the trial periods will be as follows:

1. Screening Period: up to 28 days, including a minimum 24-hour run-in period
2. Part 1 (2x48-hour period: double-blind, randomized, placebo-controlled): 96 hours
3. Part 2 (Open-label Active Treatment Period): 21 days
4. Follow-up Period: Patients who do not enter the extension trial will have a Follow-up Visit 28 days after their last dose of trial drug

The maximum trial duration for each patient is 81 days. The maximum treatment duration for each patient is 25 days.

11.2. Assessments

11.2.1. Efficacy

11.2.1.1. Plasma Glucose Monitoring

As long as the patient is receiving IV glucose, the GIR will be reviewed hourly, based on an hourly PG check performed by the StatStrip Xpress2 meter. The IV GIR will be reviewed, evaluated, and adjusted (if indicated) according to the IV GIR algorithm based on PG levels specified in Table 1. If IV glucose has been stopped, PG will be checked according to local practice, but at least 3 times daily.

Blood for PG check can be obtained either by finger or heel sticks or by an indwelling peripheral line. However, if using an indwelling peripheral line, the total blood volume drawn during a 28-day period should be considered according to Section 11.2.3.1.

At each visit, the investigator will ensure that SMPG data are downloaded from the patient's device. The investigator will check for patient compliance in SMPG measurements. The procedure for download of SMPG data will be described in the pharmacy manual/TMM.

Adjustments to the trial drug will be made as outlined in Section 10.2.

11.2.1.2. Continuous Glucose Monitoring

Dexcom CGMs, configured and labeled for use in the trial, will be provided. In Germany, only the Dexcom G6 CGM will be used. Enrollment in Germany will start only after Dexcom G6 CGM is available.

For other countries: the Dexcom G4 may be used until all required approvals for use of the Dexcom G6 are obtained, but no later than to the end of 2020, when the Dexcom G4 will be taken off the market by the supplier. Patients enrolled thereafter will all be using the Dexcom G6 CGM. Patients who started in the trial using the Dexcom G4 CGM should continue using this device throughout the trial and should not switch to the Dexcom G6 device.

The CGM will be started at least 24 hours prior to randomization and will be used during Parts 1 and 2 in a blinded mode to evaluate efficacy in terms of hypoglycemic episodes. Short pauses of 1 to 3 days due to skin irritation or discomfort are allowed during Part 2 of the trial after consultation with the investigator.

The CGM device should be calibrated and used according to the manufacturer's instructions.

At each visit, the investigator will ensure that CGM data are downloaded from the patient's device. The procedure for download of CGM data will be described in the pharmacy manual/TMM.

The contract research organization (CRO) or delegate will handle the device sourcing, configuration for use in this trial, procedures for blinded data extraction, device service, and handling of potential returns.

11.2.1.3. Diary

For patients who are discharged during the trial, the parent(s)/guardian will receive a paper diary to be completed at home. The investigator will instruct the patient's parent(s)/guardian on how to complete the diary. The following information should be recorded in the diary:

- Type and volume of fluid administered through NG tube/gastrostomy
- Hypoglycemic events, including related SMPG measurements
- Concomitant medications
- AEs
- Hospitalizations, visits to health care providers or emergency room (ER)/accident & emergency (A&E) department, and visits by paramedics
- Data regarding suspicion of NME and neurological findings

Diary entries should be reviewed at each visit and the review documented in the diary. Data from the diary entries should be transcribed to the eCRF on an ongoing basis.

11.2.2. Pharmacokinetics/Drug Exposure

Blood samples will be collected in Part 2 to measure dasiglucagon levels at steady-state (Schedule of Events; Section 18.1). The blood samples should be collected after a minimum of 4 hours without changes to the dasiglucagon infusion rate. For patients with a body weight above 4 kg, an additional PK sample should be collected at Day 5. However, the total amount of blood collected should always be considered according to Section 11.2.3.1.

Details on sampling/collection, shipment, and analysis will be provided in the laboratory manual.

11.2.3. Safety

Safety assessments will include the evaluation of AEs, clinical laboratory assessments (hematology, biochemistry, and ADAs), vital signs, physical examinations, ECGs, echocardiography, and local tolerability.

11.2.3.1. Laboratory Safety Assessments

Trial procedures require a maximum total of 8.8 to 9.2 mL of blood in a 28-day period. Where this exceeds the 2.5 mL/kg maximum[18,19], safety laboratory tests (2 mL per sampling) will be prioritized over immunogenicity (1 mL) and drug exposure (0.4 mL) samples.

All measurements described in this section are recognized standard methods.

Hematology and Biochemistry

Samples for hematology and biochemistry will be collected at the time points specified in the Schedule of Events (Section 18.1).

Hematology:	hemoglobin, hematocrit, red blood cell (RBC) count, RBC indices, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet count (or estimate), white blood cell count, including differential
Biochemistry:	albumin, total bilirubin, total protein, calcium, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, blood urea nitrogen, creatinine, glucose, sodium, potassium, chloride, eGFR, ketones (measured with PG meter)

Standard of care blood samples can be used as screening samples if they were collected within 1 week of screening.

Laboratory specimens will be analyzed at local laboratories.

Immunogenicity

Blood samples will be collected to test for antibodies against dasiglucagon at Visit 2 (Day 1, prior to dosing), Visit 7 (Day 25 ±3), and at the Follow-up Visit (Visit 8, Day 53 ±3) (Section 18.1) and processed and shipped according to instructions provided in the laboratory manual. The samples will be stored until the ADA screening and confirmatory assays have been approved by the FDA. Afterwards, the ADA samples will be analyzed in batches during the trial. The ADA samples will be analyzed at a specialty laboratory.

The clinical ADA assays specific for dasiglucagon and glucagon have been validated in accordance with existing guidelines and recommendations.[19,21,22,23]

Samples will be measured in anti-dasiglucagon antibody screening and confirmatory assays. Due to the limited sample volume, the ADA characterization of confirmed positive samples will be conducted according to the following priority:

- Establishment of anti-dasiglucagon binding titer.
- Cross-reactivity against endogenous glucagon

The neutralizing potential in samples from ADA-positive patients will be evaluated on the basis of drug exposure/PD data (steady-state exposure and PG).

The ADA samples will be analyzed in batches during the trial and any anti-dasiglucagon antibody-positive patients (treatment induced or treatment boosted, titer increase above 5-fold)

will be monitored at an additional follow-up visit, preferably 16 weeks after last ADA-positive sample. Patients who complete the trial before the ADA screening and confirmatory assays have been approved by the FDA and who do not continue treatment in the long-term extension trial will have this additional visit 16 weeks after the end of trial visit (Visit 8). Patients who are tested ADA positive after the additional Follow-up Period will be categorized as having persistent antibodies.

It will not be possible to collect back-up ADA samples for long-term storage due to limited blood volume in these patients. However, any residual serum samples may be stored until approval of market authorization since further characterization of the antibody response may be requested.

Specimen Handling Requirements

The transmission of infectious agents may occur through contact with contaminated needles and blood or blood products. Consequently, appropriate blood and body fluid precautions should be employed by all trial personnel involved in the collection of blood and handling of specimens in both the clinic and laboratory settings. Refer to current recommendations of the appropriate authorities.

In addition to appropriate handling of patient samples, specific regulations exist regarding the shipment of biologic/etiologic samples. Procedures and regulations for the packaging and shipping of infectious samples are outlined in the laboratory manual. The investigator is responsible for ensuring that all trial samples that are to be transported to another location are packed and shipped appropriately according to the applicable regulations.

Evaluation of Laboratory Values

The normal ranges of values for the laboratory assessments in this trial will be provided by the responsible laboratories and submitted to the sponsor before the beginning of the trial. They will be regarded as the reference ranges on which decisions will be made.

If a laboratory value is out of the reference range, the investigator must evaluate if the value is clinically significant and record his or her assessment in the appropriate eCRF.

All laboratory values that in the investigator's opinion are clinically relevant during or after termination of the treatment have to be reported as AEs and followed, as described in Section 12.3.

11.2.3.2. Clinical Examinations

Vital Signs

Vital signs, including blood pressure, heart rate, respiratory rate, and SpO_2 will be measured while the child is sleeping or in a calm state at times specified in the Schedule of Events (Section 18.1). Vital signs can be performed using the bedside monitoring device as per standard of care.

Twelve-lead Electrocardiogram

A standard 12-lead ECG will be performed while the child is sleeping or in a calm state at times specified in the Schedule of Events (Section 18.1). If it is not practical or possible, then a 2-lead ECG may be used.[24] If arrhythmia is detected on a 2-lead ECG, this should be followed by 12-lead ECG. All ECG recordings will be identified with the patient number, date, and time of the recording and will be attached to his or her medical record.

The ECG parameters (heart rate, PQ, QRS, QT, and QTcF) and any abnormality will be recorded and described in the eCRF, including the investigator's assessment of clinical significance (Abnormal, Clinically Significant; or Abnormal, Not Clinically Significant). At subsequent visits, any clinically significant deterioration of a pre-existing condition, as well as any new clinically significant findings, will be recorded as AEs.

Echocardiogram

An echocardiogram will be performed according to the time points specified in the Schedule of Events (Section 18.1).

Physical Examination and Neurological Examination

A complete physical examination of body systems according to standard of care and an age-appropriate neurological examination will be performed according to the Schedule of Events (Section 18.1).

Local Tolerability

Local tolerability data will be collected separately from AEs. Within the eCRF, data will be collected on the nature of any reaction (erythema, pain, swelling, etc.), the severity (mild, moderate, or severe), and any action taken (e.g., no action, interruption of infusion). The likely cause of the reaction will also be collected (e.g., insertion site, drug, adhesive dressing).

Other skin findings will be collected along other AEs. If clinical suspicion of NME is made, data describing the lesion(s) will be collected as an AE of special interest (AESI) (see Section 12.1.8), together with a photograph or series of photographs of the lesion(s) uploaded to a central repository.

Assessment of Fluid Balance for Patients Receiving IV Glucose

Fluid balance assessments are to be performed and documented at least every 8 hours as long as the patient is hospitalized and receiving IV glucose. These assessments will include administered IV and oral fluids, fluid loss from stools and urine by weighing of diapers, and vital signs of fluid balance status. Furthermore, daily body weight will also be part of the individualized assessment based on the patients overall status, at investigator's discretion. According to Section 9.5, the investigator should evaluate whether the IV glucose concentration should be increased to avoid volume overload or whether oral/gastric non-nutritional carbohydrates are to be introduced to ensure the patient's safety whilst maintaining the maximum tolerable concentration and infusion rate of IV glucose.

All details on IV GIR (including any bolus given) will be recorded in the eCRF.

11.2.3.3. Reporting of Hypoglycemia Events

All hypoglycemic episodes are to be reported via the dedicated hypoglycemia eCRF form. Hypoglycemic episodes that fulfill the definition of a serious adverse event (SAE) should also be recorded as an SAE. The following information should be collected:

- Date, start time
- PG value
- Selected symptoms (e.g., unconsciousness, seizures)

- Intervention, type, and amount of food; route of administration (oral vs. NG tube/gastrostomy); and use of marketed glucagon as rescue therapy
- Subsequent PG measurements
- End time and PG value (time when PG exceeded a threshold of 70 mg/dL [3.9 mmol/L])
- Contact to trial doctor or emergency services, paramedic visit, ER admission, hospitalization, if applicable

A single hypoglycemia episode is defined as up until 60 minutes from the start of the episode. If normoglycemia (>70 mg/dL) is not reached within this time, a new episode of hypoglycemia will be recorded.

11.2.3.4. Technical Complaints

Reporting of Technical Complaints

Technical complaints should be reported to the sponsor on any of the following products if technical issues occur between the first and last use:

- Dasiglucagon 4 mg/mL / placebo vial containing 1 mL
- Accu-Chek Spirit pump
- Accu-Chek Spirit 3.15 mL Cartridge system, Accu-Chek Flex-Link Infusion set (Accu-Check UltraFlex Infusion set in the US), and Accu-Check Rapid-D Link infusion set
- Accu-Chek Link-Assist Insertion device
- SMPG meter, StatStrip Xpress2
- Dexcom G4/G6 systems (in Germany, only the Dexcom G6 CGM will be used)

The investigator must report whether the technical complaint is associated with any AEs or SAEs. Any AE/SAE associated with a technical complaint must be reported in accordance with Section 12.2; the relationship between the technical complaint and the AE/SAE must be assessed by the investigator.

Technical complaints must be reported on a dedicated technical complaint form.

The investigator must complete the technical complaint form in the eCRF according to the following timelines, starting from the time the trial site becomes aware of the technical complaint:

- Technical complaint assessed as related to an SAE **within 24 hours**
- Technical complaint which could have led to serious medical occurrence if either suitable action had not been taken, if intervention had not been made, or if circumstances had been less fortunate within 24 hours
- All other technical complaints within **5 calendar days**

Use Trial the paper technical complaint form when reporting a technical complaint for an item that is not yet allocated to a patient. The form should be sent by e-mail to e-mail address in the Appendix A.

Collection, Storage, and Shipment of Technical Complaint Items

The investigator must collect and store the item(s) and notify the CRA (including photo documentation) **within 5 calendar days** of obtaining the item at the trial site. Upon request, the CRA must coordinate the shipment as per instruction from the sponsor.

11.2.3.5. Adverse Events

The definitions and management of and special considerations for AEs are provided in Section 12.

12. ADVERSE EVENTS

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

12.1. Definitions

12.1.1. Adverse Events

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

AEs include:

- A clinically significant worsening of a concomitant illness
- A clinical laboratory AE: a clinical abnormality which is clinically significant, i.e., any abnormality that suggests a disease and/or organ toxicity and is of a severity that requires active management. Active management includes active treatment or further investigations, e.g., change of dose or more frequent follow-up due to the abnormality.

The following should **not** be considered as AEs:

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

12.1.2. Severity

When assessing the severity of an AE, the following definitions are used:

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 find unacceptable. A severe reaction does not necessarily deem the AE as serious (SAE), and an SAE is not always severe in nature.

12.1.3. Causality

When assessing the cause of an AE, the following definitions are used:

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

Possible: A causal relationship is conceivable and cannot be dismissed

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

Not related: No relationship to product.

Causality will take into consideration whether the cause of the AE was related to the trial drug, device, or procedures.

12.1.4. Outcome

When assessing the outcome of an AE, the following definitions are used:

Recovered/resolved: The patient has fully recovered, or by medical or surgical treatment, the condition has returned to the level observed at the first trial-related activity after the patient signed the ICF

Recovering/resolving: The condition is improving and the patient is expected to recover from the event. This term is only applicable as final outcome of an event if the patient has completed the trial or has died from another AE

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, or the outcome is not known

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.

12.1.5. Serious Adverse Events

An SAE is any untoward medical occurrence that at any dose results in any of the following:

- results in death
- is life-threatening
- requires inpatient hospitalization or prolongation of existing hospitalization
- results in persistent or significant disability or incapacity
- is a congenital anomaly or birth defect
- is otherwise medically important and may not result in death, be life threatening, or require hospitalization may be considered an SAE when (based on appropriate medical judgement) it may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed in the definition of an SAE. Examples could be ER or home treatment of allergic bronchospasm or convulsion

12.1.6. Other Important Events

The following events must always be reported in the electronic data capture (EDC) system on a dedicated form, regardless of whether it is related to an AE:

- suspicion of transmission of infectious agents via the trial product
- overdose of the trial product
- medication error involving the trial product
- inadvertent or accidental exposure to the trial product

12.1.7. Nonserious Adverse Events

A nonserious AE is any AE that does not fulfill the definition of an SAE.

12.1.8. Adverse Events of Special Interest

For this trial, the following events are to be regarded as AESIs, with data collected under a specific eCRF form:

- Suspicion of NME
- Risk of liver injury defined as ALT or AST >3 x UNL AND total bilirubin >2 x UNL, where no alternative etiology exists (Hy's law).
- Loss of consciousness, partial, and generalized seizures
- Clinically significant changes in blood pressure or heart rate

12.1.9. Suspected Unexpected Serious Adverse Reactions

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

12.1.10. Adverse Events Associated with Devices

Adverse events associated with devices must be reported to the ethics committee and competent authority according to local requirements. Such events include the following:

12.1.10.1. Adverse Device Effect

An adverse device effect (ADE) is an AE related to the use of an investigational medical device. This definition includes AEs resulting from insufficient or inadequate instructions for use, deployment, installation, operation, or any malfunction of the investigational medical device. This definition also includes any event resulting from use error or from intentional misuse of the investigational device.

12.1.10.2. Serious Adverse Device Effect

A serious ADE (SADE) is an ADE that has resulted in any of the consequence characteristics of an SAE, meaning that the SAE is related to one of the investigational devices.

12.1.10.3. Unanticipated Serious Adverse Device Effect

An unanticipated SADE (USADE) is an SADE which by its nature, incidence, severity, or outcome has not been identified in the current version of the risk analysis report.

12.1.10.4. Device Deficiency

A device deficiency is the inadequacy of a medical device with respect to its identity, quality, durability, reliability, safety, or performance. It also includes malfunctions, use errors, and inadequate labelling. In this clinical trial, these are covered by the term technical complaint (see reporting requirements in Section 11.2.3.4). Reporting of technical complaints is synonymous with device deficiency. The device deficiencies will be monitored and managed by the sponsor throughout the trial.

12.2. Collection, Recording, and Reporting of Adverse Events

All AEs, whether serious or nonserious, will be reported from the time a signed and dated ICF is obtained until the end of the posttreatment follow-up period (which may include contacts for follow-up of safety) or inclusion in the ZP4207-17106 trial. Parent(s)/guardian will be asked about their child's condition by open questioning, such as "How has your child been feeling since you were last asked?" at each contact with the trial site (visit or telephone). Parent(s)/guardian will also be encouraged to spontaneously report AEs occurring at any other time during the trial. In addition, patients will be observed for any signs or symptoms.

All AEs, regardless of seriousness, severity, or presumed relationship to the trial drug, must be recorded and evaluated by the investigator. Whenever possible, diagnoses should be given when signs and symptoms are due to a common etiology. If no diagnosis can be made, the investigator should record each sign and symptom as individual AEs. Investigators must record their opinion concerning the relationship of the AE to the trial drug. All 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 eCRF within 5 days of the investigator becoming aware of the event.

All AE information should at a minimum include the following:

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

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

All SAEs, including those spontaneously reported to the investigator within 30 days after the last dose of trial drug, must be reported within 24 hours after obtaining knowledge about the event by

completing the SAE form in the EDC system. A separate SAE form should be completed for each SAE.

All SAEs will be reported in EDC; for each reported event a system-generated e-mail will be sent to the safety CRO ([redacted]), medical monitor, sponsor medical director, and trial manager.

Specific information about AESIs will be collected via the SAE form (if qualifying for an SAE), as well as via the dedicated AESI eCRF page(s). Reporting requirements for serious and nonserious AEs as described previously also apply for serious and nonserious AESIs.

Other important events (Section 12.1.6) will be reported via a dedicated eCRF page. Reporting timelines will be within 24 hours if related to an SAE, and 5 calendar days for all other events.

It is the responsibility of [redacted] to report all SUSARs that occur in this trial to competent authorities, the institutional review board (IRB), or independent ethics committee (IEC) in accordance with the local requirements in force and ICH guideline for GCP.

All SAEs, SADEs, and USADEs must be reported to the ethics committee and competent authority according to local requirements.

12.2.1. Serious Adverse Event Reporting Process in Germany

The Bundesinstitut für Arzneimittel und Medizinprodukte (BfArM) is to be informed about events as defined per Medical Device Directive 93/42/EEC, and the Ordinance on Medical Device Vigilance Section 3, Sub-section 6.

The Principal Investigator must report all SAEs and events with SADE potential to the Sponsor within 24 hours via the eCRF. Device- and/or procedure-related SAEs and events with SADE potential occurring in Germany will be reported individually by the Sponsor to the BfArM immediately by using the SAE Form available on the BfArM website. Device- or procedure-related SAEs and events with SADE potential occurring in all other countries in this trial will be reported by the Sponsor to the BfArM immediately using the European Medical Device Vigilance System (MEDDEV) 2.7/3 SAE Reporting Form. Serious adverse events deemed unrelated to the device and the procedure, occurring both in Germany and in all other countries, will be reported by the Sponsor to BfArM quarterly by using the MEDDEV 2.7/3 SAE Reporting Form next to the procedure laid out in the protocol.

12.2.2. Contact Information

Pharmacovigilance for this trial is outsourced to [redacted]; refer to Appendix A for contact details.

12.3. Follow-up of Adverse Events

The investigator must record follow-up information on the eCRF for nonserious adverse event and on the SAE form for serious adverse events. Follow-up questions to investigators regarding serious adverse events are queried directly by [redacted] to the investigator.

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

- **SAEs:** All SAEs must be followed until the outcome of the events is “recovered/resolved,” “recovered/resolved with sequelae,” or “fatal,” and until all queries have been resolved. Cases of chronic conditions, cancer, or AEs that are ongoing at time of death (where death is due to another AE) may be closed with the outcome “recovering/resolving” or “not

recovered/not resolved." Cases can be closed with the outcome of "recovering/resolving" when the patient has completed the follow-up period and is expected by the investigator to recover.

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

- **Nonserious AEs:** Nonserious AEs must be followed until the outcome of the event is "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 or if the patient continues in the extension trial.

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

The investigator must ensure that the worst-case severity and seriousness of an event is kept throughout the trial, from the start of trial product administration (i.e. the most severe of the applied severity/seriousness categories should be ascribed to the event). Accordingly, for AEs with onset after trial product administration, changes in the severity or seriousness of an event should not lead to reporting of separate AEs. In contrast, for AEs with onset before trial product administration, any worsening of severity/seriousness after trial product administration should be recorded as a separate AE, with onset date of the event corresponding to the date of the severity/seriousness upgrade. Similarly, AEs with onset during the blinded treatment that worsen after the switch to the second blinded treatment or to the unblinded treatment, should be recorded as separate AEs.

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.

12.4. Precautions

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

For further information on safety precautions for dasiglucagon, refer to the current version of the IB.

12.5. Safety Committee

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

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

12.6. Independent Data Monitoring Committee

An independent data monitoring committee (DMC) will be established for this trial and follow the procedures outlined in the DMC Charter.

13. STATISTICS

13.1. Statistical Hypotheses

This section details the null hypotheses for this trial. They are presented in the form H_{ij}, where i = trial part (i = 1, 2) and j = hypothesis number (j = 1, 2, 3, 4, 5).

The hypothesis relates to the primary endpoint of mean IV GIR in the last 12 hours of each treatment period during Part 1. Formally, the hypothesis is:

H_{11}: Mean IV GIR $_{\text{dasiglucagon}}$ = Mean IV GIR $_{\text{placebo}}$

The hypotheses relating to the key secondary endpoints (based on the last 48 hours of each treatment period in Part 1) are:

H_{12}: Total amount (g) of carbohydrates administered (regardless of the route) per day $_{\text{dasiglucagon}}$ = total amount (g) of carbohydrates administered (regardless of the route) per day $_{\text{placebo}}$

A fixed-sequence statistical strategy will test first the primary (Section 7.2.1) and then the key secondary endpoints of Part 1 (Section 7.2.2), all at the same significance level ($\alpha = 0.05$, 2-sided test), moving to the next hypothesis only after rejecting the previous null hypothesis.

The test hierarchy is:

Part 1

H_{11}: Mean IV GIR in the last 12 hours of each treatment period during Part 1 (primary endpoint)

H_{12}: Total amount (g) of carbohydrates administered (regardless of the route) per day (key secondary endpoint)

13.2. Sample Size Determination

A total of 12 patients is planned to be randomized and exposed to trial product in this crossover trial on the basis of the following considerations.

The GIR results from 40 infants treated with IV glucagon for 24 hours published in the JIMD Research Report "The Effect of Continuous Intravenous Glucagon on Glucose Requirements in Infants with Congenital Hyperinsulinism."[14]

"Overall there was a statistically significant reduction in the median (IQR) GIR during the 24h following initiation of continuous glucagon infusion compared to 24 h before initiation (18.5 (12.9, 22.8) to 11 (6.6, 17.5) mg/kg/min…"

This trial is powered to detect an effect in GIR for dasiglucagon after 48 hours compared to placebo of at least this effect size. Based on the longer infusion and titration of dasiglucagon in this trial, this is considered a conservative approach, allowing for some uncertainty when translating the published data into a clinical trial setting. Using unpublished individual patient data from the above-referenced study, the difference in GIR between the 2 treatment groups is assumed to follow a normal distribution. Assuming the true mean difference is 7.5 mg/kg/min with a standard deviation of differences of 7.36, the trial will have 89% power using a 1-sample t-test with 12 patients randomized to receive either placebo first and then dasiglucagon or vice versa.

13.3. Analysis Populations

Three analysis populations have been defined for this trial:

- The Safety Set: defined as all patients administered any randomized treatment.
- The Full Analysis Set (FAS): defined as all patients in the Safety Set who have a valid baseline efficacy assessment. Patients will be analyzed according to randomized treatment (Part 1) or overall patients (otherwise).
- Per protocol (PP) set: defined as all patients in the FAS without any major protocol deviations. Patients will be excluded from the PP set if they do not receive both treatments and/or do not have efficacy data available to evaluate the primary endpoint for both treatments.
- Pharmacokinetic Set (PK): defined as all patients in the Safety Set who have at least 1 measurement with quantifiable plasma concentration of dasiglucagon.

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

Inclusion in the analysis populations will be determined prior to database lock.

For the primary endpoint, baseline is defined as the IV GIR rate obtained at the end of the run-in period which corresponds to the last value obtained before randomization. During the 24-hour run-in period, all non-nutritional carbohydrates and carbohydrate fortification of feeds are prohibited as this might impact the GIR level and result in many updates during the run-in period. Therefore the IV GIR rate obtained at the end of the run-in period will give a better estimate of the stabilized value of GIR. For the second period of the crossover (Part 1), the IV GIR is also started at the IV GIR obtained at the end of the run-in period.For the purpose of all other efficacy analyses where applicable, baseline is defined as the last measurement prior to the start of trial drug administration on Day 1.

13.4. Statistical Analyses

This section presents a summary of the planned statistical analyses. A statistical analysis plan (SAP) that describes the details of the analyses to be conducted will be finalized prior to database lock.

Unless otherwise indicated, testing of statistical significance will be 2 sided with a significance level of $\alpha = 0.05$.

Continuous endpoints will be summarized with number (n), mean, standard deviation (SD), median, minimum, and maximum. In addition, change from baseline values will be calculated at each time point and summarized descriptively; the exception to this is the time of PG within prespecified ranges, which will not have a baseline value. For categorical endpoints, descriptive summaries will include counts and percentages.

All data will be presented in the data listings.

Immunogenicity data will be analyzed descriptively. No statistical tests are planned. Baseline ADA-positive patients will be calculated as a percentage of the total number of patients whose baseline samples were tested for ADA. Overall ADA incidence, the combined results of treatment-induced and treatment-boosted ADA-positive patients will be calculated as a percentage of the total number of evaluable patients, excluding baseline positive patients without

any samples available after drug administration. Titers will be reported as median and interquartile range.

Graphical presentations of selected endpoints will be prepared and will be outlined in the SAP.

13.4.1. Trial Patients and Demographics

13.4.1.1. Disposition and Withdrawals

The numbers of patients randomized, completing, and withdrawing, along with reasons for withdrawal, will be tabulated overall and by treatment sequence. The number of patients in each analysis population will be reported.

13.4.1.2. Protocol Deviations

Protocol deviations will be provided in a listing and summarized if appropriate.

13.4.1.3. Demographics and Other Baseline Characteristics

Demographic and baseline characteristics (including age, sex, race, ethnicity, weight, and length) at screening will be summarized using descriptive statistics. No formal statistical analyses will be performed.

Prior and concomitant medications and procedures will be summarized by treatment sequence (prior medications and procedures) or treatment and part (concomitant medications and procedures), by the number and percentage of patients taking each medication, classified using World Health Organization Drug Dictionary Anatomical Therapeutic Chemical classes and preferred term (PT).

13.4.2. Duration of Exposure and Compliance

Trial drug administration (i.e., amount administered) will be summarized in terms of each patient's mean, mode, and final dose, and in terms of duration of exposure. Descriptive statistics for these quantities, including the mean, median, SD, minimum, maximum, and quartiles, will be provided by treatment and part.

13.4.3. Efficacy Analyses

13.4.3.1. Primary Analysis

The mean IV GIR in the last 12 hours of each treatment period during Part 1 will be analyzed for the FAS and PP populations. The hypothesis:

H_{11}: Reduction in weighted mean IV GIR $_{dasiglucagon}$ = Reduction in weighted mean IV GIR $_{placebo}$

will be based the last 12 hours of each treatment period, calculated as a weighted mean across the 12 hours, taking the actual time periods between the measurements into account (corresponding to calculating the area under the curve [AUC] and dividing by the length of the time period, i.e., weighted mean IV GIR). The IV GIR endpoint is expressed as glucose in mg/kg/min, i.e., data reported from different concentrations of the glucose used for infusion will be transformed to this standardized unit prior to analysis.

The primary analysis is defined by the estimand based on the treatment policy (de-facto) strategy, where the actual GIR measurement reported irrespective of adherence to treatment or

use of subsequent therapy is used. The reduction in weighted mean IV GIR between placebo and dasiglucagon will be estimated.

The weighted mean IV GIR will be analyzed using a mixed-model regression approach, with treatment and period as fixed effects and patient as a random effect. The 2-sided 95% confidence interval (CI) for the treatment difference will be calculated from the mixed regression model.

Missing data will be imputed using the following methodology: if the weighted mean IV GIR is missing for the placebo period, it will be imputed using the baseline weighted mean IV GIR; if the weighted mean IV GIR is missing for the dasiglucagon period, it will be imputed using the placebo-weighted mean IV GIR for that patient. As a sensitivity analysis, the primary endpoint will be re-analyzed as follows: 1) using only complete cases (i.e., weighted mean IV GIR is non-missing for both placebo and dasiglucagon) and 2) imputing missing weighted mean IV GIR using the baseline weighted mean IV GIR and 3) imputing missing dasiglucagon values during one period with the placebo value for that patient and imputing missing placebo values with the mean values of placebo patients for that period.

13.4.3.2. Key Secondary Analyses

The hypothesis:

H_{12}: Total amount (g) of carbohydrates administered (regardless of the route) per day $_{dasiglucagon}$ = total amount (g) of carbohydrates administered (regardless of the route) per day $_{placebo}$

between the 2 treatment groups will be analyzed by using a mixed-model regression approach, with treatment and period as fixed effects and patient as a random effect. The total amount (g) of carbohydrates administered (regardless of the route) per day is the sum of the last 48 hours of each period in Part 1.

For the key secondary endpoint, 2-sided 95% CIs for the treatment differences will be estimated from the mixed-model. Missing dasiglucagon values during one period will be imputed with the placebo value for that patient and missing placebo values will be imputed with the mean values of placebo patients for that period. In addition, a sensitivity analysis will be performed using complete cases.

13.4.3.3. Secondary and Other Efficacy Analyses

Part 1

A mixed-model regression approach will be used to analyze the weighted mean IV GIR over 48-hour treatment period during Part 1 (dasiglucagon or placebo administration) similar to the primary analysis for the primary endpoint.

A generalized estimating equation (GEE) method with a logit link function will be used to analyze the weighted mean IV GIR below 10 mg/kg/min in the last 12 hours of each treatment period during Part 1 (yes/no) (dasiglucagon or placebo administration) to account for repeated observations in patients. In case of non-convergence, a Mc Nemar test will be used.

No sensitivity analysis will be used on these endpoints.

Part 2

Due to the potential impact of pancreatectomy on the secondary endpoints during Part 2, any endpoint assessment after pancreatectomy will not be included in the primary analyses

(endpoints set to missing after pancreatectomy). Sensitivity analyses will be run on those endpoints including all assessments in the descriptive analyses.

For Part 2 efficacy endpoints, continuous and categorical endpoints will be presented using summary statistics or frequencies, respectively; no formal testing will be performed. For the time to complete weaning off IV GIR, the time to actual hospital discharge and the time to pancreatic surgery, Kaplan-Meier curves will be produced.

13.4.4. Safety and Tolerability Analyses

Safety analyses will be conducted using data from the Safety Population (as defined in Section 13.3). Safety assessments will include the evaluation of AEs; clinical laboratory assessments (hematology, biochemistry, and ADAs); vital signs, physical examinations; ECGs, echocardiography, and local tolerability issues. No formal inferential analyses will be conducted for safety variables, unless otherwise noted.

Baseline is defined as the last nonmissing assessment before the first exposure to trial drug in the trial.

All safety analyses will be summarized by treatment received within treatment period and part and by trial visit, if applicable.

13.4.4.1. Adverse Events

Adverse events will be coded using the most current version of MedDRA.

A treatment-emergent AE is defined as an AE with an onset at the time of or following the start of treatment with the trial drug through the Follow-up Visit or Early Termination Visit, whichever occurs first.

The number and percentage of patients with AEs, as well as the number of AEs, will be displayed by system organ class, PT, and treatment group. The incidence of AEs will also be presented by severity and relationship to the trial drug. Serious AEs, AESIs and AEs resulting in discontinuation of trial drug will be summarized separately in a similar manner. Patient listings of AEs, SAEs, AESIs, and AEs causing discontinuation of trial drug and withdrawal from the trial will be produced.

13.4.4.1.1. Serious Adverse Events Associated with Devices

The number and percentage of patients with SADEs, as well as the number of SADEs, will be displayed by system organ class, PT and treatment group. Patient listing of SADEs will include information whether it was expected (yes/no), identification of the study device or a device procedure to which the SAE is considered related and will be produced in addition to listings of SADEs causing discontinuation of trial drug and withdrawal from the trial.

Similar listings will be prepared for ADEs reported in German patients.

13.4.4.2. Clinical Laboratory Evaluations

Descriptive summaries (mean, SD, median, minimum, and maximum) of actual (absolute) values and changes from baseline values will be presented for clinical laboratory values.

The number of patients with clinical laboratory values categorized as below, within, or above normal ranges will be tabulated showing change from baseline (shift tables) for each clinical laboratory analyte.

Laboratory values that are outside the normal range will also be flagged in the data listings, along with corresponding normal ranges. Any out-of-range values that are identified by the investigator as being clinically significant will also be shown in a data listing.

13.4.4.3. Vital Signs

Descriptive summaries (mean, SD, median, minimum, and maximum) of actual values and changes from baseline will be calculated for systolic blood pressure, diastolic blood pressure, heart rate, respiratory rate, and SpO_2.

The number of patients with vital signs values categorized Abnormal, Clinically Significant or Abnormal, Not Clinically Significant will be tabulated showing change from baseline (shift tables) for each parameter.

13.4.4.4. Twelve-lead Electrocardiograms

The number and percentage of patients with normal and abnormal ECG findings will be summarized. Abnormal results will be grouped as Abnormal, Clinically Significant or Abnormal, Not Clinically Significant.

13.4.4.5. Echocardiograms

The number and percentage of patients with normal and abnormal echocardiogram findings will be summarized. Abnormal results will be grouped as Abnormal, Clinically Significant or Abnormal, Not Clinically Significant.

13.4.4.6. Physical Examination Findings

The number and percentage of patients with normal and abnormal findings in the complete physical examination will be displayed.

13.4.4.7. Local Tolerability

The number and percentage of patients with local tolerability findings, collected separately from AEs, will be summarized.

13.4.5. Interim Analysis

No interim analysis is planned.

14. TRIAL CONDUCT

The accuracy and reliability of data are ensured, among others, by the selection of qualified investigators and appropriate trial sites, review of protocol procedures with the investigator and associated personnel before the trial, periodic monitoring visits, and meticulous data management.

14.1. Sponsor and Investigator Responsibilities

14.1.1. Sponsor Responsibilities

The sponsor is obligated to conduct the trial in accordance with strict ethical principles (Section 16). The sponsor reserves the right to terminate participation of a trial site at any time (Section 14.7), and/or to discontinue the trial (Section 14.6 for US studies and Section 14.6.2 for studies conducted outside of the US).

The sponsor agrees to provide the investigator with sufficient material and support to permit the investigator to conduct the trial according to the trial protocol.

14.1.2. Investigator Responsibilities

By signing the Investigator's Agreement (Section 18.2), the investigator indicates that he/she has carefully read the protocol, fully understands the requirements, and agrees to conduct the trial in accordance with the procedures and requirements described in this protocol.

The investigator also agrees to conduct this trial in accordance with all laws, regulations, and guidelines of the pertinent regulatory authorities, including the November 2016 ICH Guidance for Industry E6(R2) GCP, and in agreement with the 2013 version of the Declaration of Helsinki. While delegation of certain aspects of the trial to subinvestigators and trial coordinators is appropriate, the investigator will remain personally accountable for closely overseeing the trial and for ensuring compliance with the protocol and all applicable regulations and guidelines. The investigator is responsible for maintaining a list of all persons that have been delegated trial-related responsibilities (e.g., subinvestigators and trial coordinators) and their specific trial-related duties.

Investigators should ensure that all persons who have been delegated trial-related responsibilities are adequately qualified and trained in the protocol, trial drugs handling, and their specific duties within the context of the trial. Investigators are responsible for providing the sponsor with documentation of the qualifications, GCP training, and research experience for themselves and their staff as required by the sponsor and the relevant governing authorities.

To ensure compliance with the guidelines, the trial may be audited by an independent person. The investigator agrees, by written consent to this protocol, to cooperate fully with compliance checks by allowing access to all trial documentation by authorized individuals.

14.2. Site Initiation

Trial personnel may not screen or enroll patients into the trial until after receiving notification from the sponsor or its designee that the trial can be initiated at the trial site. The trial site will not be authorized for trial initiation until:

1. The trial site has received the appropriate IRB/IEC approval for the protocol and the appropriate ICF.
2. All regulatory documents have been submitted to and approved by the sponsor or its designee.
3. The trial site has a Clinical Trial Agreement in place.
4. Trial site personnel, including the investigator, have participated in a trial initiation meeting.

The regulatory documents must be received from the investigator before the sponsor will authorize shipment of trial drug to the trial site, Regulatory Green Light. Copies of the investigator's regulatory documents must be retained at the trial site in a secure location in the ISF. Additional documents, including a copy of the protocol and applicable amendment(s), the dasiglucagon IB, eCRF completion guidelines, copies of regulatory references, copies of IRB/IEC correspondence, and trial drug accountability records should also be retained in the ISF. It is the investigator's responsibility to ensure that copies of all required regulatory documents are organized, current, and available for inspection.

14.3. Screen Failures

Patients who fail inclusion and/or exclusion criteria may be rescreened for the trial upon approval by the sponsor and medical monitor. Patients may only be rescreened once 30 days or more after the original Screening Visit. If a patient is eligible to enter the trial after having previously failed screening, the patient will be assigned a new patient identification number.

14.4. Trial Documents

All documentation and material provided by the sponsor for this trial are to be retained in a secure location and treated as confidential material.

14.4.1. Investigator's Regulatory Documents

The regulatory documents will be maintained by the investigator in the ISF.

14.4.2. Case Report Forms

By signing the Investigator's Agreement (Section 18.2), the investigator agrees to maintain accurate eCRFs and source documentation as part of the case histories for all patients who sign an ICF.

Case report forms are considered confidential documents and should be handled and stored accordingly. The sponsor or its designee will provide the necessary training on the use of the specific eCRF system used during the trial to ensure that the trial information is captured accurately and appropriately.

To ensure data accuracy, eCRF data for individual patient visits should be completed as soon as possible after the visit. All requested information must be entered in the EDC system according to the completion guidelines provided by the sponsor or its designee.

The eCRF must be signed by the investigator or a subinvestigator when all data are entered and cleaned. These signatures serve to attest that the information contained in the eCRF is accurate and true.

14.4.3. Source Documents

Information recorded in the eCRF should be supported by corresponding source documentation. Examples of acceptable source documentation include, but are not limited to, hospital records, clinic and office charts, laboratory notes, and recorded data from automated instruments, memoranda, and pharmacy dispensing records.

Clinical laboratory data required by the protocol will be entered into the eCRF at the site.

The investigator should permit trial-related monitoring, IEC review, regulatory inspections, and sponsor audit by providing direct access to source data and documents.

14.5. Data Quality Control

The sponsor and its designees will perform quality control checks on this clinical trial.

14.5.1. Monitoring Procedures

The sponsor and/or its designee will conduct site visits to monitor the trial and ensure (i) the safety and rights of the patients are respected, (ii) compliance with the protocol, GCP, and applicable regulations and guidelines, and (iii) that accurate, valid, and complete data are collected. The assigned CRA(s) will visit the investigator and trial site at periodic intervals and maintain periodic communication, this are described in detail in the Monitoring Plan. The investigator agrees to allow the CRA(s) and other authorized sponsor personnel access to ISF and source data (original documents, data, and records). The CRA(s) will maintain current personal knowledge of the trial through observation, review of trial records and source documentation, and discussion of the conduct of the trial with the investigator and staff. While on site, the CRA(s) will review:

- regulatory documents
- entries in the EDC system compared with the source documents
- consents
- adherence to the inclusion/exclusion criteria
- AE records
- storage and accountability of trial drug and trial materials
- adherence to the protocol and ICH-GCP

The CRA(s) will ask for clarification and/or correction of any noted inconsistencies. Procedures for correcting eCRFs are described in the Trial Reference Manual. As representatives of the sponsor, CRAs are responsible for notifying project management of any noted protocol deviations.

By signing the Investigator's Agreement (Section 18.2), the investigator agrees to meet with the CRA(s) during trial site visits; to ensure that trial staff is available to the CRA(s) as needed; to provide the CRA(s) access to all trial documentation, to the clinical supplies dispensing and storage area; and to assist the monitors in their activities, if requested. Further, the investigator

agrees to allow the sponsor or designee auditors or inspectors from regulatory agencies to review records, and to assist the inspectors in their duties, if requested.

14.5.2. Data Management

The sponsor or designee will be responsible for activities associated with the data management of this trial. The standard procedures for handling and processing records will be followed per GCP and Premier standard operating procedures. A comprehensive data management plan will be developed, including a data management overview, description of database contents, annotated eCRF, pre-entry review list, self-evident correction conventions, query contacts, and consistency checks.

Trial site personnel will be responsible for providing resolutions to all data queries. The investigator will be required to document electronic data review to ensure the accuracy of the corrected and/or clarified data. Procedures for soliciting and documenting resolution to data queries are described in the trial manual.

14.5.3. Quality Assurance/Audit

This trial will be subject to audit by the sponsor/its designee or national/international regulatory authorities. Audits may be performed to check compliance with GCP guidelines, and can include:

- site audits
- trial master file audits
- database audits
- document audits (e.g., protocol and/or the clinical trial report [CTR])

The sponsor or its designee may conduct additional audits on a selection of trial sites, requiring access to patient notes, trial documentation, and facilities or laboratories used for the trial.

The trial site, facilities, all data (including source data), and documentation will be made available for audit by quality assurance auditors and for IRB/IEC or regulatory authorities according to GCP guidelines. The investigator agrees to cooperate with the auditor during the visit and will be available to supply the auditor with eCRFs or other files necessary to conduct that audit. Any findings will be strictly confidential.

If a regulatory authority informs the investigator that it intends to conduct an inspection, the investigator shall notify the sponsor immediately.

14.6. Trial Termination

The trial may be terminated at the sponsor’s discretion at any time and for any reason.

14.6.1. Regular Trial Termination

The end of this trial is defined as the date of the last visit of the last patient (last patient out or last patient last visit) participating in the trial. Within 90 days of the end of the clinical trial, the sponsor or its designee and/or site will notify the IRBs and IECs and regulatory authorities on the regular termination of the trial as required according to national laws and regulations.

14.6.2. Premature Trial Termination

The trial may be terminated prematurely for any reason and at any time by the sponsor, the IRBs/IECs, regulatory authorities, respective steering committees, or the coordinating investigator. A decision to terminate the trial prematurely is binding to all investigators at all trial sites.

Within 15 days of premature termination of a clinical trial, the sponsor or its designee and/or site will notify the IRBs/IECs and regulatory authorities on the premature termination as required according to national laws and regulations. The sponsor or its designee must clearly explain the reasons for premature termination.

If the trial is terminated prematurely, all investigators must inform their patients and take care of their appropriate follow-up and further treatment to ensure protection of their interests. Trial sites may be asked to have all patients currently participating in the trial complete all of the assessments for an Early Termination Visit.

14.7. Trial Site Closure

At the end of the trial, all trial sites will be closed. The sponsor may terminate participation of a trial site at any time. Examples of conditions that may require premature termination of a trial site include, but are not limited to, the following:

- Noncompliance with the protocol and/or applicable regulations and guidelines
- Inadequate patient enrollment

14.7.1. Record Retention

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

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

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

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

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

One copy will remain with the investigator. The investigator shall arrange for the retention of the patient identification codes, patient files, and other source data until at least 5 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region, or until at least 2 years have elapsed since the formal discontinuation of the clinical development of the product. These documents need to be retained for a longer period of time if required by applicable regulatory authorities or by agreement with the sponsor.

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

14.7.2. Sample Retention

Samples will only be used for purposes related to this trial.

All blood samples will be destroyed upon completion of the CTR, except for residual ADA samples, which will be stored until approval of market authorization because further characterization of the antibody response may be requested by the health authorities. Identifiable samples can be destroyed at any time at the request of the patient.

14.8. Changes to the Protocol

This protocol cannot be altered or changed except through a formal protocol amendment, which requires the written approval of the sponsor. The protocol amendment must be signed by the investigator and approved by the IRB/IEC before it may be implemented. Protocol amendments will be filed with the appropriate regulatory agency(s) having jurisdiction over the conduct of the trial.

14.9. Use of Information and Publication

All information concerning dasiglucagon, the sponsor's operations, patent applications, formulae, manufacturing processes, basic scientific data, and formulation information supplied by the sponsor or its designee to the investigator and not previously published, is considered confidential and remains the sole property of the sponsor. Case report forms also remain the property of the sponsor. The investigator agrees to use this information for purposes of trial execution through finalization and will not use it for other purposes without the written consent of the sponsor.

The information developed in this trial will be used by the sponsor in connection with the continued development of dasiglucagon and thus may be disclosed as required to other clinical investigators or government regulatory agencies.

The information generated by this trial is the property of the sponsor. Publication or other public presentation of dasiglucagon data resulting from this trial requires prior review and written approval of the sponsor. Abstracts, manuscripts, and presentation materials should be provided to the sponsor for review and approval at least 30 days prior to the relevant submission deadline.

It is agreed that the results of the trial will not be submitted for presentation, abstract, poster exhibition or publication by the investigator until the sponsor has reviewed and commented on such a presentation or manuscript for publication.

15. FINAL CLINICAL TRIAL REPORT

The sponsor will retain ownership of the data.

The final CTR will be prepared and reviewed in cooperation with the signatory investigator. The coordinating investigator will be appointed by the sponsor to review and sign the CTR on behalf of all participating investigators. This report will include a summary of the trial results based on a statistical evaluation and clinical assessment of the protocol-defined endpoints.

The final CTR may be submitted to the regulatory authorities.

16. ETHICAL AND LEGAL CONSIDERATIONS

16.1. Declaration of Helsinki and Good Clinical Practice

This trial will be conducted in compliance with the November 2016 ICH Guidance for Industry E6(R2) GCP (including archiving of essential trial documents), the 2013 version of the Declaration of Helsinki, and the applicable regulations of the country(ies) in which the trial is conducted.

See Appendix B for regulation and guidelines.

16.2. Patient Information and Informed Consent

According to the Declaration of Helsinki and ICH GCP, patients' parent(s)/guardian must provide their written informed consent prior to enrollment in a clinical trial and before any protocol-specified procedures are performed. Patients' parent(s)/guardian must declare their consent by personally signing and dating the ICF. The written ICF will embody the elements of informed consent as described in the Declaration of Helsinki and will also comply with local regulations.

Each patient's parent(s)/guardian should be made aware by the investigator of the nature of the trial (objectives, methods, and potential hazards and benefits) and the procedures involved using the information on the ICF. Information should be given in both oral and written form whenever possible and deemed appropriate by the IRB/IEC. Patients' relatives, or, if necessary, legal representatives must be given ample opportunity to inquire about details of the trial.

Patient information and the ICF must be in a language fully comprehensible to the prospective patient's parent(s)/guardian. The written information must be provided to the patient's parent(s)/guardian to give him or her sufficient time to understand the information and to prepare questions before being asked for his or her consent. The investigator must confirm that the text was understood by the patient's parent(s)/guardian. The patient's parent(s)/guardian will then sign and date the IRB/IEC-approved consent form indicating that he or she has given his or her consent for his or her child to participate in the trial. The signature confirms that the consent is based on information that has been understood. The form will also be signed by the investigator obtaining the consent and annotated with the trial patient number. Each signed patient parent(s)/guardian ICF must be kept on file by the investigator for possible inspection by regulatory authorities, the sponsor, and/or the sponsor's designee. Collection of informed consent has to be documented on the eCRF.

Furthermore, the patient's parent(s)/guardian will be informed that if he or she wishes to drop-out or withdraw his or her child (see Section 9.2.3) at any time during the trial, this will not have any negative consequences. Patients may be withdrawn by the investigator if any change

related to safety or ethics precludes further participation in the trial. Patients' parent(s)/guardian will be asked to agree to a final assessment in the event of an early termination of the trial.

If information becomes available that may be relevant to the patient's willingness to continue participating in the trial, the investigator must inform the patients' parent(s)/guardian in a timely manner, and a revised written informed consent must be obtained.

Patients' parent(s)/guardian will be informed that data from their children's case may be stored in a computer without inclusion of their name and that such data will not be revealed to any unauthorized third party. Data will be reviewed by the monitor, an independent auditor, and possibly by representatives of regulatory authorities and/or IRBs/IECs. The terms of the local data protection legislation will be applied as appropriate.

16.3. Approval by Institutional Review Board and Independent Ethics Committee

For Investigational New Drug studies, the minimum standards of conduct and requirements for informed consent are defined in the FDA regulations.

A valid IRB/IEC must review and approve this protocol before trial initiation. Written notification of approval is to be provided by the investigator to the sponsor's monitor and project manager before shipment of investigational drug supplies, and will include the date of the committee's approval and the chairperson's signature. This written approval must consist of a completed sponsor form, IRB/IEC Approval Form, or written documentation from the IRB/IEC containing the same information.

Until written approval by the IRB/IEC has been received by the investigator, no patient may undergo any procedure not part of routine care for the patient's condition.

Protocol amendments must also be reviewed and approved by the IRB/IEC. Written approval from the IRB/IEC, or a designee, must be received by the sponsor before implementation. This written approval will consist of a completed IRB Approval Form or written documentation from the IRB/IEC containing the same information.

16.4. Finance and Insurance

Details on finance and insurance will be provided in a separate agreement between the investigator and the sponsor.

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31. Summary of Safety And Effectiveness Data (SSED) Continuous Glucose Monitoring System-FDA PMA 05OCT2012

18. ATTACHMENTS

18.1. Schedule of Events

Table 2 Schedule of Events

Period	Screening	Part 1 (2x48-hr period: double blind, randomized, placebo controlled)				Part 2 (21-day Open-label Active)						Follow-up[a]
Visit day		1[b]	2	3	4	5	6	11[c]	18[c]	25	Telephone Call[d]	53
Time window	Day -28 to -1	0	0	0	0	0	0	±2	±2	±3		±3
Visit #	1	2		3		4		5	6	7		8
General assessments												
Informed consent	X											
Inclusion/exclusion criteria	X	X										
Randomization exclusion criteria		X										
Demography	X											
Body weight and length[e]	X[e]	X	X	X	X	X	X	X	X	X[e]		
Medical history (including current illness[f])	X											
Concomitant medication	X	X	X	X	X	X	X	X	X	X		X
Safety Assessment												
Electrocardiogram	X	X	X	X	X	X	X	X	X	X		X
Echocardiography	X[g]											X
Vital signs	X	X[h]	X[h]	X[h]	X[h]	X[h]	X[h]	X	X	X		X
Adverse events		X	X	X	X	X	X	X	X	X	X	X
Local tolerabilty		X	X	X	X	X	X	X	X	X		
Physical examination and neurological examination	X	X	X	X	X	X	X	X	X	X		X
Fluid balance assessment[i]		Continuous (when receiving IV Glucose)										
Laboratory												
Clinical laboratory tests[j]	X						X			X		X
Anti-drug antibodies		X[k]								X		X[k]

Period	**Screening**	**Part 1 (2x48-hr period: double blind, randomized, placebo controlled)**				**Part 2 (21-day Open-label Active)**						**Follow-up[a]**
Visit day		**1[b]**	**2**	**3**	**4**	**5**	**6**	**11[c]**	**18[c]**	**25**	**Telephone Call[d]**	**53**
Time window	**Day -28 to -1**	**0**	**0**	**0**	**0**	**0**	**0**	**±2**	**±2**	**±3**		**±3**
Visit #	**1**	**2**		**3**		**4**		**5**	**6**	**7**		**8**
Pharmacokinetics/drug exposure						X[l]	X			X		
Efficacy												
Continuous glucose monitoring	X (for 24 hours prior to randomization)	Continuous										
Self-monitoring plasma glucose		X										
IV GIR adjustment		Continuous (until weaned off)										
Trial Materials and Reminders												
Randomization		X										
Dispense patient diary								Upon discharge[m]				
Diary recording								Continuous after discharge from hospital				
Dispensing of trial product		X		X		X						
Drug accountability				X		X		X	X	X		

Abbreviations: GIR = glucose infusion rate; IV = intravenous; SOC = standard of care; SpO_2 = blood oxygen saturation level

Note: Unscheduled visits can occur at any time if the investigator deems it necessary for patient safety.

a The Follow-up Visit will only be performed for patients who will not enter the extension trial.

b After screening, eligible patients will complete a minimum 24-hour run-in period to confirm the IV glucose randomization requirement (≥10 mg/kg/min). All non-nutritional carbohydrates and carbohydrate fortification of feeds are prohibited during this 24-hour run-in period.

c Visits 5 (Day 11) and 6 (Day 18) can be converted to phone visits at the investigator's discretion.

d Patients who are discharged from the hospital before Day 25 will be contacted by the investigator by telephone the day after discharge. The investigator will ask the parent(s)/guardian if they have any questions about the trial procedures and if their child has experienced any AEs.

e Body length will be measured at screening and at the End of Treatment visit.

f For CHI diagnosis: Data on biochemical parameters, genotyping results, and information about PET-CT should be captured when available.

g An echocardiogram performed within 1 month of screening can be used.

h Vital signs should be measured at 6 ± 1, 12 ± 2, and 24 ± 4 hours after initiation of the trial drug and every 8 ± 2 hours hereafter..

i Fluid balance assessments are to be performed and documented every 8 hours as long as the patient is receiving IV glucose.

j Clinical laboratory tests include hematology and biochemistry.

k Blood test for anti-drug antibodies to be performed prior to dosing. Any anti-dasiglucagon antibody-positive patient (treatment induced or treatment boosted) will be monitored at an additional Follow-up Visit preferably 16 weeks after last ADA-positive sample. Patients completing the trial before the ADA screening and confirmatory assays have been approved by the FDA and who do not continue treatment in the long-term extension trial will have this additional visit 16 weeks after the end of trial visit (Visit 8).

l Sampling for drug exposure at Day 5 is only applicable for patients with a body weight of ≥4 kg. This sample should only be collected if it does not compromise the total amount of blood drawn according to Section 11.2.3.1.
m The diary should be dispensed if a patient is discharged from the hospital during Part 2. At subsequent visits, the parent(s)/guardian will return the completed diary and obtain a new one.

18.2. Investigator's Agreement

PROTOCOL NUMBER:	ZP4207-17103
PROTOCOL TITLE:	A Randomized Trial in 2 Parts: Double-Blind, Placebo-Controlled, Crossover Part 1 and Open-label Part 2, Evaluating the Efficacy and Safety of Dasiglucagon for the Treatment of Children with Congenital Hyperinsulinism
FINAL PROTOCOL:	Version 14.0, 04-Jun-2021 (Germany)

The undersigned acknowledges possession of and has read the product information (e.g., IB) on the trial drug and have discussed these data with the trial monitor. Having considered fully all the available information, the undersigned considers that it is ethically justifiable to give the trial drug to selected patients in his or her care, according to the trial protocol.

- He or she agrees to use the trial material, including trial drug, only as specified in the protocol. He or she understands that changes cannot be made to the protocol without prior written approval of Zealand.
- He or she understands that any deviation from the protocol may lead to early termination of the trial.
- He or she agrees to report to Zealand within time any clinical AE or abnormal laboratory value that is serious, whether or not considered related to the administration of trial drug.
- He or she agrees to comply with Zealand and regulatory requirements for the monitoring and auditing of this trial.

In addition, he or she agrees that the trial will be carried out in accordance with the revised Declaration of Helsinki (2013) and the local laws and regulations relevant to the use of new therapeutic agents.

I, the undersigned, have carefully read this protocol and agree that it contains all the necessary information required to conduct the trial.

Principal Investigator:

Printed Name: _____

Signature: _____

Date: _____

Investigator's name and address (stamp)

19. APPENDICES

A. Address List

B. Regulations and Good Clinical Practice Guidelines

A. Address List

Name:	[redacted]
Address:	[redacted]
	[redacted]
E-mail:	[redacted]
Telephone:	[redacted]

Technical Complaints

Name:	Zealand Pharma A/S Quality Assurance
Email:	[redacted]

B. Regulations and Good Clinical Practice Guidelines

1. Regulations

Refer to the following United States Code of Federal Regulations (CFR):

- FDA Regulations 21 CFR, Parts 50.20 – 50.27
  Subpart B – Informed Consent of Human Subjects
- FDA Regulations 21 CFR, Parts 56.107 – 56.115
  Part 56 – Institutional Review Boards
  Subpart B – Organization and Personnel
  Subpart C – IRB Functions and Operations
  Subpart D – Records and Reports
- FDA Regulations 21 CFR, Parts 312.50 – 312.70
  Subpart D – Responsibilities of Sponsors and Investigators

Refer to the following European Directives [and applicable regulations/guidances]:

- European Directive 2001/20/EC and related guidance documents
- European Directive 2005/28/EC and related guidance documents

2. Good Clinical Practice Guidelines

ICH GCP guidelines can be found at the following URL:

http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E6/E6_R2__Step_4_2016_1109.pdf

20. ADDENDUM

20.1. Administrative Information

The Coordinating Investigator for Germany is:

A list of all participating investigational sites, including name, address, and professional position of the Principal Investigator for each site is maintained in the trial master file.

20.2. Device Information

This clinical trial is being conducted solely to investigate the safety and efficacy of dasiglucagon usage in children with CHI. More information can be found in Section 6.2.

This clinical trial is not being conducted to verify claims and new intended performance of the medical devices. No risks and device effects will be formally assessed, but only collected and, if appropriate, reported to the applicable bodies. The planned devices are only being used for the administration of dasiglucagon and to measure glucose levels since no other approved devices are available as an alternative for this rare disease. The protocol will be submitted to competent authorities and ethics committees for approval. If additional requirements are imposed by these bodies, those recommendations will be followed.

20.2.1. Planned Devices

20.2.1.1. Pump System Accu-Chek Spirit Combo

The planned drug delivery pump Accu-Chek Spirit Combo System with its components as described in Section 10.3 will be used to administer the investigational drug dasiglucagon. The Accu-Chek Spirit Combo Pump is a portable, battery-operated pump indicated for subcutaneous or intraperitoneal continuous delivery of insulin for the management of diabetes mellitus in persons requiring insulin as prescribed by a physician. It is a CE-marketed Class IIb product under the EC directive 92/42/EEC and is also in conformity with the EU directive 1999/5/EC on radio and telecommunications terminal equipment. The manufacturer is Roche Diabetes Care GmbH, Sandhofer Str. 116, 68305 Mannheim - Germany.

The pump system consists of the following components:

- Programmable Accu-Chek Spirit Combo pump with a display screen
- Adapter that connects the cartridge to the infusion set
- Batteries and battery key
- Sterile disposables:
  - Accu-Chek Spirit 3.15-mL cartridge with luer-lock connection
  - Infusion sets:
    - Accu-Chek FlexLink Infusion set
    - Accu-Chek Ultraflex

- Accu-Chek LinkAssist insertion tool
- Accu-Chek Rapid-D Link Infusion set cannula
- Accu-Chek Rapid-D Transfer Set

The single-use disposable infusion sets and sterile cartridges are packed in sealed pouches and are sterilized with ethylene oxide. Pictures of the pump and system components are provided in the ZP4207-17103 Trial Materials Manual for further information. Additional available accessories for the Accu-Check Spirit Combo System are an alcantara pouch in black and a neoprene pouch in white with a belly belt.

The trial sites will be supplied with the pumps and their components and will be asked to document receipt of this equipment. Each pump will be identified by a serial number that will be used for accountability and traceability; the device will be labelled "for investigational use only." The trial sites must store the devices in a secure area with restricted access and at the storage conditions indicated in the user manual (+5°C – 45°C). After a patient is enrolled into the trial, one (1) pump device will be allocated exclusively to this patient; disposables are not accounted for. Upon completion of the trial and if the patient is not continuing in the ZP4207-17106 trial, the pump must be returned to the site. The site will ship all returned pumps (with the exception of those continuing in 17106 trial) back to the sponsor upon completion of the trial.

The Roche pump is CE-marked and marketed in Germany, and it can administer very low doses. The pump is designed to deliver 0.05 to 25.0 units of U-100 insulin per hour in basal rates, which corresponds to 2 to 1000 µg dasiglucagon per hour. This dosing accuracy allows reliable dosing of dasiglucagon between 10 and 70 µg per hour in the clinical trial. Further, this pump has been used in pediatric diabetes treatment in the EU since 2010. However, the Accu-Chek Spirit Combo pump system will not be used within its approved indication. The pump will be used for the administration of the investigational glucagon analogue dasiglucagon for the indication of CHI. Additional compatibility testing and assessments were performed to ensure that the Accu-Check Spirit Combo pump system and its components are not adversely affected by using dasiglucagon instead of insulin. Also, testing was performed to ensure that dasiglucagon is stable and effective when administered via this pump system. The general management of the device, as well as the duration of contact of the pump material and infusion sets with human tissue, will be based on the approved intended use of the device. The complete infusion set will be changed every 2 days (48 hours) for the Accu-Chek Rapid-D link infusion set, and every 3 days (72 hours) as a maximum for the Accu-Chek FlexLink infusion set. Only the application and dosage of dasiglucagon via the Accu-Chek pump system requires an off-label change in practice compared to the procedure described in the general instruction for use for Accu-Chek Spirit Combo pump system. The pump is programmed to reflect doses of insulin in units (U), whilst dasiglucagon requires weight-based dose administration (µg). The site staff and the patient's parent(s)/guardian(s) will be instructed on the procedures for filling the content of the trial drug vials into the cartridge to be used in the infusion pump and how to convert dasiglucagon (µg) into units (U) as shown on the pump display. For this purpose an extensive, trial-specific instruction for use was developed to accommodate the training of site staff and the patient's parent(s)/guardian(s) as described in Section 10.12.

20.2.1.2. Self-Monitoring Plasma Glucose StatStrip Xpress2

To assess plasma glucose levels and events of hypoglycemia, SMPG measurements will be taken. The plasma glucose level will be assessed at least 3 times daily and in cases of suspected hypoglycemia throughout the trial.

The hand-held StatStrip Xpress2 meter GLU/KET from Nova Biomedical is to be used to measure glucose and ketone levels in the design of the trial. The ketone measurement will only be performed by trial staff when the patient is in the clinic.

The SMPG meter manufactured by Nova Biomedical is a small (size of 98.0 × 64.0 × 22.9 mm and a weight of 78.5 g) battery-powered meter. Blood sample strips are inserted and glucose (in mg/dl or mmol/L) and ketone values (in mmol/l) will be displayed on the screen. Function and data selection are done by choosing 1 of 3 buttons. Up to 400 measurements can be stored in the device memory.

The test strip is designed with an electrode that measures glucose levels. Glucose in the blood sample mixes with reagent on the test strip, which produces an electric current. The amount of current that is produced depends on the amount of glucose in the blood. The electrical current is detected by the monitor and displayed as the glucose value. The test strip is designed such that when a drop of blood touches the end of the strip, the blood is drawn into the reaction space via capillary action. Test strips are available in cartons of 100 strips: 50 strips/vial. Additionally a QC control glucose/ketone solution is provided to test device functionality.

The manufacturer Nova Biomedical Corporation, 200 Prospect Street, Waltham, MA 02454-9141 – USA (European Authorized Representative Nova Biomedical U.K.; Innovation House Aston Lane South, Runcorn, Cheshire WA7 3FY, UK) have self-declared conformity with the EU Directive 98/79/EC In Vitro Diagnostic Medical Device Directive. This SMPG device is marketed as an In Vitro Diagnostic Device. The SMPG device is intended for in-vitro diagnostic use by health care professionals and for point-of-care usage in the quantitative measurement of glucose in fresh capillary, venous, arterial, and neonate whole blood; it can also be used for quantitative determination of beta-hydroxybutyrate-ketone in fresh capillary and venous blood samples. It is not intended for diagnosing or screening for diabetes.

The SMPG device is reusable with single-use test strips. The SMPG device is not sterile; however, the strips themselves are in sterile packaging. Once the vial containing the strips is opened, the single strips may be used for 180 days or until the expiration date printed on the label has been reached, whichever comes first. The patient's blood will be drawn via single-use lancing devices and a drop is applied to the strip. The strip is then inserted into the device and the glucose level is displayed.

The rationale for selecting this device for the clinical trial is that this blood glucose meter is the only one approved for use in neonates in an intensive care unit setting, based on its accuracy and extensive testing for possible concomitant medication interference. No BG meter for use by lay person in the home care setting is approved for the age group <1 year. To ensure consistency of data between the hospitalized and home-care periods in the trial, it was deemed preferable to continue with the same BG meter, rather than change to ones approved for use by lay persons in home-care settings, but that were not approved for this specific age group.

The trial sites will be supplied with the StatStrip Glucose Xpress2 meter and the StatStrip Glucose and ketone test strips by the sponsor, and are asked to document the receipt of the

material. Each SMPG is identified by a serial number that will be used for accountability and traceability and the device is labelled as "for investigational use only." The trial sites are asked to store the devices in a secure area with restricted access at the storage conditions indicated in the user manual (+1°C – 30°C); the QC Control GLU/KET solution must be stored between +15 – 30°C. After a patient is enrolled into the trial, 1 SMPG device is exclusively allocated to that patient; disposable accessories are not accounted for. Upon completion of the trial and if the patient is not continuing in the ZP4207-17106 trial, the SMPG device has to be returned to the site. The site will ship all returned SMPG devices back to the sponsor upon completion of the trial.

The same SMPG StatStrip Glucose Xpress2 meter will be used for each enrolled patient during the hospital stay and at home. The home assessments will be performed by the parent(s)/guardian(s); however, the use of the device by a lay-person is outside the intended purpose. The risk resulting from this deviation from the intended purpose will be minimized by offering extensive training of the parent(s)/guardian(s) by the site staff, before independent measurements are performed.

Upon return to the site, the SMPG data will be uploaded to a tablet provided to the site personnel. The trial tablet is loaded with the applicable software for sending the SMPG data to the Vitalograph Web Portal.

20.2.1.3. Dexcom G6 Continuous Glucose Monitoring System

In Germany, only the Dexcom G6 CGM will be used. The Dexcom G6 is indicated for detecting trends and tracking patterns in patients (aged 2 and older) with diabetes. The system is intended for use by patients at home and in healthcare facilities. It is designed to replace fingerstick blood glucose testing for diabetes treatment decisions. The system aids in the detection of episodes of hyperglycemia and hypoglycemia, facilitating both acute and long-term therapy adjustments, which may minimize their excursions. Interpretation of CGM results should be based on the trends and patterns noted in several sequential readings over time.

The G6 system components include the following:

- **Sensor (Disposable)** – comprises a sensor applicator, an adhesive pad, transmitter mount, and the sensor probe. The sensor can be worn for up to 10 days. The sensor is a sterile device inserted by the user into the subcutaneous tissue of the abdomen or buttucks using the applicator for that purpose. The applicator is attached to the surface of the skin with a standard medical grade adhesive pad. The applicator is a 26-gauge introducer needle that contains the sensor probe. The needle is not exposed, or even visible, to the user during the insertion process. After deployment of the introducer needle, the needle is retracted back into the applicator. The sensor probe remains beneath the surface of the skin and uses the enzyme glucose oxidase to convert the glucose in the interstitial fluid around the sensor into an electrical current proportional to the ambient glucose concentration. The applicator is detached and discarded by the user, exposing a transmitter mount ready for placement of the transmitted current and signal.

- **Transmitter** – The G6 transmitter transmits data with Bluetooth wireless technology. After sensor insertion and removal of the applicator, the user manually places the transmitter into the transmitter mount on the adhesive pad already attached to the skin. The transmitter contains all the electrical circuitry necessary for the operation of the electrochemical sensor and to transmit the sensor signal to the receiver via Bluetooth wireless technology. The transmitter collects the small electrical current from the sensor and transmits the sensor signal wirelessly to the receiver at regular 5-minute intervals. The transmitter is reusable and can be used for repeated 10-day sessions by a single user for up to three months.
- **Receiver** – The G6 receiver is a small hand-held device that receives the Bluetooth wireless technology signals from the transmitter. The receiver contains a rechargeable battery. In typical use, the receiver may last approximately 2 days before requiring recharging. The user must maintain the receiver within 6 meters or less of the transmitter, which is attached to the sensor on the body. The receiver also contains calibration and signal processing algorithms required to convert the sensor electrical signal to glucose values.

Other accessories include the following:

- Receiver USB charging / download cable
- Alternating current (AC) power adapter
- User's guides
- Training checklist

The manufacturer is Dexcom, Inc. located at 6340 Sequence Drive – San Diego, CA 92121 United States of America. The European Authorized Representative is MediTech Strategic Consultants B.V., Maastrichterlaan 127-129, 6291 EN Vaals, the Netherlands. The following device classifications and rules were applied to demonstrate compliance with the Medical Device Directives:

- Sensor: Class IIb: Rule 8 according to Annex IX of the MDD 93/42/EEC & 2007/47/EC
- Transmitter: Class IIa, Rule 10 according to Annex IX of the MDD 93/42/EEC and 2007/47/EC
- Receiver: Class IIb, Rule 10 according to Annex IX of the MDD 93/42/EEC and 2007/47/EC
- Dexcom G6 Mobile System: Class IIb, Rule 8 according to Annex IX of the MDD 93/42/EEC and 2007/47/EC

The associate notified body is the British Standards Institution. The device has been marketed since 2018.

The system components (applicator, transmitter housing, insertion needle and sensor are sterilized via electron beam radiation using the (VD)max[25] method. Transmitters and receivers are not sterile products.

The trial sites will be supplied with the Dexcom CGM system by the sponsor and are asked to document the receipt of the material. Each CGM is identified by a serial number which will be used for accountability and traceability and the device is labelled as "for investigational use

only." The trial sites are asked to store the devices in a secure area with restricted access at the storage conditions indicated in the user manual:

- Sensors (+2°C – 25°C)
- Transmitter and receiver (0°C – 45°C)

After the patient is enrolled into the trial, a CGM device is allocated exclusively to this patient; disposable materials are not accounted for. Upon completion of the trial, the CGM must be returned to the site. The site will ship all returned CGMs back to the sponsor upon completion of this trial.

Congenital hyperinsulinism is characterized by frequent, often severe episodes of hypoglycemia due to over-secretion of insulin regardless of blood glucose. Therefore, CHI closely mimics the exogenous insulin overdose occurring in insulin-dependent diabetes management. The CGM device is being used in this clinical trial to confirm potential treatment effects on a continuous basis.

The rationale for selecting the Dexcom G6 CGM device for this clinical trial is that it can be used in a blinded mode. By using the blinded mode, the patient's parent(s)/guardian(s) and the investigator will not be able to make any treatment decisions or change behavior based on the CGM readings.

The Dexcom G6 CGM system will be used by the patient during the hospital stay, as well as at home. For the purpose of this trial the device will be used outside the intended purpose in regard to the age of the patients (7 days to 12 months instead of 2 years and older) and in regard to the indication (CHI instead of Diabetes). No mechanical or design changes have been made to the device. The CGM device is used as intended according to the CE mark, except for the age group and the disease. The essential requirements testing performed for CE marking is assessed applicable for proposed use. Additionally, literature shows that CGMs have been successfully used in infants and neonates.[26] The risk resulting from this deviation from the intended purpose, in terms of age, will be minimized by offering extensive training to parent(s)/guardian(s) by the site staff, before the device and its components are used independently.

The results of the CGM assessments are blinded for review by patient(s)/parent(s)/guardians and trial staff. The display will be visible and the menu functions available for the user, but the display will not show any measurements nor will any alarm go off based on the measurements. The Dexcom G6 CGM does not require calibration.

At every patient's onsite visit, the site staff will transfer data of the CGM device via the CENDUIT Data Agent, after the device has been initially registered within the CENDUIT IRT system. Full instructions on data transfer are available in the CENDUIT user manual.

20.2.1.4. Other Medical Devices

The following medical devices will be used within their intended purpose during this trial:

- BD Lancet – BD Microtainer® CAL – contact-activated lancet
- Disinfecting wipes (alcohol pads)
- Disinfecting wipes – Diabete ezy

The provided devices are disposable; they will be provided to the patient but not accounted for.

20.2.2. Risk Evaluation for Off-label Use of Devices

20.2.2.1. Anticipated Clinical Benefit of Planned Devices

The main aim of this clinical trial is to assess the safety and efficacy of the investigational drug dasiglucagon. To achieve this, the Accu-Chek Spirit Combo Pump system, the Self-Monitoring Plasma Glucose StatStrip Xpress2 and Dexcom G6 CGM device are utilized in this trial outside their intended use. There is no direct clinical benefit anticipated by using the Accu-Chek Spirit Combo Pump except the administration of dasiglucagon and therefore, only an indirect benefit can be anticipated, resulting from the drug administration as referenced in Section 6.3.5. The clinical benefit of the SMPG is to help to assess if patients undergo hyper/hypoglycemic events requiring adjustments to gastric and oral feeds or dose. For the usage of the CGM, there is no direct clinical benefit anticipated for the patients, but the CGM data will help to confirm treatment effects independent from parents'/guardians' ability to suspect hypoglycemia.

20.2.2.2. Risks Associated with the Planned Devices and their Control

A risk analysis according to EN ISO 14971:2012 was performed specifically for risks related to the off-label use of the planned devices, to identify and mitigate potential risks due to the off-label use of the devices. The analysis rated risks based on their probability of occurrence and the severity of their consequence. Unacceptable risk levels were defined and risks, meeting this definition were mitigated as far as possible to get to an acceptable level. Results of the risk analysis were entered into a risk management report. The identified risks were evaluated and risk mitigation measures were and will be implemented throughout this trial. The main task to mitigate the risks is to educate site staff, parents and guardians on the proper use of the devices to minimize the risks associated with the off-label use.

The following residual risks were identified, which will be specifically addressed in the planned training of the users and thereby risks will be mitigated:

Accu-Chek® Spirit Combo Pump System and Self-Monitoring Plasma Glucose (SMPG) meter (Nova Biomedical / Xpress2 GLU/KET)

Training will cover the following aspects:

- Recognition of hypo- and hyperglycemia in the patient
- Response to hypo- and hyperglycemia
- Usage of the devices according to the instructions for use in the trial
- Communication between patients/parents/guardians and the medical professionals of the trial team.

Dexcom G6 Continuous Glucose Monitoring (CGM) device

The training will cover the following aspects:

- Avoid to cover the sensor by the diaper in babies
- Avoid that the transmitter is grabbed by baby by covering it with bandage or clothes
- Avoid that the transmitter is misplaced under diaper area or child pollutes around diaper area

Additionally, the assessment identified other risks caused by potential Accu-Chek® Spirit Combo pump system/drug interaction e.g. unintentional misappropriation of drug delivery or hazards caused by direct drug/device interactions. These potential risks were assessed by additional compatibility and in-use stability testing and no unacceptable risk was noticed. Flow-rate assessments were performed and a conversion table for the use of dasiglucagon in the Accu-Chek® Spirit Combo pump system was developed and tested in a Human Factor trial. This conversion table was included with further instructions in a trial specific "Instruction for Use" for the combination of dasiglucagon with the pump system. By this measure the risk of misappropriation of drug delivery was reduced to an acceptable level.

20.2.2.3. Possible Interactions with Concomitant Medical Treatment

The use of the planned devices is not expected to interfere with other concomitant medical treatments, except those treatments referenced in Section 10.11.2.

20.2.2.4. Risk/Benefit Assessment

At the present time there are no known CE Marked devices intended for the delivery of dasiglucagon for the treatment of CHI. Additionally, there are no known devices intended to monitor glycemic control in children with CHI. Since there are no devices currently approved to treat this condition, off-label use of the Accu-Chek Combo Insulin pump system, Dexcom G6 CGM and Nova Biomedical / Xpress2 GLU/KET for the purpose of delivering dasiglucagon and monitoring glucose levels potentially presents a beneficial alternative to the standard clinical practices of current treatment options, continuous gastric infusion and pancreatectomy for controlling CHI.

The most relevant risks resulting from the off-label use in terms of their potential to cause harm in relationship to their probability are the following:

- Lay persons using devices which are not used according to their intended use e.g. perform and interpret blood glucose measurements
- Lay person applying devices which require invasive procedures at home for very young children in skin areas which might be contaminated due to the expectable uncleanliness of babies and the fact that they might carry diapers.

To evaluate the risks and the benefits of the clinical trial, the duration of the trial and the effect of the foreseeable risks during the trial period are compared to the current standard of care (SoC) treatment for CHI. This comparison is done without taking the possible positive results of the trial into account. A positive trial outcome would most likely result in a change in the treatment of CHI towards a continuous delivery of dasiglucagon with devices that are proven to be save in use by children.

In general, the current SoC treatment for patients with CHI bears higher risks than risks associated with the clinical trial participation. The current risks associated with the SoC treatment result from:

- Continuous application of glucose rich liquids via intravenous infusion (infection, skin injury, thrombi, contamination, dosage and content errors, risk of volume overload)
- Frequent tube feedings or gastric infusions
- For patients on diazoxide treatment, the more common side effects comprise hypertrichosis, fluid retention, and gastrointestinal symptoms
- For patients on somatostatin analog (octreotide or lanreotide [long acting]) possible side effects include necrotizing enterocolitis, gallstones, and hepatitis
- Pancreatectomy (and its related exo/endocrine complications, particularly the development of insulin-dependent diabetes)
- Local wound infection

Irrespective if the patient participates in the planned clinical trial or is treated by SoC, parent(s)/guardian(s) need to be able to detect and respond to hypoglycemic events, therefore the risk of not noticing such a situation is not increased by participating in the trial. The blood glucose measurements are performed by SMPGs, where the general functionality of the device can be considered as being equivalent to the proposed Nova Biomedical SMPG. It can be concluded, that there will be no additional risks for the clinical trial participants by using the Nova Biomedical SMPG compared to patients who do not take part in the clinical trial, provided the users are adequately trained.

The primary objective of this clinical trial is to evaluate the efficacy of dasiglucagon in reducing glucose requirements in children with persistent CHI requiring continuous IV glucose administration to prevent/manage hypoglycemia. The clinical trial aims to determine practicable procedures for treating CHI with dasiglucagon that can eliminate, minimize or postpone the need for continuous application of glucose via intravenous infusions, frequent tube feedings or continuous gastric infusions and finally, pancreatectomy in the treatment of children with CHI. This will significantly reduce the risks for the patient's health. The nature of the residual risks introduced by the off-label use of the devices selected for the clinical trial is similar to the risks which already exist in the treatment of these patients and their severity is only minor. The most relevant risks result from the usage of the CGM device which is not intended to be used directly for the patient care in the trial. However, the duration of the trial will be shorter than the duration of the disease or of its long-term side-effects (either of the disease or the treatment). Thus, the potential benefit of a positive trial outcome is estimated higher than the risks introduced, especially when taking into account the risks which are present by the current SoC treatment of CHI.

20.3. Device-related Safety Section

20.3.1. Anticipated Adverse Device Effects

The following anticipated ADEs may be associated with the use of these medical devices, together with those associated with the application of dasiglucagon. All AEs and ADEs will be assessed if they meet the requirements for competent authority reporting.

The following device-related possible complications/AEs are anticipated:

20.3.2. Pump System Accu-ChekSpirit Combo

- Ketoacidosis, hypo- or hyperglycemic events resulting from incorrect pump usage, a damaged pump, or infusion set blockage[27,28]
- At infusion site:
  - Local infection
  - Irritation
  - Local pain
  - Redness
  - Swelling
  - Lumps
  - Heat
- Strangulation if tubing becomes wrapped around the neck

20.3.3. Self-monitored Plasma Glucose StatStrip Xpress2

Since the StatStrip Xpress 2 Blood Glucose Monitoring System is intended for use outside the body, only the following limited ADEs are expected:

- Hypo- or hyperglycemic events due to incorrect usage or damaged meter or strips
- Indirectly related to SMPG: Local infections caused by the single-use lancing devices to obtain blood

20.3.4. Continuous Glucose Monitoring Dexcom G6

The following events are possible ADEs of inserting a sensor or wearing the adhesive patch:

- Local infection
- Inflammation
- Pain or discomfort
- Bleeding at the glucose sensor inserting site
- Bruising
- Itching
- Scarring or skin discoloration
- Hematoma
- Tape irritation
- Sensor or needle fracture during insertion, wear of removal

The following risks are inherent to the device, but not anticipated for the trial since the device will be used in a blinded manner and no treatment decisions will be based on the CGM results. However, there are potential risks due to missed alerts, false alerts, false-negative hypoglycemia, hyperglycemic readings, false-positive hypoglycemia, and hyperglycemia readings by the device. There are additional possible risks if the system inaccurately calculates the rate of change of glucose.[29]

20.4. Protocol Clarifications

20.4.1. Informed Consent

A patient is considered enrolled in the trial as soon as the patients' parent(s)/guardian(s) (according to local law) have signed the EC-approved ICF.

20.4.2. Vulnerable Population

As discussed in Section 8.1 children between the ages of 7 days and 12 months with CHI will be enrolled in this clinical trial. The informed consent process is referenced in Section 9.2.1, Inclusion Criteria, Section 11, Trial Procedures, and Section 16.2, Patient Information and Informed Consent. No vulnerable patients other than children are planned to be enrolled in this trial and no enrollment under an emergency situation is allowed.

20.4.3. Addition to Statistical Section

- **Drop-out rate**: The drop-out rate is expected to be low as the trial period is short and the primary endpoint is evaluated within the first 96 hrs after randomization.
- **Pass/fail criteria:** No pass/fail criteria were defined since no trial endpoints are device related.
- **Criteria for termination of trial on statistical grounds:** N/A. Please refer to Section 13.4.5 Interim Analysis.
- **Procedures for reporting deviation from statistical plan:** Since no medical device data will be evaluated statistically, no reporting of deviations from statistical plan are anticipated in regard to the devices.
- **Enrollment rate per investigational site:** Anticipated enrollment for the German clinical trial sites is 0 – 5 patients per German site. For analysis involving trial site, if the number of patients per site is small, sites may be pooled for analysis, or omitted from statistical models. The final determination will be made prior to database lock.

20.4.4. Protocol Deviations

A protocol deviation is defined as an event where the investigator or site personnel did not conduct the trial according to the protocol. Investigators are not allowed to deviate from the protocol unless it is necessary to protect the life or physical well-being of a patient in an emergency situation. Those emergency situations or other unforeseen circumstances that are beyond the investigator's control, e.g., the patient did not attend scheduled visits or blood samples were lost by the laboratory, are still considered deviations. Deviations will be reported to the sponsor, regardless of whether or not they are medically justifiable or done to protect the patient in an emergency. All deviations will be reported in a timely manner on a protocol deviation form.

In addition, the investigator is required to adhere to the ethics committee procedures for reporting protocol deviations. International regulatory body regulations require that investigators maintain accurate, complete, and current records, including documents showing the dates of and reasons for each deviation from the trial protocol. The site will receive a list of all site-specific deviations upon completion of the trial.

Deviations include, but are not limited to the following list:

- Failure to obtain informed consent prior to conducting trial-specific activities
- Incorrect version of patient information and/or ICF used
- Patient did not attend a visit or the visit was outside the required time frame
- Assessments as detailed in the Schedule of Events (Table 2) were not conducted or were performed incorrectly
- Adverse events and deficiency with SADE potential not reported by investigators within the required timeframe as specified in the protocol
- Source data were permanently lost

As addressed in Section 14.5.1 of the protocol, the CRA will review the site compliance with regard to deviations at each monitoring visit. The monitor will discuss any deviations directly with the investigator and will summarize the findings in a follow-up letter to the site.

If a trial site deviates from the protocol, those deviations will be analyzed and re-training on the particular topic(s) will be initiated as appropriate, e.g., training on the informed consent process, training on visit window adherence, and planned assessments, etc. Such trainings will be documented on the training logs for the applicable roles. If despite all training efforts an investigational site continues to deviate from the trial protocol, a site can be discontinued from the trial as agreed upon by the Principal Investigator according to his or her signature on the Investigator's Agreement in Section 18.2.

PROTOCOL

PRODUCT NAME/NUMBER:	Dasiglucagon
PROTOCOL NUMBER:	ZP4207-17103
IND NUMBER:	135869
EUDRACT NUMBER:	2017-004545-24
DEVELOPMENT PHASE:	Phase 2/3
PROTOCOL TITLE:	A Randomized Trial in 2 parts: Double-Blind, Placebo-Controlled, Crossover Part 1 and Open-label Part 2, Evaluating the Efficacy and Safety of Dasiglucagon for the Treatment of Children with Congenital Hyperinsulinism
PROTOCOL DATE:	Original Protocol Version 1.0, 14-Dec-2017 Final Version 13.0, 04-Jun-2021 (All countries except Germany)
SPONSORED BY:	Zealand Pharma A/S Sydmarken 11 2860 Soeborg (Copenhagen) Denmark Telephone: +45 88 77 36 00
CONTRACT RESEARCH ORGANIZATION:	Premier Research One Park Drive Suite 150 Durham, NC 27709 USA

1. REVISION HISTORY

PROTOCOL TITLE:	A Randomized Trial in 2 parts: Double-Blind, Placebo-Controlled, Crossover Part 1 and Open-label Part 2, Evaluating the Efficacy and Safety of Dasiglucagon for the Treatment of Children with Congenital Hyperinsulinism
PROTOCOL DATE:	Original Protocol Version 1.0, 14-Dec-2017
	This updated protocol version 13.0 is applicable for all countries except Germany, and includes:
AMENDMENT No. 1	Final Version 2.0, 20-Jun-2018 (All countries)
AMENDMENT No. 2	Final Version 3.0, 25-Jul-2018 (France, Germany, Israel, United Kingdom)
AMENDMENT No. 3	Final Version 4.0, 15-Apr-2019 (All countries)
AMENDMENT No. 4	Final Version 5.0, 03-Jun-2019 (All countries)
AMENDMENT No. 5	Final Version 6.0, 05-Jul-2019 (Germany)
AMENDMENT No. 5.1	Final Version 6.1, 05-Mar-2020 (Germany)
AMENDMENT No. 6	Final Version 7.0, 19-Sep-2019 (All countries except Germany)
AMENDMENT No. 7	Final Version 8.0, 11-Oct-2019 (Germany)
AMENDMENT No. 8	Final Version 9.0, 29-Apr-2020 (Germany)
AMENDMENT No. 9	Final Version 10, 08-Jul-2020 (All countries except Germany)
AMENDMENT No. 10	Final Version 11.0, 27-Aug-2020 (Germany)
AMENDMENT No. 11	Final Version 12.0, 05-Nov-2020 (Germany)
AMENDMENT No. 12	Final Version 13.0, 04-Jun-2021 (All countries except Germany)

2. APPROVAL SIGNATURES

PROTOCOL NUMBER:	ZP4207-17103
PROTOCOL TITLE:	A Randomized Trial in 2 parts: Double-Blind, Placebo-Controlled, Crossover Part 1 and Open-label Part 2, Evaluating the Efficacy and Safety of Dasiglucagon for the Treatment of Children with Congenital Hyperinsulinism

I, the undersigned, have read this protocol and confirm that to the best of my knowledge it accurately describes the planned conduct of the trial.

[redacted]

05-Jun-2021 | 11:37:02 CEST

Date

Clinical Trial Manager
Zealand Pharma A/S

[redacted]

10-Jun-2021 | 02:06:01 PDT

Date

[redacted]
Medical Director
Zealand Pharma A/S

[redacted]

07-Jun-2021 | 00:56:01 EDT

Date

[redacted]
Senior Manager, Biostatistics
Premier Research

3. SYNOPSIS

PRODUCT NAME/NUMBER	Dasiglucagon
PROTOCOL NUMBER	ZP4207-17103
EUDRACT NUMBER	2017-004545-24
DEVELOPMENT PHASE	Phase 2/3
PROTOCOL TITLE	A Randomized Trial in 2 parts: Double-Blind, Placebo-Controlled, Crossover Part 1 and Open-label Part 2, Evaluating the Efficacy and Safety of Dasiglucagon for the Treatment of Children with Congenital Hyperinsulinism
INDICATION	Congenital hyperinsulinism (CHI)
OBJECTIVES	Primary: To evaluate the efficacy of dasiglucagon in reducing glucose requirements in children with persistent CHI requiring continuous IV glucose administration to prevent/manage hypoglycemia. Secondary: To evaluate the safety and tolerability of dasiglucagon administered as a subcutaneous (SC) infusion in patients with CHI.
TRIAL DESIGN	This is a combined phase 2 and 3, randomized, multinational trial to evaluate the efficacy and safety of individually titrated dasiglucagon in children ≥7 days and <1 year of age who have been diagnosed with CHI, comprising 2 parts, a crossover (2 periods, 48 hours each), double-blind, placebo-controlled Part 1, and an open-label, single-arm Part 2 of 21 days. After screening, eligible patients will complete a minimum 24-hour run-in period to confirm the IV glucose randomization requirement (≥10 mg/kg/min). All non-nutritional carbohydrates and carbohydrate fortification of feeds are prohibited during this 24-hour run-in period. After the run-in period, patients will be randomly assigned in a double-blind fashion to receive dasiglucagon or placebo for 48 hours, after which they will be crossed over to the other trial treatment for an additional 48 hours. At the time of crossover, the trial drug will be initiated from the starting dose of 10 µg/hr, and IV glucose infusion rate (GIR) will be set to the rate obtained at the end of the run-in period and titrated accordingly. There will be no washout between the 2 periods in Part 1 to limit the length of time in which patients are reliant on IV GIR as their only means of preventing/treating hypoglycemia. This is deemed acceptable because: • The half-life (t½) of dasiglucagon is short (~30 minutes for the 0.6 mg dose and potentially shorter for lower doses, ~22 minutes) relative to the 48-hour duration of each crossover period. Moreover, for the primary endpoint, only the last 12 hours of the 48-hour period will be used for endpoint assessment. • Assuming a positive effect of dasiglucagon, the absence of washout is more conservative with respect to key secondary endpoints since a potential carry over effect of dasiglucagon in Period 2 is attributed to placebo. During Part 1, the trial drug and IV GIR should be adjusted according to the protocol-specified algorithm. Non-nutritional carbohydrates and/or carbohydrate fortification of feeds during Part 1 is only allowed when the maximum tolerable volume and concentration of IV glucose for the patient is reached, and should be limited to the minimum needed to ensure the patient's safety. All feedings (administered as parenteral nutrition, by nasogastric [NG] tube, gastrostomy, or normal route), will be recorded

	during this period. Safety assessments will be performed daily after initiation of the trial drug (active or placebo). After Part 1, patients will continue in open-label Part 2 to receive dasiglucagon for 21 days. Additional CHI treatments can be introduced during Part 2 if needed, if up-titration of dasiglucagon is not possible because of undesirable side effects or if the maximum dose level (70 µg/hr) has been reached. Gradual transfer from IV glucose to oral and gastric carbohydrates should be initiated in Part 2, enabling weaning of IV glucose and hospital discharge. Patients will continue to be hospitalized until IV GIR is weaned off; however, as soon as local site criteria for discharge are met, patients can be discharged to continue the treatment period at home. Visits are planned at Day 11 and Day 18 in Part 2. These visits can be converted to telephone visits if appropriate, at the investigator's discretion. On Day 25, the End of Treatment Visit will take place, and based on the investigator's confirmation of continued positive benefit-risk balance, patients will be offered the opportunity to participate in the long-term extension trial (Trial ZP4207-17106) to continue dasiglucagon treatment. Patients who do not enter the long-term extension trial will have a Follow-up Visit 28 days after their last dose of trial drug.
PLANNED NUMBER OF PATIENTS	A total of 12 patients is planned to be randomized 1:1 to receive either placebo first and then dasiglucagon or vice versa.
TRIAL ENTRY CRITERIA	Eligible patients will be ≥7 days and <12 months of age at screening with a body weight of ≥2.0 kg (4.4 lbs.) and a diagnosis of CHI established based on the following: • Hyperinsulinemia (plasma insulin above the limit of detection of the assay documented during an event of hypoglycemia), and/or • Hypofattyacidemia (plasma free fatty acid <1.7 mmol/L), and/or • Hypoketonemia (beta-hydroxy-butyrate <1.8 mmol/L), and/or • Glycemic response (increase in plasma glucose [PG] of >30 mg/dL after 1 mg IV or intramuscular [IM] glucagon administration). Eligible patients will be dependent on continuous IV glucose to prevent hypoglycemia.
INVESTIGATIONAL PRODUCTS	Dasiglucagon injection 4 mg/mL in a 3 mL vial containing 1 mL for injection. Matching placebo for dasiglucagon injection in a 3 mL vial containing 1 mL for injection.
REFERENCE PRODUCT	None
TREATMENT REGIMENS	As long as the patient is receiving IV glucose, PG will be measured and reviewed hourly using a hand-held PG meter. The IV GIR will be titrated to achieve glycemia of at least 70 mg/dL (3.9 mmol/L) (minimum GIR will be established as the rate up-titrated after the patient drops at least once below 70 mg/dL, or 3.9 mmol/L). When the patient is no longer on IV GIR, the PG will be checked according to local practice, but at least 3 times daily. The PG will be measured using the same trial-supplied hand-held PG meter during the entire trial. Additionally, blinded continuous glucose monitoring (CGM) will be started 24 hours prior to randomization (using the Dexcom G4 or G6 system) and continued until the end of Part 2. **Dosing of trial drug** Dosing of dasiglucagon will approximate continuous infusion by delivering small doses at frequent intervals via a trial-supplied infusion pump. The adjustment of trial drug dosing is closely linked to the PG level achieved, which in turn will determine the adjustments to the IV GIR.

	Part 1 (Crossover, double-blind, randomized, placebo-controlled) The starting dose of trial drug is 10 µg/hr at t=0. Every 2 hours (t=2, 4, 6h, etc.), the dose will be increased by an additional 10 µg/hr until either: • The patient is totally weaned off IV glucose, or • PG during the last 2 hours was constantly above 120 mg/dL (6.7 mmol/L), or • IV GIR has not decreased despite 2 sequential dose increases (in this situation the dose of trial drug product should be maintained until the IV GIR can be further decreased or until crossover or the end of the treatment period), or • The maximum dose of 70 µg/hr is reached, or • Adverse events (AEs) emerge that are considered to be related to the trial drug (e.g., nausea/change in feeding patterns or increased vomiting) and that are limiting further dose escalation. The 2-hour dose-adjustment interval will allow drug plasma levels to approach steady state before the dose is further increased. The cumulative dose will not exceed 1.26 mg over the first 24 hours and 1.68 mg for the subsequent 24-hour periods. At the time of crossover from the first 48-hour period to the second 48-hour period, the trial drug will again be titrated from the starting dose of 10 µg/hr, and IV GIR will be set to the rate obtained at the end of the run-in period and titrated accordingly. Part 2 (Open-label) After Part 1, all patients will continue in open-label Part 2 to receive dasiglucagon for an additional 21 days. Since treatment allocation during Part 1 remains blinded, all patients are required to initiate dasiglucagon dosing at 10 µg/hr in Part 2, while IV GIR in Part 2 should be started at the rate obtained at the end of the run-in period. During Part 2, the dasiglucagon dose, the amount and route of administration of carbohydrates (IV GIR, parenteral nutrition, gastric carbohydrate infusions, and carbohydrates from oral feeds) can be adjusted at the discretion of the investigator. Plasma glucose should be monitored at least hourly to adjust the IV GIR as appropriate and to avoid hypoglycemia. Additional concomitant medications, including somatostatin analogs and/or sirolimus/mechanistic target of rapamycin (mTOR) inhibitors, may be introduced at the investigator's discretion if the maximum dose level of dasiglucagon (70 µg/hr) has been reached or if further up-titration is not possible due to undesirable side effects. Adjustment of total carbohydrates administered (IV glucose, parenteral nutrition, gastric carbohydrate infusions, and carbohydrates from oral feeds) should continue throughout this period according to local practice to maintain PG levels in the range of 70 mg/dL to 120 mg/dL (3.9-6.7 mmol/L). The aim will be to obtain stable glucose levels with minimum rescue/hypoglycemia-prevention carbohydrates administered by invasive routes and to encourage/optimize oral feeds, and achieve weaning off IV glucose. Regardless of their discharge status, all patients will be offered the opportunity to participate in the long-term safety extension trial (ZP4207-17106), providing the investigator attests to the positive benefit-risk balance of continued dasiglucagon treatment.
PLANNED TRIAL SITES	Five to 7 sites in the United States, Europe, and Israel

ENDPOINTS	Primary endpoint *Part 1 (Day 1 to 4)* • Mean IV GIR in the last 12 hours of each treatment period during Part 1 (dasiglucagon or placebo administration) Key secondary endpoints *Part 1 (Day 1 to 4, for each 48-hour treatment period)* • Total amount (g) of carbohydrates administered (regardless of the route) per day.
STATISTICAL METHODS	Continuous endpoints will be summarized with number (n), mean, standard deviation (SD), median, minimum, and maximum. In addition, change from baseline values will be calculated at each time point and summarized descriptively; the exception to this is the time of PG within prespecified ranges, which will not have a baseline value. For categorical endpoints, descriptive summaries will include counts and percentages. All data will be presented in the data listings. Analysis Populations Four analysis populations have been defined for this trial: Safety analysis set: defined as all patients administered any randomized treatment. Full analysis set (FAS): defined as all patients in the Safety Set who have a valid baseline efficacy assessment. Patients will be analyzed according to randomized treatment (Part 1) or overall patients (otherwise). Per protocol (PP) set: defined as all patients in the FAS without any major protocol deviations. Patients will be excluded from the PP set if they do not receive both treatments and/or do not have efficacy data available to evaluate the primary endpoint for both treatments. Pharmacokinetic Set (PK): defined as all patients in the Safety Set who have at least 1 measurement with quantifiable plasma concentration of dasiglucagon. Efficacy Analyses The primary analyses of the primary and the key secondary endpoint will be based on the FAS. Supportive analyses of the primary endpoint will be based on the PP. *Primary Endpoint* *Part 1, Day 1 to 4* The mean IV GIR in the last 12 hours of each treatment period will be calculated as a weighted mean across the 12 hours, taking the actual time periods between the measurements into account (corresponding to calculating the area under the curve (AUC) and dividing it by the length of the time period, i.e., weighted mean IV GIR). The IV GIR endpoint is expressed as glucose in mg/kg/min, i.e., data reported from different concentrations of the glucose used for infusion will be transformed to this standardized unit prior to analysis. The primary analysis is defined by the estimand based on the treatment policy (de-facto) strategy, where the actual GIR measurement reported irrespective of adherence to treatment or use of subsequent therapy is used. The reduction in weighted mean IV GIR, as determined as the difference in weighted mean IV GIR between placebo and dasiglucagon, will be estimated. The weighted mean IV GIR will be analyzed using a mixed-model regression approach, with treatment and period as fixed effects and patient as a random effect. The 2-sided 95% confidence interval (CI) for the treatment difference will be calculated from the mixed regression model.

	Handling of missing data and sensitivity analyses are defined in the body of the protocol. *Key Secondary Endpoint* *Part 1, Day 1 to 4* The key secondary endpoint of total amount of gastric carbohydrates administered will be analyzed by using a mixed-model regression approach, with treatment and period as fixed effects and patient as a random effect. <u>Safety Analyses</u> Adverse events will be coded using the latest version of the Medical Dictionary for Regulatory Activities (MedDRA). Adverse events that begin after the first dose of trial drug will be defined as treatment emergent. The overall incidence of AEs will be displayed by system organ class, preferred term, and treatment. The incidence of AEs will also be presented by severity and by relationship to the trial drug. Vital signs, clinical laboratory measures (including hematology, biochemistry, and incidence of anti-drug antibodies [ADAs]), 12-lead electrocardiograms (ECGs), echocardiography, physical examinations, and local tolerability data will be summarized by treatment, where applicable. Out-of-range safety endpoints may be categorized as low or high, where applicable. Safety results will be summarized by treatment received within treatment period and part and by trial visit, if applicable. A formal statistical analysis plan (SAP) will be prepared to provide further details on the methods for statistical analysis. No interim analysis is planned.
SAMPLE SIZE DETERMINATION	A total of 12 patients will be randomized in this crossover trial on the basis of the following considerations: The GIR results from 40 infants treated with IV glucagon for 24 hours published in the JIMD Research Report "The Effect of Continuous Intravenous Glucagon on Glucose Requirements in Infants with Congenital Hyperinsulinism" (Hawkes et al 2019). "Overall there was a statistically significant reduction in the median (IQR) GIR during the 24h following initiation of continuous glucagon infusion compared to 24 h before initiation (18.5 (12.9, 22.8) to 11 (6.6, 17.5) mg/kg/min…" This trial is powered to detect an effect in GIR for dasiglucagon after 48 hours compared to placebo of at least this effect size. Based on the longer infusion and titration of dasiglucagon in this trial, this is considered a conservative approach, allowing for some uncertainty when translating the published data into a clinical trial setting. Using unpublished individual patient data from the above-referenced study, the difference in GIR between the 2 treatment groups is assumed to follow a normal distribution. Assuming the true mean difference is 7.5 mg/kg/min with a standard deviation of differences of 7.36, the trial will have 89% power using a one-sample t-test with 12 patients randomized to receive either placebo first and then dasiglucagon or vice versa.
TRIAL AND TREATMENT DURATION	The sequence and maximum duration of the trial periods will be as follows: 1. Screening Period: up to 28 days, including a minimum 24-hour run-in period 2. Part 1 (2x48-hour period: double-blind, randomized, placebo-controlled): 96 hours 3. Part 2 (Open-label Active Treatment Period): 21 days 4. Follow-up Period: Patients who do not enter the extension trial will have a Follow-up Visit 28 days after their last dose of trial drug The maximum trial duration for each patient is 81 days. The maximum treatment duration for each patient is 25 days.

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5. LIST OF ABBREVIATIONS

ADA	anti-drug antibody
A&E	accident and emergency
AE	adverse event
AESI	adverse event of special interest
ANOVA	analysis of variance
AUC	area under the curve
$AUC_{0\text{-}inf}$	area under the plasma concentration-time curve from time zero to infinity
$AUC_{glucose}$	area over the glucose curve
CGM	continuous glucose monitoring
CHI	congenital hyperinsulinism
CI	confidence interval
C_{max}	maximum observed concentration
CRA	clinical research associate
CRF	case report form
CRO	contract research organization
CTR	clinical trial report
DMC	independent data monitoring committee
ECG	electrocardiogram
eCRF	electronic case report form
EDC	electronic data capture
ER	emergency room
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GI	gastrointestinal
GIR	glucose infusion rate
GLMM	generalized linear mixed-model
H	hypothesis
ICF	informed consent form
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IEC	independent ethics committee

IM	intramuscular(ly)
IRB	institutional review board
ISF	investigator site file
IV	intravenous(ly)
MedDRA	Medical Dictionary for Regulatory Activities
mTOR	mechanistic target of rapamycin
NG	nasogastric
NME	necrolytic migratory erythema
PD	pharmacodynamic(s)
PG	plasma glucose
PK	pharmacokinetic(s)
PT	preferred term
RSI	Reference Safety Information
SAE	serious adverse event
SAP	statistical analysis plan
SC	subcutaneous(ly)
SMPG	self-monitored plasma glucose
SpO_2	peripheral capillary oxygen saturation
SUSAR	suspected unexpected serious adverse reaction
t1/2	half-life
TMM	trial materials manual
Zealand	Zealand Pharma A/S

6. INTRODUCTION

6.1. Background and Rationale

Congenital hyperinsulinism (CHI) is a rare and challenging disorder in which β-cells in the pancreas secrete insulin irrespective of plasma glucose (PG) concentration, resulting in persistent and often severe hypoglycemia.[1] Congenital hyperinsulinism affects up to 1 in 50,000 newborns. It is typically diagnosed on the basis of signs and symptoms of hypoglycemia during the neonatal period or in infancy; however, the diagnosis may be made later in childhood. Mutations in several different β-cell genes have been described to cause CHI. Hypoglycemia that results from CHI is of particular concern because it is an important cause of brain injury in neonates, infants, and children with this disease, which leads to long-term neurological impairments.[1,2] Up to approximately 50% of children with CHI experience neurodevelopmental abnormalities caused by severe hypoglycemia that results from inadequate treatment and/or delays in diagnosis.[1,3] Severe brain damage is the consequence of severe hypoglycemia, which presents as coma and/or long-lasting epileptic seizures in neonates. Major intellectual disability is, therefore, most frequent in patients with neonatal initial onset, whereas hypoglycemia is usually less severe and brain damage less frequent in children with CHI diagnosed later in childhood.[1] Since symptoms and severity of hypoglycemia can vary and pose a diagnostic challenge in neonates, infants, and children with CHI, prompt recognition and treatment of hypoglycemia is critical to reduce the risk of long-term neurological consequences.

6.2. Current Treatment and Unmet Medical Need

Medical treatment for CHI is focused on chronic therapies to avoid hypoglycemia, as well as on rescue therapy during acute episodes of severe hypoglycemia. Available medical therapies (mainly diazoxide, octreotide, or glucagon alone or in combination with glucose infusion) are often inadequate and accompanied by inability to control PG, as reflected in a large proportion of patients requiring sub-total pancreatectomy.[4,5,6] With the exception of surgery for focal CHI, which is curative in the vast majority of patients, sub-total pancreatectomy for diffuse CHI has substantial inadequacies. A recent retrospective chart review[7] showed that 60% of patients who underwent near-total pancreatectomy had persistent hypoglycemia after surgery. Moreover, 96% had developed insulin-dependent diabetes within 11 years after surgery.

First-line medical treatment of CHI is diazoxide, which is the only EU- and US-approved drug for treatment of hyperinsulinemic hypoglycemia. Diazoxide acts to open K_{ATP} channels of the pancreatic β-cells, thereby inhibiting insulin secretion. Unfortunately, many patients with CHI are resistant to diazoxide because of mutations in the genes encoding the K_{ATP} channel of the pancreatic β-cells.[6] For those who respond to diazoxide treatment, the more common side effects comprise hypertrichosis, fluid retention, and gastrointestinal (GI) symptoms; however, side effects are usually not severe. In diazoxide nonresponders, second-line (and off-label) treatment is a somatostatin analog (octreotide or lanreotide [long acting]), which (among other effects) inhibits secretion of insulin and glucagon from the pancreas and suppresses GLP-1 secretion. Factors that limit their use comprise tachyphylaxis, as well as possible side effects, including necrotizing enterocolitis, gallstones, and hepatitis.[6]

The glycogenolytic effect of glucagon and its ability to increase PG levels has been confirmed in children with CHI or neonatal hypoglycemia.[8,9] Administration of marketed glucagon (intravenous [IV] or subcutaneous [SC] injection) is often used in the diagnostic phase of CHI to

stabilize patients before surgery or initiation of other medical treatments.[10] In a retrospective review of 55 neonates with resistant hypoglycemia, native glucagon IV infusion achieved a median rise in plasma glucose of 45 mg/dL (2.5 mmol/L) after an hour of administration, regardless of the underlying cause for the persistent neonatal hypoglycemia.[9] Furthermore, glucagon is administered as single SC doses to treat severe hypoglycemic episodes. While IV administration of glucagon to patients with CHI is used short-term in the hospital setting, e.g., before pancreatectomy,[2,10,11] long-term glucagon treatment is challenging since currently available glucagon products are unstable and form fibrils within hours after reconstitution.[12] Catheter obstruction and occlusion because of glucagon instability were observed daily to 2 to 3 times weekly in a retrospective review of 9 patients with CHI receiving continuous SC infusion of glucagon for weeks or months.[11] In another series of patients, 60% of the patients treated with short-term SC glucagon experienced catheter occlusion.[6] Instability of currently available glucagon products and the associated risk of dosing errors contributing to hypoglycemic events is a major barrier for using glucagon for long-term treatment of patients with CHI.

When successfully administered, long-term home treatment with subcutaneously infused glucagon over extended periods (years) has been beneficial, with a potentially good safety profile as compared to diazoxide and octreotide.[6,11,13]

6.3. Dasiglucagon for the Treatment of Congenital Hyperinsulinism

6.3.1. Dasiglucagon

Dasiglucagon is a peptide analog of human glucagon in development for the treatment and prevention of hypoglycemia in patients with diabetes mellitus and CHI via SC or intramuscular (IM) administration. Dasiglucagon is a stable analog of glucagon that has been specifically designed to overcome the issues with fibril formation and instability in solution observed with marketed glucagon products. Compared to native human glucagon, dasiglucagon also comprises 29 amino acids. As a result of chemical modifications (7 amino acid substitutions compared to human glucagon), the pronounced tendency of glucagon to form fibrils and aggregate has been effectively prevented in dasiglucagon. In addition, the chemical stability in aqueous media at physiological pH has been improved.

To support the use of dasiglucagon in the pump for this trial, compatibility/in-use studies have been performed with dasiglucagon 4 mg/mL in Roche Accu-Chek® Combo pump using the Accu-Chek® Spirit 3.15 mL cartridge system and the Accu-Chek® FlexLink infusion set. The studies support an in-use time for up to 6 days at 37ºC.

Dasiglucagon has been granted orphan drug designation by the European Commission on 20 June 2017 for the '*treatment of congenital hyperinsulinism*.' Furthermore, the FDA granted an orphan drug designation for the '*treatment of hypoglycemia in patients with congenital hyperinsulinism (CHI)*' on 10 August 2017.

6.3.2. Nonclinical Experience

The completed nonclinical pharmacology program has determined that dasiglucagon is a specific glucagon receptor agonist with comparable in vitro potency to glucagon, promoting a rapid onset of PG increase in both normoglycemic and insulin-induced hypoglycemic animals, similar to that of glucagon.

Results of the toxicity studies with dasiglucagon are comparable to what has been reported for glucagon. Those from chronic toxicity studies with dasiglucagon in rats and dogs are in line with the results of short-term toxicity studies, indicating that long-term treatment with dasiglucagon is safe and that the pharmacodynamic (PD) effects noted do not adversely affect organ function following chronic use.

6.3.3. Clinical Experience

Dasiglucagon is being developed to manage patients with CHI 1) as an initial short-term therapy to stabilize PG levels and reduce glucose infusion needs, and 2) as a long-term treatment to help maintain euglycemia. Dasiglucagon has not previously been studied in patients with CHI. Clinical experience with dasiglucagon comes from the following studies in healthy subjects and type 1 diabetics.

The safety, tolerability, pharmacokinetic (PK), and PD characteristics of dasiglucagon were investigated in 2 phase 1 trials, 3 phase 2 trials, and 2 phase 3 trials.

ZP4207-14013 was a phase 1 randomized, double-blind, 2-part, single-dose trial designed to evaluate the pharmacokinetics, PD, safety, and tolerability of dasiglucagon compared to GlucaGen®. Part 1 consisted of SC and IM administration of single ascending doses of dasiglucagon in 64 adult healthy volunteers. Part 2 consisted of 20 adult patients with type 1 diabetes using a crossover design with IM administration of 0.7 mg dasiglucagon. GlucaGen 1.0 mg was used as a comparator in both parts of the trial.

ZP4207-15007 was a phase 1 randomized, placebo-controlled, double-blind, multiple-dose trial designed to evaluate the PK, PD, safety, and tolerability of repeated doses of dasiglucagon SC in 24 healthy adult volunteers. The trial included 3 dose cohorts of 8 healthy volunteers each. In each cohort, patients were randomly assigned in a 3:1 ratio to receive 5 repeated daily doses of 0.1, 0.3, and 1.0 mg dasiglucagon (n=6) or placebo (n=2).

After the 2 phase 1 trials were completed, the composition of the dasiglucagon formulation was optimized with respect to its excipients (buffer, tonicity adjusting agent, and stabilizer). The PK and PD properties, safety, and tolerability of the optimized formulation were evaluated in the phase 2 trials.

ZP4207-15126 was a phase 2 randomized, double-blind, single-dose trial designed to evaluate the PK, PD, safety, and tolerability of a single SC dose of dasiglucagon in the optimized formulation (0.1, 0.3, 0.6, and 1.0 mg) or GlucaGen (0.5 and 1.0 mg) in 58 adult patients with type 1 diabetes.

ZP4207-16051 was a phase 2 open-label, randomized, crossover trial designed to evaluate the feasibility of using dasiglucagon 1 mg/mL SC in conjunction with insulin in a pump setting using an iLet algorithm as part of an automated dual hormone pump delivery system in 12 patients with type 1 diabetes. The primary objective was to evaluate the safety and tolerability of dasiglucagon compared to marketed glucagon (Lilly Glucagon™). The trial also aimed to demonstrate the functionality and accuracy of the iPhone-based bionic pancreas in a controlled,

clinical setting. Patients were randomly assigned to two 1-day treatment arms: dasiglucagon or Lilly Glucagon in the iPhone-based bionic pancreas.

ZP4207-16098 was a phase 2 randomized, crossover, double-blind trial designed to evaluate the safety, tolerability, PK, and PD of dasiglucagon in 23 patients with type 1 diabetes using a 4 mg/mL formulation of dasiglucagon (0.03 mg, 0.08 mg, 0.2 mg, and 0.6 mg) compared to an active comparator, Lilly Glucagon.

ZP4207-16136 was a phase 3 randomized, double-blind parallel-group safety trial designed to evaluate the immunogenicity of dasiglucagon compared to GlucaGen in 111 adult patients with type 1 diabetes.

ZP4207-16137 was a phase 3 randomized, double-blind, parallel-group trial designed to confirm the clinical efficacy and safety of a single dose of dasiglucagon (0.6 mg) for the rescue treatment of hypoglycemia compared to placebo and with reference to GlucaGen (1 mg) in 168 adult patients with type 1 diabetes.

Overall, no safety concerns were observed for dasiglucagon at the doses investigated in these clinical trials. The most frequently reported adverse event (AE) in each trial was nausea, which is a known side effect after administration of glucagon, and appeared at a similar frequency to marketed glucagon, which was used as active comparator in some of the trials. No local tolerability issues were reported. Injection site reactions were observed only sporadically after administration with dasiglucagon, placebo, or marketed glucagon in trials with SC or IM administration, and all events were mild and transient. No treatment-induced or treatment-boosted anti-drug antibodies (ADAs) were noted in any of the reported trials, except for trial ZP4207-16098, in which 1 patient tested positive for both anti-dasiglucagon and anti-glucagon antibodies. Due to the crossover nature of this trial, the induction of ADAs could not be associated with a specific treatment.

Dose proportionality for dasiglucagon PK using the 1 mg/mL formulation was confirmed in the clinical trials, with doses ranging between 0.1 and 1.0 mg, characterized by a fast absorption with a peak plasma concentration obtained after 35 minutes. The median time to maximum concentration was later for dasiglucagon compared to marketed glucagon (GlucaGen) (35 vs 20 minutes). In the phase 2 trial ZP4207-15126, doses of 0.3 mg dasiglucagon and 0.5 mg GlucaGen and also 0.6 mg dasiglucagon and 1.0 mg GlucaGen were similar with regard to maximum observed concentration (C_{max}). The total exposure in terms of area under the concentration-time curve (AUC) from time zero to infinity ($AUC_{0\text{-}inf}$) was consistently higher for dasiglucagon compared to GlucaGen. This was likely due to a higher bioavailability of dasiglucagon since nonclinical data consistently show that the bioavailability of dasiglucagon is higher compared to marketed glucagon formulations. For the 4 mg/mL formulation, dose-proportionality of dasiglucagon doses between 0.03 and 0.6 mg was indicated for $AUC_{0\text{-}240min}$ and $AUC_{0\text{-}inf}$ in trial ZP4207-16098. Pharmacokinetic parameters were all similar for dasiglucagon administration under both euglycemic and hypoglycemic conditions. An approximately 30 to 40% greater exposure in terms of $AUC_{0\text{-}inf}$ and C_{max} was observed with the 4 mg/mL formulation as compared with the 1 mg/mL formulation.

In the phase 2 trial ZP4207-15126, all patients achieved a PG level of at least 70 mg/dL (3.9 mmol/L) at all dose levels of dasiglucagon after insulin-induced hypoglycemia, as well as an increase in PG by at least 20 mg/dL (1.1 mmol/L) within 30 minutes postdose. The PD responses of 0.3 mg of dasiglucagon and 0.5 mg of marketed glucagon (GlucaGen) were similar in the first 30 minutes postdose, as were the PD responses of 0.6 mg of dasiglucagon and 1.0 mg of

GlucaGen. The PD response over the entire observation time (0-360 minutes) was significantly greater after dosing with dasiglucagon than with GlucaGen for all prespecified dose comparisons. This was likely an effect of the higher total drug exposure ($AUC_{0\text{-}inf}$) of dasiglucagon mentioned previously.

In the phase 3 trial ZP4207-16137, the primary endpoint was time to plasma glucose recovery after insulin-induced hypoglycemia, which was defined as first increase in plasma glucose of ≥20 mg/dL (1.1 mmol/L) from baseline. Superiority was shown for dasiglucagon relative to placebo, with a median time to plasma glucose recovery of 10 versus 40 minutes. The median time to recovery for GlucaGen was 12 minutes. All patients in the dasiglucagon and GlucaGen treatment arms recovered from insulin-induced hypoglycemia within 30 minutes. All patients but one receiving dasiglucagon and one receiving GlucaGen had recovered within 20 minutes (dasiglucagon: 99% and GlucaGen: 98%). At 15 minutes, 99% of patients receiving dasiglucagon and 95% of those receiving GlucaGen had recovered. At 10 minutes, 65% of patients receiving dasiglucagon and 49% of those receiving GlucaGen had recovered.

No major differences in PD responses were observed between dasiglucagon doses under euglycemic or hypoglycemic conditions in phase 2 trial ZP4207-16098. Under euglycemic conditions, all patients achieved an increase in PG of at least 20 mg/dL (1.1 mmol/L) within 30 minutes postdose with 0.08 mg dasiglucagon and above, while this target was reached only for 0.2 mg of marketed glucagon (Lilly Glucagon). Under hypoglycemic conditions, dasiglucagon met the target at doses of 0.2 mg and above. The PD response over the entire observation time of 240 minutes was significantly higher with dasiglucagon than with Lilly Glucagon at the same doses. In the bionic pancreas feasibility trial ZP4207-16051, results demonstrated comparable autonomous glycemic control with dasiglucagon compared to Lilly Glucagon. This was despite the stressed conditions of the trial in terms of fasted patients being on up to twice their basal insulin rate to stimulate glucagon use during the 8-hour test, and inclusion of a structured exercise period. With dasiglucagon, patients obtained a glucose value within target (70-180 mg/dL [3.9-10.0 mmol/L]) 70.9% of the time vs 65.6% with Lilly Glucagon.

6.3.4. Literature Data

In a retrospective review of 223 cases of diffuse or focal CHI, glucagon was reported to be used in 55% of patients with diffuse CHI and in 31% of patients with focal CHI.[10] In an observational trial of 55 newborns who received glucagon because of hypoglycemia after birth, applied doses were mainly in the range of 0.5 to 1.0 mg/day, and results indicated an increase in PG from a mean of 36.3 md/dL to a mean of 93.0 mg/dL, observed within 4 hours after the start of glucagon infusion.[9] The frequency of hypoglycemic episodes was significantly reduced, and no further episodes of severe hypoglycemia were observed.

In another retrospective chart review of 40 children with CHI who received continuous IV glucagon for prevention of hypoglycemia,[14] a median (inter quartile range) glucagon dose of 205 (178, 235) mcg/kg/day over a median duration of 5 (3, 9) days enabled a glucose infusion rate (GIR) reduction from 18.5 (12.9, 22.8) to 11 (6.6, 17.5) mg/kg/min per 24 h ($p < 0.001$), and reduced hypoglycemia frequency from 1.9 (1.3, 2.9) to 0.7 (0.3, 1.2) episodes per day.

The long-term use of glucagon in patients with CHI is limited by the instability of marketed glucagon after reconstitution. A literature review on the long-term medical treatment of CHI revealed that only 1% of 619 patients identified received glucagon as part of their medical management.[6] A retrospective review of 9 children with CHI who received continuous SC

infusion of glucagon for weeks or months showed that introduction of glucagon allowed the reduction or discontinuation of central glucose infusion in all patients.[11] Six of 9 patients were discharged with continued glucagon therapy that their parents were able to continue without further symptomatic hypoglycemia, convulsions, or unconsciousness. In 3 children, glucagon therapy was continued for 1 to 4 years, which led to stable euglycemia.

The data reported on marketed glucagon use in patients with CHI indicate that continuous SC infusion of a glucagon agonist could provide therapeutic benefit to patients by stabilizing PG levels and reducing the frequency of hypoglycemic episodes.[5,6,9,10,11]

6.3.5. Anticipated Medical Benefit of Dasiglucagon in the Treatment of CHI

With its physio-chemical stability in liquid formulation, dasiglucagon could provide significant added benefit in the treatment of CHI relative to currently marketed glucagon by enabling long-term reliable IV infusion. Long-term subcutaneous infusion of dasiglucagon through a pump may be an attractive alternative or addition to diazoxide and octreotide since it may reduce the dependency on intensive nutritional support, both IV and gastric (NG tube or gastrostomy), whilst maintaining euglycemia by harnessing physiological mechanisms for combating hypoglycemia. Furthermore, if long-term euglycemia is achieved with medical therapy, pancreatectomy for the treatment of diffuse CHI could eventually be avoided. In one cohort of nonsurgically treated children, the mean clinical remission rate was 5 (1.5-12) years for diffuse CHI.[5] This suggests that a significant proportion of infants with CHI could avoid surgery if medical treatment allowed for the effective long-term control of hyperinsulinism.

The target population in this trial is children with CHI who are especially challenging to treat, and who need continuous IV glucose infusion to prevent or treat hypoglycemia, necessitating ongoing hospitalization. These children have limited options in terms of other treatments, and represent very high unmet medical need group within already ultra-rare CHI population.

6.3.6. Anticipated Risks of Dasiglucagon in the Treatment of CHI

So far, there is no previous clinical experience with dasiglucagon SC infusion for the treatment of CHI. The following anticipated risks are derived from other clinical trials with dasiglucagon conducted in other indications, from marketed glucagon labelling information, and from native glucagon's physiological effects.

In clinical trials with dasiglucagon conducted up to now, the most frequent treatment-related AEs were nausea and vomiting, followed by headache.

Glucagon exerts positive inotropic and chronotropic effects and may, therefore, cause tachycardia and hypertension. Transient increases in heart rate and hypertension have been observed in clinical trials with dasiglucagon. Additionally, several episodes of hypotension and bradycardia have been noted in the clinical program, often in association with nausea and/or vomiting.

Accidental overdose may occur due to inappropriate handling of the infusion pump or due to pump malfunction. Overdose may result in nausea, vomiting, inhibition of GI tract motility, short-term increase in heart rate or blood pressure, and/or hypokalemia. Symptomatic care for nausea and vomiting, as well as monitoring of heart rate, blood pressure, and hypokalemia is advised.

Injection site reactions are seen with many injectable peptides. Injection site reactions have been reported across trials, with similar frequency in all treatment groups (dasiglucagon, marketed

glucagons, and placebo), irrespective of dose. The injection site reactions were generally mild and transient.

Overall, the clinical trial data indicate that dasiglucagon has a low risk for induction of ADAs. Out of more than 350 doses of dasiglucagon administered to more than 200 patients, only 1 low-titer transient ADA incident has been reported to date, in a patient following administration of 11 SC doses of dasiglucagon or Lilly Glucagon ranging from 0.03 to 0.6 mg, with low binding and low *in vitro* neutralizing capability. No apparent clinical effects on PK, PD, or AEs were noted. Due to the crossover trial design, the ADA induction could not be ascribed to a specific treatment. Based on the current clinical experience and the product characteristics, the risk of dasiglucagon inducing an ADA response is considered low.

Administration of glucagon or dasiglucagon may be associated with a risk of allergic reactions similar to those observed for other therapeutic peptides or proteins.

Data on the risks of chronic administration of dasiglucagon are not available. From sporadic reports of extended SC/IV infusion of marketed glucagon and in glucagonoma patients,[15] sustained exposure to high levels of glucagon may lead to development of the skin condition necrolytic migratory erythema (NME), a highly specific migrating, erythematous rash with predilection for perioral, perianal, and lower leg distribution.[11]

For further information on risks, please refer to the current version of the investigator's brochure (IB).

6.3.7. Summary of Potential Benefits and Risks

As with all treatment interventions, the anticipated benefits to trial patients should be balanced against the potential risks. The accumulated experience from nonclinical studies and clinical trials with dasiglucagon supports that dasiglucagon is a specific glucagon receptor agonist and is well tolerated. Glucagon and its analogs belong to a well-known drug class with known mode of action. The clinical investigators involved in the trial will all have had experience with use of glucagon in patients with CHI.

The investigator will inform the patients/parent(s)/guardian of the potential risks of dasiglucagon treatment and other trial-related procedures before they enter the trial. The investigator must become familiar with all sections of the dasiglucagon IB before the start of the trial.

In this trial population, the major and clinically relevant benefit is the expected reduction and eventually elimination of need for IV glucose, together with the reduction in gastric nutritional interventions while avoiding hypoglycemia. The reduced volume of nutritional interventions should limit the risk of volume overload. Furthermore, by removing the invasive methods of nutrition, the children should better retain or re-establish orality – the intake of nutrients by mouth.

In summary, with its marked improvements in stability in solution and solubility in aqueous media compared to currently marketed glucagon products, dasiglucagon is expected to have significant clinical benefits in the treatment of CHI and to substantially reduce the disease burden in these patients. This includes discharge from the hospital by enabling convenient and reliable long-term treatment via a pump device in a home setting, together with the potential to delay and ultimately avoid pancreatectomy and its related exo/endocrine complications, particularly the development of insulin-dependent diabetes.

Overall, the benefit to risk ratio for patients entering the ZP4207-17103 trial is considered acceptable.

7. OBJECTIVES AND ENDPOINTS

7.1. Objectives

7.1.1. Primary Objective

To evaluate the efficacy of dasiglucagon in reducing glucose requirements in children with persistent CHI requiring continuous IV glucose administration to prevent/manage hypoglycemia.

7.1.2. Secondary Objectives

To evaluate the safety and tolerability of dasiglucagon administered as an SC infusion in patients with CHI.

7.2. Endpoints

7.2.1. Primary Endpoint

Part 1 (Day 1 to 4)

- Mean IV GIR in the last 12 hours of each treatment period during Part 1 (dasiglucagon or placebo administration)

7.2.2. Key Secondary Efficacy Endpoint

Part 1 (Day 1 to 4, for each 48-hour treatment period)

- Total amount (g) of carbohydrates administered (regardless of the route) per day.

7.2.3. Secondary Efficacy Endpoints

Part 1 (Day 1 to 4, for each 48-hour treatment period)

- Mean IV GIR for each 48-hour treatment period during Part 1 (dasiglucagon or placebo administration)
- Mean IV GIR below 10 mg/kg/min in the last 12 hours of each treatment period during Part 1 (yes/no) (dasiglucagon or placebo administration)

Part 2 (Day 5 to 25, assessed from the start of treatment in part 2)

- Time to complete weaning off IV GIR.
- Hypoglycemia event rate, defined as number of hypoglycemic events (PG <70 mg/dL or 3.9 mmol/L), as detected by SMPG.
- Clinically significant hypoglycemia event rate, defined as number of events <54 mg/dL (3.0 mmol/L), as detected by SMPG.
- Time to actual hospital discharge.
- Time to pancreatic surgery (sub-total or total pancreatectomy).
- Total amount (g) of carbohydrates administered (regardless of the route) per day, together with amounts (g) of carbohydrates administered per day:
  - via IV glucose infusion or bolus (not as part of total parental nutrition),
  - as part of total parenteral nutrition (if applicable),

  - via oral route, and
  - via NG tube or gastrostomy.
- CGM percent time in range 70-180 mg/dL (3.9-10.0 mmol/L).
- CGM percent time in hypoglycemia (<70 mg/dL or 3.9 mmol/L).
- CGM percent time in clinically significant hypoglycemia (<54 mg/dL or 3.0 mmol/L).
- Rate of hypoglycemia episodes, defined as number of episodes <70 mg/dL (3.9 mmol/L) for 15 min or more, as measured by CGM.
- Rate of clinically significant hypoglycemia episodes, defined as number of episodes <54 mg/dL (3.0 mmol/L) for 15 min or more, as measured by CGM.
- Extent of hypoglycemia (area over the glucose curve [$AOC_{glucose}$] below 70 mg/dL [3.9 mmol/L]) as measured by CGM.
- Extent of clinically significant hypoglycemia ($AOC_{glucose}$ below 54 mg/dL [3.0 mmol/L]) as measured by CGM.
- CGM percent time in hyperglycemia (>180 mg/dL or 10.0 mmol/L).

7.2.4. Safety Endpoints

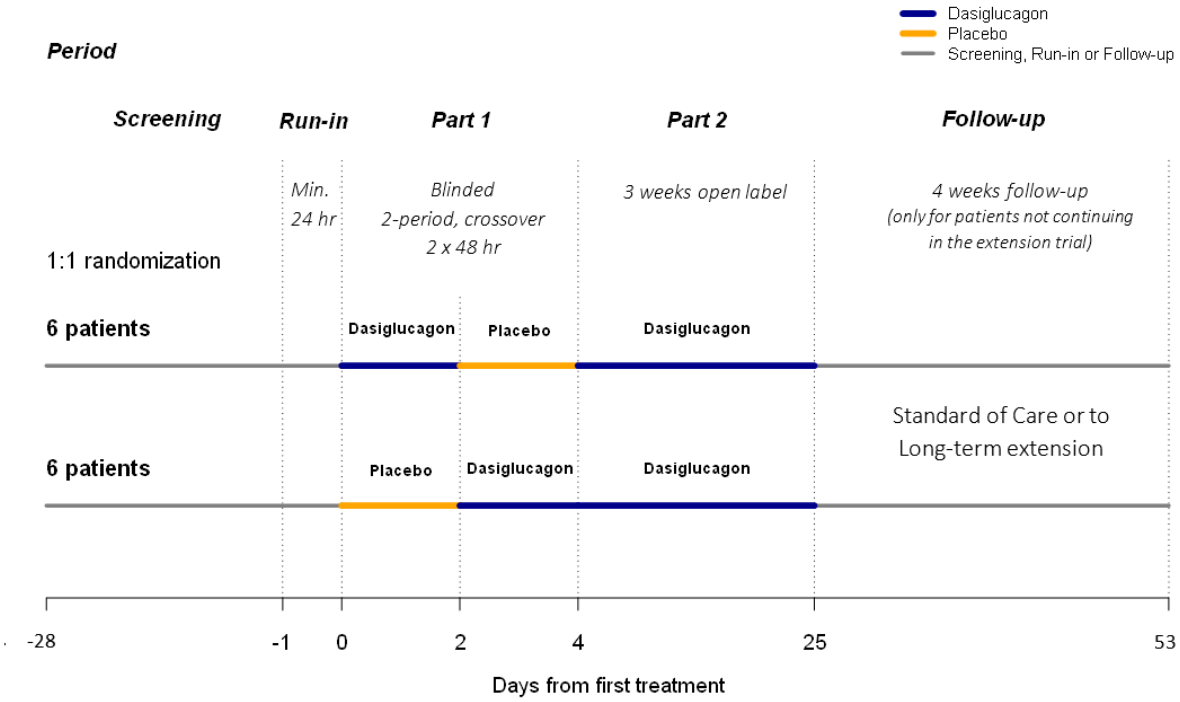
By Part 1 or 2 of the trial:

- Adverse events
- Changes in clinical evaluations:
  - Vital signs
  - Physical examination
  - 12-lead ECG
  - Echocardiogram
- Changes in clinical laboratory assessments:
  - Hematology
  - Biochemistry
- ADA

8. TRIAL DESIGN

8.1. Overall Trial Design and Plan

This is a combined phase 2 and 3, randomized, multinational trial to evaluate the efficacy and safety of individually titrated dasiglucagon in children ≥7 days and <1 year of age who have been diagnosed with CHI, comprising 2 parts, a crossover (2 periods, 48 hours each), double-blind, placebo-controlled Part 1, and an open-label, single-arm Part 2 of 21 days (Figure 1).



Abbreviations: Min = minimum

Figure 1 Design of Trial ZP4207-17103

After screening, eligible patients will complete a minimum 24-hour run-in period to confirm the IV glucose randomization requirement (≥10 mg/kg/min). All non-nutritional carbohydrates and carbohydrate fortification of feeds are prohibited during this 24-hour run-in period. After the run-in period, patients will be randomly assigned in a double-blind fashion to receive dasiglucagon or placebo for 48 hours, after which they will be crossed over to the other trial treatment for an additional 48 hours. At the time of crossover, the trial drug will be initiated from the starting dose of 10 µg/hr, and IV GIR will be set to the rate obtained at the end of the run-in period and titrated accordingly.

There will be no washout between the 2 periods in Part 1 to limit the length of time in which patients are reliant on IV GIR as their only means of preventing/treating hypoglycemia. This is deemed acceptable because:

- The half-life ($t_{½}$) of dasiglucagon is short (~30 minutes, for the 0.6 mg dose, and potentially shorter for lower doses, ~22 minutes) relative to the 48-hour duration of each crossover

period. Moreover, for the primary endpoint, only the last 12 hours of the 48-hour period will be used for endpoint assessment.

- Assuming a positive effect of dasiglucagon, the absence of washout is conservative with respect to key secondary endpoints since a potential carry over effect of dasiglucagon in Period 2 is attributed to placebo.

During Part 1, the trial drug and IV GIR should be adjusted according to the protocol-specified algorithm (see Section 10.2). Non-nutritional carbohydrates and/or carbohydrate fortification of feeds during Part 1 is only allowed if the maximum tolerable volume and concentration of IV glucose for the patient is reached, and should be limited to the minimum needed to ensure the patient's safety (Section 9.5). All feedings (administered as parenteral nutrition, by NG tube, gastrostomy, or normal route), will be recorded during this period. Safety assessments will be performed daily after initiation of the trial drug (active or placebo).

After Part 1, patients will continue in open-label Part 2 to receive dasiglucagon for 21 days. Additional CHI treatments can be introduced during Part 2 if needed, if up-titration of dasiglucagon is not possible due to undesirable side effects or if the maximum dose level (70 µg/hr) has been reached. Gradual transfer from IV glucose to oral and gastric carbohydrates should be initiated in Part 2, enabling weaning of IV glucose and hospital discharge. Patients will continue to be hospitalized until IV GIR is weaned off; however, as soon as local site criteria for discharge are met, patients can be discharged to continue the treatment period at home. Visits are planned on Day 11 and Day 18 in Part 2. These visits can be converted to telephone visits if appropriate, at the investigator's discretion. On Day 25, the End of Treatment Visit will take place, and based on the investigator's confirmation of continued positive benefit-risk balance, patients will be offered the opportunity to enter the long-term extension trial (Trial ZP4207-17106) to continue dasiglucagon treatment. Patients who do not enter the long-term extension trial will have a Follow-up Visit 28 days after their last dose of trial drug.

8.2. Discussion of Trial Design

The initial 2x48-hour treatment period (Part 1) is double-blind and placebo-controlled to avoid bias in the assessment of the effect of dasiglucagon on GIR. A crossover design has been chosen to limit the influence of inherent variability of the population, which allows for recruitment of fewer patients compared to a parallel-group design, reflecting the ultra-rare status of the disease.

All patients will receive dasiglucagon in open-label Part 2. According to International Council for Harmonisation (ICH) guidelines, for the conduct of clinical trials in rare diseases, where the anticipated likelihood of considerable benefit is high, such design has some potential efficiencies because all patients will be exposed to test drug, which is of particular importance in rare diseases.[16]

8.3. Trial Sites

The trial will take place at 5 to 7 sites in the US and Europe that are experienced in the treatment of CHI. A total of 12 patients with CHI is planned to be randomized and exposed to the trial product. Patients who withdraw prematurely will not be replaced.

8.4. Point of Contact

A point of contact will be identified to provide information to each patient's parent(s)/guardian about where to obtain information on the trial, the patient's rights, and whom to contact in case of trial-related injury. This information will be provided in the patient information and informed consent form (ICF).

9. PATIENT POPULATION

9.1. Selection of Trial Population

A screening log of potential trial candidates must be maintained at each trial site.

9.2. Trial Entry Criteria

9.2.1. Inclusion Criteria

A patient will be eligible for trial participation if he or she meets all of the following criteria:

1. CHI diagnosis established based on the following:
   a. Hyperinsulinemia: plasma insulin above the limit of detection of the assay documented during an event of hypoglycemia, and/or
   b. Hypofattyacidemia: plasma free fatty acid <1.7 mmol/L, and/or
   c. Hypoketonemia: Beta-hydroxybutyrate <1.8 mmol/L, and/or
   d. Glycemic response: an increase in PG of >30 mg/dL (1.7 mmol/L) after 1 mg IV or IM glucagon administration
2. Male or female, age ≥7 days and <12 months at screening
3. Body weight of ≥2.0 kg (4.4 lbs.)
4. Continuous IV glucose requirement to prevent hypoglycemia
5. One or both parents* or guardians of the patient must provide signed informed consent before any trial related activity is performed. (*according to local regulations)

9.2.2. Exclusion Criteria

A patient will be excluded from the trial if he or she meets any of the following criteria:

1. Is suspected of having a transient form of CHI (e.g., transient hyperinsulinism due to maternal diabetes or perinatal stress)
2. Was born preterm below 34 weeks of gestational age
3. Presence of hypertension or hypotension, including circulatory instability requiring supportive medication or presence of pheochromocytoma
4. Known or suspected presence of severe brain damage
5. Evidence of metabolic, endocrine, or syndromic causes of hypoglycemia not due to hyperinsulinism
6. Use of systemic corticosteroids, e.g., hydrocortisone >20 mg/m^2 body surface area or equivalent within 5 days before screening

7. Prior use of lanreotide, sirolimus (mechanistic target of rapamycin [mTOR inhibitors]), anti-inflammatory biological agents, or other immune-modulating agents. Prior use of octreotide is allowed after a minimum of 48-hour washout before randomization.
8. Aspartate aminotransferase (AST) or alanine aminotransferase (ALT) >2.5 X the upper limit of normal (ULN), or estimated glomerular filtration rate (eGFR) <30 mL/min/1.73m^2 adjusted by a pediatric formula (e.g., Schwartz formula)
9. Any clinically significant abnormality identified on echocardiogram that in the opinion of the investigator would affect the subject's ability to participate in the trial
10. Known history of laboratory test results obtained before screening that show presence of HIV, hepatitis B surface antigen, hepatitis C antibody, or hepatitis A immunoglobulin M
11. Any recognized clotting or bleeding disorder
12. Previous administration of dasiglucagon (previously referred to as ZP4207)
13. Known or suspected allergy to the trial drug or related products
14. Previous participation (randomization) in the clinical program (Trials ZP4207-17103 or -17109)
15. Has participated in an interventional clinical trial (investigational or marketed product) within 30 days of screening, or plans to participate in another clinical trial, except for 18F-Dopa PET CT/MRI investigation (where performed as a part of a research trial), which is allowed for diagnosis of focal CHI
16. The use of prescription or non-prescription medications known to cause QT prolongation

9.2.3. Randomization Exclusion Criteria

A screened patient will not be randomized if:

1. Mean IV glucose requirement is <10 mg/kg/min to maintain glycemia above 70 mg/dL (3.9 mmol/L) during the previous 24 hours prior to randomization
2. Use of glucagon within 24 hours before randomization.
3. Use of additional enteral glucose within 24 hours before randomization. Patients should be transitioned to IV glucose infusions only at least 24 hours before randomization.
4. Is not sufficiently clinically stable on IV GIR only (± diazoxide, as applicable), in the opinion of the investigator, to undergo the placebo-controlled randomized Part 1 of the trial (2x48 hours).

The Randomization Visit may be rescheduled once if Randomization Exclusion Criterion 2 is met.

9.3. Premature Patient Withdrawal

Randomization day exclusion criteria are described in Section 9.2.3.

Patients' parent(s)/guardian 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. Every reasonable attempt should be made by the investigator to keep patients in the trial to obtain data for the primary endpoint in Part 1 and for safety follow-up even if trial treatment has been discontinued. However, patients must be withdrawn from the trial if their parent(s)/guardian withdraw consent to participate.

Investigators must attempt to contact patients' parent(s)/guardian who fail to attend scheduled visits by telephone or other means to exclude the possibility of an AE being the cause of withdrawal. Attempts to contact the patient must be documented. At least 3 phone calls and 3 written attempts to contact the patient will be made prior to considering them lost to follow-up. Should an AE be the cause of withdrawal, it must be documented, reported, and followed up as described in Section 12.3.

If a patient/parent(s)/guardian withdraws consent, the reason for withdrawal and the date of withdrawal will be recorded on the appropriate page of the electronic case report form (eCRF). Whenever possible and reasonable, the evaluations that were to be conducted at the completion of the trial should be performed at the time of premature withdrawal.

9.4. Treatment Discontinuation

To prevent missing data, patients should, to the extent possible, be kept in the trial; therefore, treatment discontinuation is often the preferred option in case of, e.g., substantial noncompliance with trial procedures or initiation of prohibited treatment that interferes with the efficacy and safety evaluation. If it is an investigator's decision to discontinue the patient's treatment, the investigator should, whenever possible, discuss the potential discontinuation of the treatment with the medical monitor. If the patient is discontinued from trial treatment by the investigator or by parent/guardian's decision, the reason for treatment discontinuation and the date of treatment discontinuation will be recorded on the appropriate page of the eCRF. The patient should be asked to continue in the trial by following the planned visit schedule and to have trial assessments performed according to the Schedule of Events. This is especially important during Part 1 of the trial where the primary endpoint is evaluated. As a minimum, the patient will be asked to attend the Follow-up Visit 28 days (± 3 days) after discontinuation of trial treatment.

9.5. Rescue Criteria

During Part 1, the IV glucose concentration and rate of infusion can be increased to avoid hypoglycemia. When the maximum (or maximum tolerable) dose of trial drug has been reached and the necessary amount of GIR to prevent hypoglycemia is likely to cause (in the investigator's opinion) a moderate or severe AE (e.g., volume overload), oral/gastric non-nutritional carbohydrates can be introduced to ensure the patient's safety whilst maintaining the maximum tolerable concentration and infusion rate of IV glucose.

During Part 2, patients are allowed to initiate and titrate oral/gastric non-nutritional carbohydrates, and initiate any CHI treatment.

9.6. Patient Replacement Criteria

Patients who withdraw from the trial prematurely will not be replaced. If a substantial number of patients withdraw prematurely, the sponsor will evaluate the need for developing replacement criteria.

Enrolled patients (defined as randomized) who are subsequently withdrawn from the trial may not re-enter. The patient number for a withdrawn patient will not be reassigned to another patient.

10. TREATMENTS

10.1. Identification of Investigational Product

Dasiglucagon Injection, 4 mg/mL will be supplied by the sponsor in a 3 mL vial containing 1 mL of drug product.

Matching placebo for dasiglucagon injection will be supplied by the sponsor in a 3 mL vial containing 1 mL of drug product.

Dasiglucagon and matching placebo will be provided in the form of solution for injection for subcutaneous administration through an infusion pump.

The trial drug product, as applicable, must be transferred from the vial to the Accu-Chek Spirit Cartridge. The amount of drug product dosed via the pump will vary among patients.

Cartridges and infusion sets should be filled and replaced as indicated in the instructions for use.

10.1.1. Packaging and Labeling

Trial drug products will be packaged and labeled by the sponsor.

Part 1 dispensing unit configuration: 3 vials containing dasiglucagon, 4 mg/mL or placebo for dasiglucagon, packed in an outer carton. The vials and carton will be packaged and labeled in local language without revealing the treatment.

Part 2 dispensing unit configuration: 3 vials containing dasiglucagon, 4 mg/mL, packed in an outer carton. The vials and carton will be packaged and labeled in local language, revealing the treatment.

The storage conditions for trial drug products will be described on the trial drug product label. The labels will supply no information about patients. Each dispensing unit (3 vials) will have a unique Dispensing Unit Number for drug allocation, drug accountability, and traceability purposes.

Labelling will be performed according to Annex 13 of the Good Manufacturing Practice guidelines of the European Commission, ICH Good Clinical Practice (GCP) guidelines, local laws, and regulations.

10.2. Treatments Administered

As long as the patient is receiving IV glucose, the PG will be measured and reviewed hourly using a hand-held PG meter. The IV GIR will be titrated to achieve glycemia of at least 70 mg/dL (3.9 mmol/L) (a minimum GIR will be established as the rate up-titrated after the patient drops at least once below 70 mg/dL [3.9 mmol/L]). When the patient is no longer on IV GIR, the PG will be checked according to local practice, but at least 3 times daily. The PG will be measured using the same trial-supplied hand-held PG meter during the entire trial.

Additionally, blinded CGM will be started 24 hours prior to randomization (using the Dexcom G4 or G6 system) and continued until the end of Part 2.

Dosing of dasiglucagon will approximate continuous infusion by delivering small doses at frequent intervals via the infusion pump. The adjustment of trial drug dosing is closely linked to the PG level achieved, which in turn will govern the IV GIR. In Part 1 the algorithm in Table 1 should be used.

The pump administers 0.000025 mL/dose ~ 0.1 µg/dose (4 mg/mL formulation):

- 10 µg/hr ~ 0.5 µg every 3 min
- 20 µg/hr ~ 1 µg every 3 min
- 30 µg/hr ~ 1.5 µg every 3 min
- 40 µg/hr ~ 2µg every 3 min
- 50 µg/hr ~ 2.5µg every 3 min
- 60 µg/hr ~ 3µg every 3 min
- 70 µg/hr ~ 3.5µg every 3 min

Table 1 Algorithm to Maintain Plasma Glucose

Plasma Glucose (mg/dL)	Plasma Glucose (mmol/L)	Action
<50	<2.8	Give 200 mg/kg of dextrose as a bolus and increase IV GIR by 2 mg/kg/min
50-59	2.8-3.3	Increase IV GIR by 2 mg/kg/min
60-69	3.3-3.9	Increase IV GIR by 1 mg/kg/min
70-80	3.9-4.4	No change
81-90	4.4-5.0	Reduce IV GIR by 0.5 mg/kg/min
91-100	5.0-5.5	Reduce IV GIR by 1 mg/kg/min
101-120	5.5-6.7	Reduce IV GIR by 1.5 mg/kg/min
>120	>6.7	Reduce IV GIR by 2 mg/kg/min

Abbreviations: GIR = glucose infusion rate; IV = intravenous

The IV GIR will be reviewed, evaluated, and adjusted (if indicated) every hour according to the above PG-driven algorithm to maintain a PG of >70 mg/dL (>3.9 mmol/L). Rechecks of PG are allowed.

Part 1 (Crossover, double-blind, randomized, placebo-controlled)

The starting dose of trial drug is 10 µg/hr at t=0. Every 2 hours (t=2, 4, 6, etc.), the dose will be increased by an additional 10 µg/hr until either:

- The patient is totally weaned off IV glucose, or
- Plasma glucose during the last 2 hours was constantly above 120 mg/dL (6.7 mmol/L), or
- IV GIR has not decreased despite 2 sequential dose increments of trial drug (in this situation, the dose of trial drug should be maintained until the IV GIR can be further decreased or until crossover or the end of Part 1), or
- The maximum dose of 70 µg/hr is reached, or
- AEs emerge that are considered to be related to dasiglucagon (e.g., nausea/change in feeding pattern or increased vomiting) that are limiting further dose escalation.

The 2-hour dose-adjustment interval will allow drug plasma levels to approach steady-state before the dose is further increased. The cumulative dose will not exceed 1.26 mg over the first 24 hours and 1.68 mg for each of the subsequent 24-hour periods.

At the time of crossover from the first 48-hour period to the second 48-hour period, the trial drug will again be titrated from the starting dose of 10 µg/hr, and IV GIR will be set to the rate obtained at the end of the run-in period and titrated accordingly.

Part 2 (Open-label)

After Part 1, patients will continue in open-label Part 2 to receive dasiglucagon for a further 21 days. Since treatment allocation during Part 1 remains blinded, all patients are required to initiate dasiglucagon dosing at 10 µg/hr in Part 2, while IV GIR should be started at the rate obtained at the end of the run-in period.

During Part 2, the dasiglucagon dose, the amount and route of administration of carbohydrates (IV GIR, parenteral nutrition, gastric carbohydrate infusions, and carbohydrates from oral feeds) can be adjusted at the discretion of the investigator. The PG should be monitored at least hourly to adjust the IV GIR as appropriate and to avoid hypoglycemia. Additional concomitant medications, including somatostatin analogs and/or sirolimus/mTOR inhibitors, may be introduced at the investigator's discretion if the maximum dose level of dasiglucagon (70 µg/hr) has been reached or if further up-titration is not possible due to undesirable side effects.

Adjustment of total carbohydrate administered (IV glucose, parenteral nutrition, carbohydrate infusions, and carbohydrates from oral feeds) should continue throughout this period according to local practice to maintain PG levels in the range of 70 mg/dL to 120 mg/dL (3.9-6.7 mmol/L).

The aim will be to obtain stable glucose levels with minimum rescue/hypoglycemia-preventative carbohydrates administered by invasive routes, and to encourage/optimize oral feeds, and achieve weaning off IV glucose. Regardless of their discharge status, all patients will be offered the opportunity to participate in the long-term safety extension trial (ZP4207-17106), providing the investigator attests to the positive benefit-risk balance of continued dasiglucagon treatment.

10.3. Trial Supplies

The device and ancillaries listed in the following table will be supplied by the sponsor throughout the trial. Trained trial personnel will train parent(s)/guardian on the use of the devices.

Instructions for the use of all these supplies will be provided in separate manuals.

Item	Name	Manufacturer
Pump	Accu-Chek Spirit Combo	Hoffman-La Roche AG, Basel, Switzerland
Cartridge	Accu-Chek Spirit 3.15 mL Cartridge system	Hoffman-La Roche AG, Basel, Switzerland
Infusion sets	Accu-Chek FlexLink Infusion set (Accu-Check UltraFlex Infusion set in US) and Accu-Chek Rapid-D Link Infusion set	Hoffman-La Roche AG, Basel, Switzerland
Infusion set inserter	Accu-Chek LinkAssist Insertion device (can be used with FlexLink & UltraFlex)	Hoffman-La Roche AG, Basel, Switzerland
SMPG	StatStrip Xpress2	Nova Biomedical, Waltham, MA, USA
CGM	Dexcom G4 Dexcom G6	Dexcom Inc., San Diego, CA, USA

The infusion pump system is CE-marked for the management of diabetes mellitus in persons requiring insulin, as prescribed by a physician. In this trial, the pump system is used outside of the CE-marked intended use since the pump system will be delivering dasiglucagon to patients with CHI. The PG meter is used as intended according to the CE mark, except for the use by a lay person in a home care setting. The CGM devices are used as intended according to the CE mark, except for the age group and the disease.

The pump, the SMPG, and the CGM will be labeled for use in an investigational trial.

10.4. Dispensing and Storage

The trial drug products supplied by the sponsor is to be dispensed exclusively to patients randomized in this clinical trial according to the instructions of this protocol and the pharmacy manual/trial materials manual (TMM). The investigator is responsible for dispensing the trial drug product according to the dosage scheme. Trial drug products will be allocated using an interactive automated web response system (IWRS), according to Section 10.5.

Dasiglucagon and placebo for dasiglucagon injection must be stored at 2–8°C in a refrigerator.

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

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

The investigator must contact the clinical research associate (CRA) in case of temperature deviations outside the acceptable range.

Please see the pharmacy manual/TMM for additional information on handling of the trial drug.

The investigator must maintain adequate records showing the receipt, dispensing, return, or other disposition of the trial drug, including the date, quantity, batch or code number, and identification of patients (patient number) who received the trial drug. The investigator will not supply the trial drug to any person except subinvestigators, designated trial personnel, and patients in this trial. The trial drug may not be relabeled or reassigned for use by other patients. If any of the trial drug is not dispensed, is lost, stolen, spilled, unusable, or is received in a damaged container, this information must be documented and reported to the sponsor and the appropriate regulatory agencies as required.

10.5. Method of Assigning Patients to Treatment Groups

For Part 1, patients will be randomly assigned 1:1 in a double-blind fashion to 1 of 2 sequences:

- Dasiglucagon 4 mg/mL for 48 hours, followed by placebo for dasiglucagon for 48 hours, or
- Placebo for dasiglucagon for 48 hours, followed by dasiglucagon 4 mg/mL for 48 hours.

Randomization will be performed using a block randomization scheme stratified by region (US/non-US). The randomization scheme will be generated prior to the initiation of the trial by an independent statistician/programmer who will not be a member of the trial team; investigators will not be aware of the block size of the randomization scheme.

Patients will be randomly assigned to a trial treatment sequence using an IWRS that has been validated for the intended use under the International Society of Pharmaceutical Engineers Good automated manufacturing practice guidelines, 21CFR Part 11 (FDA regulation for Electronic Records and Electronic Signatures), and the ICH Guidance E6 for Industry on Good Clinical Practice.

After completing Part 1, patients will continue in Part 2 to receive active treatment for a maximum of 21 days.

10.6. Blinding and Unblinding Treatment Assignment

Trial personnel will endeavor to safeguard the integrity of the trial blind to minimize bias in the conduct of the trial. Treatment unblinding is discouraged if knowledge of the treatment assignment will not materially change the planned management of a medical emergency. Unblinding will be permitted in a medical emergency that requires immediate knowledge of the patient's treatment assignment.

For emergency unblinding, the investigator will use the measures provided through the IWRS. Unblinding should be discussed in advance with the medical monitor if possible. If the investigator is not able to discuss treatment unblinding in advance, then he or she must notify the medical monitor as soon as possible about the unblinding incident without revealing the patient's treatment assignment.

The investigator or designee must record the date and reason for unblinding on the appropriate eCRF page for that patient.

10.7. Selection of Doses in the Trial

Both the starting dose and the maximum allowed doses are based on experience with marketed glucagon products in this patient population.[11]

At present, no pediatric PK data are available for dasiglucagon. However, an approximate estimation of expected drug concentration in a 3 kg patient has been made by extrapolation of a previously made PK model for pediatric patients with weights between 25 and 45 kg.[17]

In this trial, dasiglucagon will be titrated to meet the needs of the individual patient on the basis of PD effects in regards to efficacy and tolerability, and it is expected that the majority of patients will be adequately treated with a dasiglucagon infusion rate of approximately 20 to 40 µg/hr. The up-titration will stop when IV glucose is completely weaned, if undesirable side effects arise, or when no additional PD effects are observed as the dasiglucagon infusion rate is increased.

Based on the current PK/PD model, the predicted plasma concentration is expected to result in a medium PD response at the lowest dose level and be approaching maximum effect at the highest dose level. The PD response of patients will be monitored closely in this trial, and the appropriateness of the applied titration algorithm will be evaluated.

Although the PK of dasiglucagon has been shown to be dose-proportional in adults and in all toxicologically tested species over a wide range of doses, limited PK sampling at each dose level is planned based on the limited available blood volume in this population.

10.8. Selection of Timing of Dose for Each Patient

Dosing details are provided in Section 10.2.

10.9. Dose Adjustment Criteria

Dose adjustment criteria are presented in Section 10.2.

10.10. Treatment Compliance

Compliance data will be collected. Infusion details will be recorded in the patient's eCRF and drug accountability will be performed as detailed in the pharmacy manual/TMM.

10.11. Permitted and Prohibited Therapies

All concomitant medications used (including over-the-counter medications and herbal supplements) will be recorded in the source document and on the appropriate eCRF.

Caution is advised when beta-blockers, indomethacin, anticholinergic drugs, and warfarin are prescribed due to reports of interaction with marketed glucagon products.

10.11.1. Permitted Therapies

Regarding diazoxide, patients entering the trial either:

- Have tried diazoxide, but discontinued due to lack of efficacy. Minimum of 6 days' washout is required prior to randomization.
- Have tried diazoxide, but discontinued due to adverse events, or family choice, even if efficacy was established. Minimum of 6 days' washout is required prior to randomization.
- Have initiated diazoxide prior to screening, and continue on the treatment. Diazoxide dose should remain stable during Part 1 of the trial. Diazoxide should not be initiated during screening.
- Have never tried diazoxide. Diazoxide should not be initiated during screening or Part 1 of the trial.

Prior use of octreotide is allowed after a minimum 48-hour washout prior to randomization. Washout is required to eliminate the effect of octreotide during Part 1, where adjustment of GIR has to follow the protocol-defined PG-driven algorithm (Table 1) and the primary endpoint on mean GIR is assessed.

Somatostatin analogs and/or diazoxide can be introduced during Part 2 if needed, at the investigator's discretion, when the maximal limit of dasiglucagon (70 µg/hr) has been reached or if further up-titration is not possible due to undesirable side effects.

Addition of other CHI-specific treatments (e.g., sirolimus/mTOR inhibitors) during Part 2 need to be discussed with the medical monitor.

10.11.2. Prohibited Therapies

The following therapies are prohibited:

During the entire trial:

- Systemic corticosteroids, e.g., hydrocortisone >20 mg/m^2 body surface area or equivalent within 5 days before screening
- Anti-inflammatory biological agents or other immune-modulating agents
- Use of paracetamol/acetaminophen is strongly discouraged for the duration of trial when patients are using the Dexcom G4 CGM because it interferes with the accuracy of the device. Parent(s)/guardian should contact the trial site before dosing child with

paracetamol/acetaminophen. Both the site staff and the parent(s)/guardian should explore other options of treating fever and mild pain before deciding paracetamol/acetaminophen is needed

When patients are using the Dexcom G6 CGM the use of paracetamol/acetaminophen is allowed

- Other investigational agents.
- Marketed glucagon products within the last 24 hours prior to randomization and throughout the trial unless necessary for rescue therapy in case of severe hypoglycemia, as per local standard of care.
- Prescription or non-prescription medications known to cause QT prolongation.

During Screening and Part 1:

- Somatostatin analogs and sirolimus (mTOR inhibitors). No new CHI treatment is to be initiated during screening and Part 1. Octreotide is allowed in the screening period if discontinued at least 48 hours before randomization.

During Part 2 and follow-up:

- Initiation of somatostatin analogs, diazoxide and sirolimus (mTOR inhibitors) unless the maximum dose level of dasiglucagon (70 µg/hr) has been reached or further dasiglucagon up-titration is not possible due to undesirable side effects

10.12. Discharge of Patients for Home Treatment

Patients can be discharged during Part 2 to complete the treatment at home as soon as IV GIR has been weaned off and local site criteria for discharge are met. Discharge for home treatment is contingent on the parent(s)/guardian being capable of taking care of the administration of investigative product via pump at home, can use SMPG and CGM devices, and are able to attend planned assessment visits at the hospital.

Before discharge of patients from hospital care, the investigator will ensure:

- Appropriate training of patient's parent(s)/guardian in the use of dasiglucagon in the Accu-Chek Spirit Combo pump based on the training material provided. The first pump infusion set change should be performed at the hospital
- Parent(s)/guardian are trained appropriately on the handling of CGM device
- Parent(s)/guardian are trained appropriately on how to perform SMPG measurements and how to complete the diary. They will check their child's SMPG at least 3 times daily (preferably before main meals) and in case of suspected hypoglycemia
- Parent(s)/guardian are instructed not to change the dose of trial drug without prior consultation with the investigator
- Parent(s)/guardian are instructed how to recognize and handle signs of hypoglycemia
- Parent(s)/guardian are instructed to call the investigator/site staff in case of questions

Patients who are discharged from hospital before Day 25 will be contacted by the investigator by telephone the day after discharge. The investigator will ask the parent(s)/guardian if they have any questions about the trial procedures and if their child has experienced any AEs.

10.13. Treatment After End of Trial

On Day 25, the End of Treatment Visit will take place. Based on the investigator's confirmation of continued positive benefit-risk balance, patients will be offered the opportunity to enter the long-term extension trial (Trial ZP4207-17106) to continue dasiglucagon treatment. Patients who do not enter the long-term extension trial will have a Follow-up Visit 28 days after their last dose of trial drug and return to standard of care as per investigator discretion. No further treatment with dasiglucagon will be offered.

11. TRIAL PROCEDURES

Patients' parent(s)/guardian (according to local law) must provide written informed consent before any trial-related procedures are initiated, including the cessation of prohibited concomitant therapy.

For the timing of assessments and procedures throughout the trial, refer to the Schedule of Events (Section 18.1). Throughout the trial, trial personnel should make every reasonable effort to follow the timing of assessments and procedures in Section 18.1 for each patient. If a patient misses a trial visit for any reason, it should be rescheduled as soon as possible.

11.1. Trial Duration

The sequence and maximum duration of the trial periods will be as follows:

1. Screening Period: up to 28 days, including a minimum 24-hour run-in period
2. Part 1 (2x48-hour period: double-blind, randomized, placebo-controlled): 96 hours
3. Part 2 (Open-label Active Treatment Period): 21 days
4. Follow-up Period: Patients who do not enter the extension trial will have a Follow-up Visit 28 days after their last dose of trial drug

The maximum trial duration for each patient is 81 days. The maximum treatment duration for each patient is 25 days.

11.2. Assessments

11.2.1. Efficacy

11.2.1.1. Plasma Glucose Monitoring

As long as the patient is receiving IV glucose, the GIR will be reviewed hourly, based on an hourly PG check performed by the StatStrip Xpress2 meter. The IV GIR will be reviewed, evaluated, and adjusted (if indicated) according to the IV GIR algorithm based on PG levels specified in Table 1. If IV glucose has been stopped, PG will be checked according to local practice, but at least 3 times daily.

Blood for PG check can be obtained either by finger or heel sticks or by an indwelling peripheral line. However, if using an indwelling peripheral line, the total blood volume drawn during a 28-day period should be considered according to Section 11.2.3.1.

At each visit, the investigator will ensure that SMPG data are downloaded from the patient's device. The investigator will check for patient compliance in SMPG measurements. The procedure for download of SMPG data will be described in the pharmacy manual/TMM.

Adjustments to the trial drug will be made as outlined in Section 10.2.

11.2.1.2. Continuous Glucose Monitoring

Dexcom CGMs (Dexcom G4 and Dexcom G6), configured and labeled for use in the trial, will be provided. The Dexcom G4 will be taken off the market by the supplier during 2020 and patients enrolled thereafter will be using the Dexcom G6 CGM. Patients using the Dexcom G4 CGM will continue using this device throughout the trial and should not switch to the Dexcom G6 device.

The CGM will be started at least 24 hours prior to randomization and will be used during Parts 1 and 2 in a blinded mode to evaluate efficacy in terms of hypoglycemic episodes. Short pauses of 1 to 3 days due to skin irritation or discomfort are allowed during Part 2 of the trial after consultation with the investigator.

The CGM devices should be calibrated and used according to the manufacturer's instructions; the Dexcom G4 should be calibrated 2 times per day, the Dexcom G6 does not require calibration.

At each visit, the investigator will ensure that CGM data are downloaded from the patient's device. The procedure for download of CGM data will be described in the pharmacy manual/TMM.

The contract research organization (CRO) or delegate will handle the device sourcing, configuration for use in this trial, procedures for blinded data extraction, device service, and handling of potential returns.

11.2.1.3. Diary

For patients who are discharged during the trial, the parent(s)/guardian will receive a paper diary to be completed at home. The investigator will instruct the patient's parent(s)/guardian on how to complete the diary. The following information should be recorded in the diary:

- Type and volume of fluid administered through NG tube/gastrostomy
- Hypoglycemic events, including related SMPG measurements
- Concomitant medications
- AEs
- Hospitalizations, visits to health care providers or emergency room (ER)/accident & emergency (A&E) department, and visits by paramedics
- Data regarding suspicion of NME and neurological findings

Diary entries should be reviewed at each visit and the review documented in the diary. Data from the diary entries should be transcribed to the eCRF on an ongoing basis.

11.2.2. Pharmacokinetics/Drug Exposure

Blood samples will be collected in Part 2 to measure dasiglucagon levels at steady-state (Schedule of Events; Section 18.1). The blood samples should be collected after a minimum of 4 hours without changes to the dasiglucagon infusion rate. For patients with a body weight above 4 kg, an additional PK sample should be collected at Day 5. However, the total amount of blood collected should always be considered according to Section 11.2.3.1.

Details on sampling/collection, shipment, and analysis will be provided in the laboratory manual.

11.2.3. Safety

Safety assessments will include the evaluation of AEs, clinical laboratory assessments (hematology, biochemistry, and ADAs), vital signs, physical examinations, ECGs, echocardiography, and local tolerability.

11.2.3.1. Laboratory Safety Assessments

Trial procedures require a maximum total of 8.8 to 9.2 mL of blood in a 28-day period. Where this exceeds the 2.5 mL/kg maximum,[18,19] safety laboratory tests (2 mL per sampling) will be prioritized over immunogenicity (1 mL) and drug exposure (0.4 mL) samples.

All measurements described in this section are recognized standard methods.

Hematology and Biochemistry

Samples for hematology and biochemistry will be collected at the time points specified in the Schedule of Events (Section 18.1).

Hematology:	hemoglobin, hematocrit, red blood cell (RBC) count, RBC indices, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet count (or estimate), white blood cell count, including differential
Biochemistry:	albumin, total bilirubin, total protein, calcium, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, blood urea nitrogen, creatinine, glucose, sodium, potassium, chloride, eGFR, ketones (measured with PG meter)

Standard of care blood samples can be used as screening samples if they were collected within 1 week of screening.

Laboratory specimens will be analyzed at local laboratories.

Immunogenicity

Blood samples will be collected to test for antibodies against dasiglucagon at Visit 2 (Day 1, prior to dosing), Visit 7 (Day 25 ±3), and at the Follow-up Visit (Visit 8, Day 53 ±3) (Section 18.1) and processed and shipped according to instructions provided in the laboratory manual.

The samples will be stored until the ADA screening and confirmatory assays have been approved by the FDA. Afterwards, the ADA samples will be analyzed in batches during the trial. The ADA samples will be analyzed at a specialty laboratory.

The clinical ADA assays specific for dasiglucagon and glucagon have been validated in accordance with existing guidelines and recommendations.[19,21,22,23]

Samples will be measured in anti-dasiglucagon antibody screening and confirmatory assays. Due to the limited sample volume, the ADA characterization of confirmed positive samples will be conducted according to the following priority:

- Establishment of anti-dasiglucagon binding titer.
- Cross-reactivity against endogenous glucagon.

The neutralizing potential in samples from ADA-positive patients will be evaluated on the basis of drug exposure/PD data (steady-state exposure and PG).

The ADA samples will be analyzed in batches during the trial and any anti-dasiglucagon antibody-positive patients (treatment induced or treatment boosted, titer increase above 5-fold) will be monitored at an additional Follow-up Visit, preferably 16 weeks after last ADA-positive sample. Patients who complete the trial before the ADA screening and confirmatory assays have been approved by the FDA and who do not continue treatment in the long-term extension trial will have this additional visit 16 weeks after the end of trial visit (Visit 8). Patients who are tested ADA-positive after the additional Follow-up Period will be categorized as having persistent antibodies.

It will not be possible to collect back-up ADA samples for long-term storage due to limited blood volume in these patients. However, any residual serum samples may be stored until approval of market authorization since further characterization of the antibody response may be requested.

Specimen Handling Requirements

The transmission of infectious agents may occur through contact with contaminated needles and blood or blood products. Consequently, appropriate blood and body fluid precautions should be employed by all trial personnel involved in the collection of blood and handling of specimens in both the clinic and laboratory settings. Refer to current recommendations of the appropriate authorities.

In addition to appropriate handling of patient samples, specific regulations exist regarding the shipment of biologic/etiologic samples. Procedures and regulations for the packaging and shipping of infectious samples are outlined in the laboratory manual. The investigator is responsible for ensuring that all trial samples that are to be transported to another location are packed and shipped appropriately according to the applicable regulations.

Evaluation of Laboratory Values

The normal ranges of values for the laboratory assessments in this trial will be provided by the responsible laboratories and submitted to the sponsor before the beginning of the trial. They will be regarded as the reference ranges on which decisions will be made.

If a laboratory value is out of the reference range, the investigator must evaluate if the value is clinically significant and record his or her assessment in the appropriate eCRF.

All laboratory values that in the investigator's opinion are clinically relevant during or after termination of the treatment have to be reported as AEs and followed, as described in Section 12.3.

11.2.3.2. Clinical Examinations

Vital Signs

Vital signs, including blood pressure, heart rate, respiratory rate, and SpO_2 will be measured while the child is sleeping or in a calm state at times specified in the Schedule of Events (Section 18.1). Vital signs can be performed using the bedside monitoring device as per standard of care.

Twelve-lead Electrocardiogram

A standard 12-lead ECG will be performed while the child is sleeping or in a calm state at times specified in the Schedule of Events (Section 18.1). If it is not practical or possible, then a 2-lead ECG may be used.[24] If arrhythmia is detected on a 2-lead ECG, this should be followed by

12-lead ECG. All ECG recordings will be identified with the patient number, date, and time of the recording and will be attached to his or her medical record.

The ECG parameters (heart rate, PQ, QRS, QT, and QTcF) and any abnormality will be recorded and described in the eCRF, including the investigator's assessment of clinical significance (Abnormal, Clinically Significant; or Abnormal, Not Clinically Significant). At subsequent visits, any clinically significant deterioration of a pre-existing condition, as well as any new clinically significant findings, will be recorded as AEs.

Echocardiogram

An echocardiogram will be performed according to the time points specified in the Schedule of Events (Section 18.1).

Physical Examination and Neurological Examination

A complete physical examination of body systems according to standard of care and an age-appropriate neurological examination will be performed according to the Schedule of Events (Section 18.1).

Local Tolerability

Local tolerability data will be collected separately from AEs. Within the eCRF, data will be collected on the nature of any reaction (erythema, pain, swelling, etc.), the severity (mild, moderate, or severe), and any action taken (e.g., no action, interruption of infusion). The likely cause of the reaction will also be collected (e.g., insertion site, drug, adhesive dressing).

Other skin findings will be collected along other AEs. If clinical suspicion of NME is made, data describing the lesion(s) will be collected as an AE of special interest (AESI) (see Section 12.1.8), together with a photograph or series of photographs of the lesion(s) uploaded to a central repository.

Assessment of Fluid Balance for Patients Receiving IV Glucose

Fluid balance assessments are to be performed and documented at least every 8 hours as long as the patient is hospitalized and receiving IV glucose. These assessments will include administered IV and oral fluids, fluid loss from stools and urine by weighing of diapers, and vital signs of fluid balance status. Furthermore, daily body weight will also be part of the individualized assessment based on the patients overall status, at investigator's discretion. According to Section 9.5, the investigator should evaluate whether the IV glucose concentration should be increased to avoid volume overload or whether oral/gastric non-nutritional carbohydrates are to be introduced to ensure the patient's safety whilst maintaining the maximum tolerable concentration and infusion rate of IV glucose.

All details on IV GIR (including any bolus given) will be recorded in the eCRF.

11.2.3.3. Reporting of Hypoglycemia Events

All hypoglycemic episodes are to be reported via the dedicated hypoglycemia eCRF form. Hypoglycemic episodes that fulfill the definition of a serious adverse event (SAE) should also be recorded as an SAE. The following information should be collected:

- Date, start time
- PG value

- Selected symptoms (e.g., unconsciousness, seizures)
- Intervention, type, and amount of food; route of administration (oral vs. NG tube/gastrostomy); and use of marketed glucagon as rescue therapy
- Subsequent PG measurements
- End time and PG value (time when PG exceeded a threshold of 70 mg/dL [3.9 mmol/L])
- Contact to trial doctor or emergency services, paramedic visit, ER admission, hospitalization, if applicable

A single hypoglycemia episode is defined as up until 60 minutes from the start of the episode. If normoglycemia (>70 mg/dL) is not reached within this time, a new episode of hypoglycemia will be recorded.

11.2.4. Technical Complaints

Reporting of Technical Complaints

Technical complaints should be reported to the sponsor on any of the following products if technical issues occur between the first and last use:

- Dasiglucagon 4 mg/mL / placebo vial containing 1 mL
- Accu-Chek Spirit pump
- Accu-Chek Spirit 3.15 mL Cartridge system, Accu-Chek Flex-Link Infusion set (Accu-Check UltraFlex Infusion set in the US), and Accu-Check Rapid-D Link infusion set
- Accu-Chek Link-Assist Insertion device
- SMPG meter, StatStrip Xpress2
- Dexcom G4/G6 systems

The investigator must report whether the technical complaint is associated with any AEs or SAEs. Any AE/SAE associated with a technical complaint must be reported in accordance with Section 12.2; the relationship between the technical complaint and the AE/SAE must be assessed by the investigator.

Technical complaints must be reported on a dedicated technical complaint form.

The investigator must complete the technical complaint form in the eCRF according to the following timelines, starting from the time the trial site becomes aware of the technical complaint:

- Technical complaint assessed as related to an SAE **within 24 hours**
- All other technical complaints within **5 calendar days**

Use the Trial paper technical complaint form when reporting a technical complaint for an item that is not yet allocated to a patient. The form should be sent by e-mail to e-mail address in the Appendix A.

Collection, Storage, and Shipment of Technical Complaint Items

The investigator must collect and store the item(s) and notify the CRA (including photo documentation) **within 5 calendar days** of obtaining the item at the trial site. Upon request, the CRA must coordinate the shipment as per instruction from the sponsor.

11.2.4.1. Adverse Events

The definitions and management of and special considerations for AEs are provided in Section 12.

12. ADVERSE EVENTS

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

12.1. Definitions

12.1.1. Adverse Events

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

AEs include:

- A clinically significant worsening of a concomitant illness
- A clinical laboratory AE: a clinical abnormality which is clinically significant, i.e., any abnormality that suggests a disease and/or organ toxicity and is of a severity that requires active management. Active management includes active treatment or further investigations, e.g., change of dose or more frequent follow-up due to the abnormality.

The following should **not** be considered as AEs:

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

12.1.2. Severity

When assessing the severity of an AE, the following definitions are used:

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 find unacceptable. A severe reaction does not necessarily deem the AE as serious (SAE), and an SAE is not always severe in nature.

12.1.3. Causality

When assessing the cause of an AE, the following definitions are used:

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

Possible: A causal relationship is conceivable and cannot be dismissed

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

Not related: No relationship to product.

Causality will take into consideration whether the cause of the AE was related to the trial drug, device, or procedures.

12.1.4. Outcome

When assessing the outcome of an AE, the following definitions are used:

Recovered/resolved: The patient has fully recovered, or by medical or surgical treatment, the condition has returned to the level observed at the first trial-related activity after the patient signed the ICF

Recovering/resolving: The condition is improving and the patient is expected to recover from the event. This term is only applicable as final outcome of an event if the patient has completed the trial or has died from another AE

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, or the outcome is not known

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.

12.1.5. Serious Adverse Events

An SAE is any untoward medical occurrence that at any dose results in any of the following:

- results in death
- is life-threatening
- requires inpatient hospitalization or prolongation of existing hospitalization
- results in persistent or significant disability or incapacity
- is a congenital anomaly or birth defect
- is otherwise medically important and may not result in death, be life-threatening, or require hospitalization may be considered an SAE when (based on appropriate medical judgement) it may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed in the definition of an SAE. Examples could be ER or home treatment of allergic bronchospasm or convulsion

12.1.6. Other Important Events

The following events must always be reported in the electronic data capture (EDC) system on a dedicated form, regardless of whether it is related to an AE:

- suspicion of transmission of infectious agents via the trial product
- overdose of the trial product
- medication error involving the trial product
- inadvertent or accidental exposure to the trial product

12.1.7. Nonserious Adverse Events

A nonserious AE is any AE that does not fulfill the definition of an SAE.

12.1.8. Adverse Events of Special Interest

For this trial, the following events are to be regarded as AESIs, with data collected under a specific eCRF form:

- Suspicion of NME
- Risk of liver injury defined as ALT or AST >3 x UNL AND total bilirubin >2 x UNL, where no alternative etiology exists (Hy's law)
- Loss of consciousness, partial, and generalized seizures
- Clinically significant changes in blood pressure or heart rate

12.1.9. Suspected Unexpected Serious Adverse Reactions

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

12.2. Collection, Recording, and Reporting of Adverse Events

All AEs, whether serious or nonserious, will be reported from the time a signed and dated ICF is obtained until the end of the posttreatment follow-up period (which may include contacts for follow-up of safety) or inclusion in the ZP4207-17106 trial. Parent(s)/guardian will be asked about their child's condition by open questioning, such as "How has your child been feeling since you were last asked?" at each contact with the trial site (visit or telephone). Parent(s)/guardian will also be encouraged to spontaneously report AEs occurring at any other time during the trial. In addition, patients will be observed for any signs or symptoms.

All AEs, regardless of seriousness, severity, or presumed relationship to the trial drug, must be recorded and evaluated by the investigator. Whenever possible, diagnoses should be given when signs and symptoms are due to a common etiology. If no diagnosis can be made, the investigator should record each sign and symptom as individual AEs. Investigators must record their opinion concerning the relationship of the AE to the trial drug. All 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 eCRF within 5 days of the investigator becoming aware of the event.

All AE information should at a minimum include the following:

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

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

All SAEs, including those spontaneously reported to the investigator within 30 days after the last dose of trial drug, must be reported within 24 hours after obtaining knowledge about the event by completing the SAE form in the EDC system. A separate SAE form should be completed for each SAE.

All SAEs will be reported in EDC; for each reported event a system-generated e-mail will be sent to the safety CRO ([redacted]), medical monitor, sponsor medical director, and trial manager.

Specific information about AESIs will be collected via the SAE form (if qualifying for an SAE), as well as via the dedicated AESI eCRF page(s). Reporting requirements for serious and nonserious AEs as described previously also apply for serious and nonserious AESIs.

Other important events (Section 12.1.6) will be reported via a dedicated eCRF page. Reporting timelines will be within 24 hours if related to an SAE, and 5 calendar days for all other events.

It is the responsibility of [redacted] to report all SUSARs that occur in this trial to competent authorities, the institutional review board (IRB), or independent ethics committee (IEC) in accordance with the local requirements in force and ICH guideline for GCP.

12.2.1. Contact Information

Pharmacovigilance for this trial is outsourced to [redacted]; refer to Appendix A for contact details.

12.3. Follow-up of Adverse Events

The investigator must record follow-up information on the eCRF for nonserious adverse event and on the SAE form for serious adverse events. Follow-up questions to investigators regarding serious adverse events are queried directly by [redacted] to the investigator.

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

- **SAEs:** All SAEs must be followed until the outcome of the events is “recovered/resolved,” “recovered/resolved with sequelae,” or “fatal,” and until all queries have been resolved. Cases of chronic conditions, cancer, or AEs that are ongoing at time of death (where death is due to another AE) may be closed with the outcome “recovering/resolving” or “not recovered/not resolved.” Cases can be closed with the outcome of “recovering/resolving”

when the patient has completed the follow-up period and is expected by the investigator to recover.

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

- **Nonserious AEs:** Nonserious AEs must be followed until the outcome of the event is "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 or if the patient continues in the extension trial.

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

The investigator must ensure that the worst-case severity and seriousness of an event is kept throughout the trial, from the start of trial product administration (i.e. the most severe of the applied severity/seriousness categories should be ascribed to the event). Accordingly, for AEs with onset after trial product administration, changes in the severity or seriousness of an event should not lead to reporting of separate AEs. In contrast, for AEs with onset before trial product administration, any worsening of severity/seriousness after trial product administration should be recorded as a separate AE, with onset date of the event corresponding to the date of the severity/seriousness upgrade. Similarly, AEs with onset during the blinded treatment that worsen after the switch to the second blinded treatment or to the unblinded treatment, should be recorded as separate AEs.

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.

12.4. Precautions

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

For further information on safety precautions for dasiglucagon, refer to the current version of the IB.

12.5. Safety Committee

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

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

12.6. Independent Data Monitoring Committee

An independent data monitoring committee (DMC) will be established for this trial and follow the procedures outlined in the DMC Charter.

13. STATISTICS

13.1. Statistical Hypotheses

This section details the null hypotheses for this trial. They are presented in the form H_{ij}, where i = trial part (i = 1, 2) and j = hypothesis number (j = 1, 2, 3, 4, 5).

The hypothesis relates to the primary endpoint of mean IV GIR in the last 12 hours of each treatment period during Part 1. Formally, the hypothesis is:

H_{11}: Mean IV GIR $_{dasiglucagon}$ = Mean IV GIR $_{placebo}$

The hypotheses relating to the key secondary endpoints (based on the last 48 hours of each treatment period in Part 1) are:

H_{12}: Total amount (g) of carbohydrates administered (regardless of the route) per day $_{dasiglucagon}$ = total amount (g) of carbohydrates administered (regardless of the route) per day $_{placebo}$

A fixed-sequence statistical strategy will test first the primary (Section 7.2.1) and then the key secondary endpoint of Part 1 (Section 7.2.2), all at the same significance level ($\alpha = 0.05$, 2-sided test), moving to the next hypothesis only after rejecting the previous null hypothesis.

The test hierarchy is:

Part 1

H_{11}: Mean IV GIR in the last 12 hours of each treatment period during Part 1 (primary endpoint)

H_{12}: Total amount (g) of carbohydrates administered (regardless of the route) per day (key secondary endpoint)

13.2. Sample Size Determination

A total of 12 patients is planned to be randomized and exposed to trial product in this crossover trial on the basis of the following considerations.

The GIR results from 40 infants treated with IV glucagon for 24 hours published in the JIMD Research Report "The Effect of Continuous Intravenous Glucagon on Glucose Requirements in Infants with Congenital Hyperinsulinism."[14]

"Overall there was a statistically significant reduction in the median (IQR) GIR during the 24h following initiation of continuous glucagon infusion compared to 24 h before initiation (18.5 (12.9, 22.8) to 11 (6.6, 17.5) mg/kg/min…"

This trial is powered to detect an effect in GIR for dasiglucagon after 48 hours compared to placebo of at least this effect size. Based on the longer infusion and titration of dasiglucagon in this trial, this is considered a conservative approach, allowing for some uncertainty when translating the published data into a clinical trial setting. Using unpublished individual patient data from the above-referenced study, the difference in GIR between the 2 treatment groups is assumed to follow a normal distribution. Assuming the true mean difference is 7.5 mg/kg/min with a standard deviation of differences of 7.36, the trial will have 89% power using a 1-sample t-test with 12 patients randomized to receive either placebo first and then dasiglucagon or vice versa.

13.3. Analysis Populations

Three analysis populations have been defined for this trial:

- The Safety Set: defined as all patients administered any randomized treatment.
- The Full Analysis Set (FAS): defined as all patients in the Safety Set who have a valid baseline efficacy assessment. Patients will be analyzed according to randomized treatment (Part 1) or overall patients (otherwise).
- Per protocol (PP) set: defined as all patients in the FAS without any major protocol deviations. Patients will be excluded from the PP set if they do not receive both treatments and/or do not have efficacy data available to evaluate the primary endpoint for both treatments.
- Pharmacokinetic Set (PK): defined as all patients in the Safety Set who have at least 1 measurement with quantifiable plasma concentration of dasiglucagon.

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

Inclusion in the analysis populations will be determined prior to database lock.

For the primary endpoint, baseline is defined as the IV GIR rate obtained at the end of the run-in period which corresponds to the last value obtained before randomization. During the 24-hour run-in period, all non-nutritional carbohydrates and carbohydrate fortification of feeds are prohibited as this might impact the GIR level and result in many updates during the run-in period. Therefore the IV GIR rate obtained at the end of the run-in period will give a better estimate of the stabilized value of GIR. For the second period of the crossover (Part 1), the IV GIR is also started at the IV GIR obtained at the end of the run-in period.For the purpose of all other efficacy analyses where applicable, baseline is defined as the last measurement prior to the start of trial drug administration on Day 1.

13.4. Statistical Analyses

This section presents a summary of the planned statistical analyses. A statistical analysis plan (SAP) that describes the details of the analyses to be conducted will be finalized prior to database lock.

Unless otherwise indicated, testing of statistical significance will be 2 sided with a significance level of $\alpha = 0.05$.

Continuous endpoints will be summarized with number (n), mean, standard deviation (SD), median, minimum, and maximum. In addition, change from baseline values will be calculated at each time point and summarized descriptively; the exception to this is the time of PG within prespecified ranges, which will not have a baseline value. For categorical endpoints, descriptive summaries will include counts and percentages.

All data will be presented in the data listings.

Immunogenicity data will be analyzed descriptively. No statistical tests are planned. Baseline ADA-positive patients will be calculated as a percentage of the total number of patients whose baseline samples were tested for ADA. Overall ADA incidence, the combined results of treatment-induced and treatment-boosted ADA-positive patients will be calculated as a percentage of the total number of evaluable patients, excluding baseline positive patients without

any samples available after drug administration. Titers will be reported as median and interquartile range.

Graphical presentations of selected endpoints will be prepared and will be outlined in the SAP.

13.4.1. Trial Patients and Demographics

13.4.1.1. Disposition and Withdrawals

The numbers of patients randomized, completing, and withdrawing, along with reasons for withdrawal, will be tabulated overall and by treatment sequence. The number of patients in each analysis population will be reported.

13.4.1.2. Protocol Deviations

Protocol deviations will be provided in a listing and summarized if appropriate.

13.4.1.3. Demographics and Other Baseline Characteristics

Demographic and baseline characteristics (including age, sex, race, ethnicity, weight, and length) at screening will be summarized using descriptive statistics. No formal statistical analyses will be performed.

Prior and concomitant medications and procedures will be summarized by treatment sequence (prior medications and procedures) or treatment and part (concomitant medications and procedures), by the number and percentage of patients taking each medication, classified using World Health Organization Drug Dictionary Anatomical Therapeutic Chemical classes and preferred term (PT).

13.4.2. Duration of Exposure and Compliance

Trial drug administration (i.e., amount administered) will be summarized in terms of each patient's mean, mode, and final dose, and in terms of duration of exposure. Descriptive statistics for these quantities, including the mean, median, SD, minimum, maximum, and quartiles, will be provided by treatment and part.

13.4.3. Efficacy Analyses

13.4.3.1. Primary Analysis

The mean IV GIR in the last 12 hours of each treatment period during Part 1 will be analyzed for the FAS and PP populations. The hypothesis:

H_{11}: Reduction in weighted mean IV GIR $_{dasiglucagon}$ = Reduction in weighted mean IV GIR $_{placebo}$

will be based the last 12 hours of each treatment period, calculated as a weighted mean across the 12 hours, taking the actual time periods between the measurements into account (corresponding to calculating the area under the curve [AUC] and dividing by the length of the time period, i.e., weighted mean IV GIR). The IV GIR endpoint is expressed as glucose in mg/kg/min, i.e., data reported from different concentrations of the glucose used for infusion will be transformed to this standardized unit prior to analysis.

The primary analysis is defined by the estimand based on the treatment policy (de-facto) strategy, where the actual GIR measurement reported irrespective of adherence to treatment or

use of subsequent therapy is used. The reduction in weighted mean IV GIR between placebo and dasiglucagon will be estimated.

The weighted mean IV GIR will be analyzed using a mixed-model regression approach, with treatment and period as fixed effects and patient as a random effect. The 2-sided 95% confidence interval (CI) for the treatment difference will be calculated from the mixed regression model.

Missing data will be imputed using the following methodology: if the weighted mean IV GIR is missing for the placebo period, it will be imputed using the baseline weighted mean IV GIR; if the weighted mean IV GIR is missing for the dasiglucagon period, it will be imputed using the placebo-weighted mean IV GIR for that patient. As a sensitivity analysis, the primary endpoint will be re-analyzed as follows: 1) using only complete cases (i.e., weighted mean IV GIR is non-missing for both placebo and dasiglucagon) and 2) imputing missing weighted mean IV GIR using the baseline weighted mean IV GIR and 3) imputing missing dasiglucagon values during one period with the placebo value for that patient and imputing missing placebo values with the mean values of placebo patients for that period..

13.4.3.2. Key Secondary Analyses

The hypothesis:

H_{12}: Total amount (g) of carbohydrates administered (regardless of the route) per day $_{dasiglucagon}$ = total amount (g) of carbohydrates administered (regardless of the route) per day $_{placebo}$

between the 2 treatment groups will be analyzed by using a mixed-model regression approach, with treatment and period as fixed effects and patient as a random effect. The total amount (g) of carbohydrates administered (regardless of the route) per day is the sum of the last 48 hours of each period in Part 1.

For the key secondary endpoint, 2-sided 95% CIs for the treatment differences will be estimated from the mixed-model. Missing dasiglucagon values during one period will be imputed with the placebo value for that patient and missing placebo values will be imputed with the mean values of placebo patients for that period. In addition, a sensitivity analysis will be performed using complete cases.

13.4.3.3. Secondary and Other Efficacy Analyses

Part 1

A mixed-model regression approach will be used to analyze the weighted mean IV GIR over 48-hour treatment period during Part 1 (dasiglucagon or placebo administration) similar to the primary analysis for the primary endpoint.
A generalized estimating equation (GEE) method with a logit link function will be used to analyze the weighted mean IV GIR below 10 mg/kg/min in the last 12 hours of each treatment period during Part 1 (yes/no) (dasiglucagon or placebo administration) to account for repeated observations in patients. In case of non-convergence, a Mc Nemar test will be used.
No sensitivity analysis will be used on these endpoints.

Part 2

Due to the potential impact of pancreatectomy on the secondary endpoints during Part 2, any endpoint assessment after pancreatectomy will not be included in the primary analyses

(endpoints set to missing after pancreatectomy). Sensitivity analyses will be run on those endpoints including all assessments in the descriptive analyses.
For Part 2 efficacy endpoints, continuous and categorical endpoints will be presented using summary statistics or frequencies, respectively; no formal testing will be performed. For the time to complete weaning off IV GIR, the time to actual hospital discharge and the time to pancreatic surgery, Kaplan-Meier curves will be produced.

13.4.4. Safety and Tolerability Analyses

Safety analyses will be conducted using data from the Safety Population (as defined in Section 13.3). Safety assessments will include the evaluation of AEs; clinical laboratory assessments (hematology, biochemistry, and ADAs); vital signs, physical examinations; ECGs, echocardiography, and local tolerability issues. No formal inferential analyses will be conducted for safety variables, unless otherwise noted.

Baseline is defined as the last nonmissing assessment before the first exposure to trial drug in the trial.

All safety analyses will be summarized by treatment received within treatment period and part and by trial visit, if applicable.

13.4.4.1. Adverse Events

Adverse events will be coded using the most current version of MedDRA.

A treatment-emergent AE is defined as an AE with an onset at the time of or following the start of treatment with the trial drug through the Follow-up Visit or Early Termination Visit, whichever occurs first.

The number and percentage of patients with AEs, as well as the number of AEs, will be displayed by system organ class, PT, and treatment group. The incidence of AEs will also be presented by severity and relationship to the trial drug. Serious AEs, AESIs and AEs resulting in discontinuation of trial drug will be summarized separately in a similar manner. Patient listings of AEs, SAEs, AESIs, and AEs causing discontinuation of trial drug and withdrawal from the trial will be produced.

13.4.4.2. Clinical Laboratory Evaluations

Descriptive summaries (mean, SD, median, minimum, and maximum) of actual (absolute) values and changes from baseline values will be presented for clinical laboratory values.

The number of patients with clinical laboratory values categorized as below, within, or above normal ranges will be tabulated showing change from baseline (shift tables) for each clinical laboratory analyte.

Laboratory values that are outside the normal range will also be flagged in the data listings, along with corresponding normal ranges. Any out-of-range values that are identified by the investigator as being clinically significant will also be shown in a data listing.

13.4.4.3. Vital Signs

Descriptive summaries (mean, SD, median, minimum, and maximum) of actual values and changes from baseline will be calculated for systolic blood pressure, diastolic blood pressure, heart rate, respiratory rate, and SpO_2.

The number of patients with vital signs values categorized as Abnormal, Clinically Significant or Abnormal, Not Clinically Significant will be tabulated showing change from baseline (shift tables) for each parameter.

13.4.4.4. Twelve-lead Electrocardiograms

The number and percentage of patients with normal and abnormal ECG findings will be summarized. Abnormal results will be grouped as Abnormal, Clinically Significant or Abnormal, Not Clinically Significant.

13.4.4.5. Echocardiograms

The number and percentage of patients with normal and abnormal echocardiogram findings will be summarized. Abnormal results will be grouped as Abnormal, Clinically Significant or Abnormal, Not Clinically Significant.

13.4.4.6. Physical Examination Findings

The number and percentage of patients with normal and abnormal findings in the complete physical examination will be displayed.

13.4.4.7. Local Tolerability

The number and percentage of patients with local tolerability findings, collected separately from AEs, will be summarized.

13.4.5. Interim Analysis

No interim analysis is planned.

14. TRIAL CONDUCT

The accuracy and reliability of data are ensured, among others, by the selection of qualified investigators and appropriate trial sites, review of protocol procedures with the investigator and associated personnel before the trial, periodic monitoring visits, and meticulous data management.

14.1. Sponsor and Investigator Responsibilities

14.1.1. Sponsor Responsibilities

The sponsor is obligated to conduct the trial in accordance with strict ethical principles (Section 16). The sponsor reserves the right to terminate participation of a trial site at any time (Section 14.7), and/or to discontinue the trial (Section 14.6 for US studies and Section 14.6.2 for studies conducted outside of the US).

The sponsor agrees to provide the investigator with sufficient material and support to permit the investigator to conduct the trial according to the trial protocol.

14.1.2. Investigator Responsibilities

By signing the Investigator's Agreement (Section 18.2), the investigator indicates that he/she has carefully read the protocol, fully understands the requirements, and agrees to conduct the trial in accordance with the procedures and requirements described in this protocol.

The investigator also agrees to conduct this trial in accordance with all laws, regulations, and guidelines of the pertinent regulatory authorities, including the November 2016 ICH Guidance for Industry E6(R2) GCP, and in agreement with the 2013 version of the Declaration of Helsinki. While delegation of certain aspects of the trial to subinvestigators and trial coordinators is appropriate, the investigator will remain personally accountable for closely overseeing the trial and for ensuring compliance with the protocol and all applicable regulations and guidelines. The investigator is responsible for maintaining a list of all persons that have been delegated trial-related responsibilities (e.g., subinvestigators and trial coordinators) and their specific trial-related duties.

Investigators should ensure that all persons who have been delegated trial-related responsibilities are adequately qualified and trained in the protocol, trial drugs handling, and their specific duties within the context of the trial. Investigators are responsible for providing the sponsor with documentation of the qualifications, GCP training, and research experience for themselves and their staff as required by the sponsor and the relevant governing authorities.

To ensure compliance with the guidelines, the trial may be audited by an independent person. The investigator agrees, by written consent to this protocol, to cooperate fully with compliance checks by allowing access to all trial documentation by authorized individuals.

14.2. Site Initiation

Trial personnel may not screen or enroll patients into the trial until after receiving notification from the sponsor or its designee that the trial can be initiated at the trial site. The trial site will not be authorized for trial initiation until:

1. The trial site has received the appropriate IRB/IEC approval for the protocol and the appropriate ICF.
2. All regulatory documents have been submitted to and approved by the sponsor or its designee.
3. The trial site has a Clinical Trial Agreement in place.
4. Trial site personnel, including the investigator, have participated in a trial initiation meeting.

The regulatory documents must be received from the investigator before the sponsor will authorize shipment of trial drug to the trial site, Regulatory Green Light. Copies of the investigator's regulatory documents must be retained at the trial site in a secure location in the ISF. Additional documents, including a copy of the protocol and applicable amendment(s), the dasiglucagon IB, eCRF completion guidelines, copies of regulatory references, copies of IRB/IEC correspondence, and trial drug accountability records should also be retained in the ISF. It is the investigator's responsibility to ensure that copies of all required regulatory documents are organized, current, and available for inspection.

14.3. Screen Failures

Patients who fail inclusion and/or exclusion criteria may be rescreened for the trial upon approval by the sponsor and medical monitor. Patients may only be rescreened once 30 days or more after the original Screening Visit. If a patient is eligible to enter the trial after having previously failed screening, the patient will be assigned a new patient identification number.

14.4. Trial Documents

All documentation and material provided by the sponsor for this trial are to be retained in a secure location and treated as confidential material.

14.4.1. Investigator's Regulatory Documents

The regulatory documents will be maintained by the investigator in the ISF.

14.4.2. Case Report Forms

By signing the Investigator's Agreement (Section 18.2), the investigator agrees to maintain accurate eCRFs and source documentation as part of the case histories for all patients who sign an ICF.

Case report forms are considered confidential documents and should be handled and stored accordingly. The sponsor or its designee will provide the necessary training on the use of the specific eCRF system used during the trial to ensure that the trial information is captured accurately and appropriately.

To ensure data accuracy, eCRF data for individual patient visits should be completed as soon as possible after the visit. All requested information must be entered in the EDC system according to the completion guidelines provided by the sponsor or its designee.

The eCRF must be signed by the investigator or a subinvestigator when all data are entered and cleaned. These signatures serve to attest that the information contained in the eCRF is accurate and true.

14.4.3. Source Documents

Information recorded in the eCRF should be supported by corresponding source documentation. Examples of acceptable source documentation include, but are not limited to, hospital records, clinic and office charts, laboratory notes, and recorded data from automated instruments, memoranda, and pharmacy dispensing records.

Clinical laboratory data required by the protocol will be entered into the eCRF at the site.

The investigator should permit trial-related monitoring, IEC review, regulatory inspections, and sponsor audit by providing direct access to source data and documents.

14.5. Data Quality Control

The sponsor and its designees will perform quality control checks on this clinical trial.

14.5.1. Monitoring Procedures

The sponsor and/or its designee will conduct site visits to monitor the trial and ensure (i) the safety and rights of the patients are respected, (ii) compliance with the protocol, GCP, and applicable regulations and guidelines, and (iii) that accurate, valid, and complete data are collected. The assigned CRA(s) will visit the investigator and trial site at periodic intervals and maintain periodic communication, this are described in detail in the Monitoring Plan. The investigator agrees to allow the CRA(s) and other authorized sponsor personnel access to ISF and source data (original documents, data, and records). The CRA(s) will maintain current personal knowledge of the trial through observation, review of trial records and source documentation, and discussion of the conduct of the trial with the investigator and staff. While on site, the CRA(s) will review:

- regulatory documents
- entries in the EDC system compared with the source documents
- consents
- adherence to the inclusion/exclusion criteria
- AE records
- storage and accountability of trial drug and trial materials
- adherence to the protocol and ICH GCP

The CRA(s) will ask for clarification and/or correction of any noted inconsistencies. Procedures for correcting eCRFs are described in the Trial Reference Manual. As representatives of the sponsor, CRAs are responsible for notifying project management of any noted protocol deviations.

By signing the Investigator's Agreement (Section 18.2), the investigator agrees to meet with the CRA(s) during trial site visits; to ensure that trial staff is available to the CRA(s) as needed; to provide the CRA(s) access to all trial documentation, to the clinical supplies dispensing and storage area; and to assist the monitors in their activities, if requested. Further, the investigator

agrees to allow the sponsor or designee auditors or inspectors from regulatory agencies to review records, and to assist the inspectors in their duties, if requested.

14.5.2. Data Management

The sponsor or designee will be responsible for activities associated with the data management of this trial. The standard procedures for handling and processing records will be followed per GCP and Premier standard operating procedures. A comprehensive data management plan will be developed, including a data management overview, description of database contents, annotated eCRF, pre-entry review list, self-evident correction conventions, query contacts, and consistency checks.

Trial site personnel will be responsible for providing resolutions to all data queries. The investigator will be required to document electronic data review to ensure the accuracy of the corrected and/or clarified data. Procedures for soliciting and documenting resolution to data queries are described in the trial manual.

14.5.3. Quality Assurance/Audit

This trial will be subject to audit by the sponsor/its designee or national/international regulatory authorities. Audits may be performed to check compliance with GCP guidelines, and can include:

- site audits
- trial master file audits
- database audits
- document audits (e.g., protocol and/or the clinical trial report [CTR])

The sponsor or its designee may conduct additional audits on a selection of trial sites, requiring access to patient notes, trial documentation, and facilities or laboratories used for the trial.

The trial site, facilities, all data (including source data), and documentation will be made available for audit by quality assurance auditors and for IRB/IEC or regulatory authorities according to GCP guidelines. The investigator agrees to cooperate with the auditor during the visit and will be available to supply the auditor with eCRFs or other files necessary to conduct that audit. Any findings will be strictly confidential.

If a regulatory authority informs the investigator that it intends to conduct an inspection, the investigator shall notify the sponsor immediately.

14.6. Trial Termination

The trial may be terminated at the sponsor’s discretion at any time and for any reason.

14.6.1. Regular Trial Termination

The end of this trial is defined as the date of the last visit of the last patient (last patient out or last patient last visit) participating in the trial. Within 90 days of the end of the clinical trial, the sponsor or its designee and/or site will notify the IRBs and IECs and regulatory authorities on the regular termination of the trial as required according to national laws and regulations.

14.6.2. Premature Trial Termination

The trial may be terminated prematurely for any reason and at any time by the sponsor, the IRBs/IECs, regulatory authorities, respective steering committees, or the coordinating investigator. A decision to terminate the trial prematurely is binding to all investigators at all trial sites.

Within 15 days of premature termination of a clinical trial, the sponsor or its designee and/or site will notify the IRBs/IECs and regulatory authorities on the premature termination as required according to national laws and regulations. The sponsor or its designee must clearly explain the reasons for premature termination.

If the trial is terminated prematurely, all investigators must inform their patients and take care of their appropriate follow-up and further treatment to ensure protection of their interests. Trial sites may be asked to have all patients currently participating in the trial complete all of the assessments for an Early Termination Visit.

14.7. Trial Site Closure

At the end of the trial, all trial sites will be closed. The sponsor may terminate participation of a trial site at any time. Examples of conditions that may require premature termination of a trial site include, but are not limited to, the following:

- Noncompliance with the protocol and/or applicable regulations and guidelines
- Inadequate patient enrollment

14.7.1. Record Retention

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

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

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

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

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

One copy will remain with the investigator. The investigator shall arrange for the retention of the patient identification codes, patient files, and other source data until at least 5 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region, or until at least 2 years have elapsed since the formal discontinuation of the clinical development of the product. These documents need to be retained for a longer period of time if required by applicable regulatory authorities or by agreement with the sponsor.

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

14.7.2. Sample Retention

Samples will only be used for purposes related to this trial.

All blood samples will be destroyed upon completion of the CTR, except for residual ADA samples, which will be stored until approval of market authorization because further characterization of the antibody response may be requested by the health authorities. Identifiable samples can be destroyed at any time at the request of the patient.

14.8. Changes to the Protocol

This protocol cannot be altered or changed except through a formal protocol amendment, which requires the written approval of the sponsor. The protocol amendment must be signed by the investigator and approved by the IRB/IEC before it may be implemented. Protocol amendments will be filed with the appropriate regulatory agency(s) having jurisdiction over the conduct of the trial.

14.9. Use of Information and Publication

All information concerning dasiglucagon, the sponsor's operations, patent applications, formulae, manufacturing processes, basic scientific data, and formulation information supplied by the sponsor or its designee to the investigator and not previously published, is considered confidential and remains the sole property of the sponsor. Case report forms also remain the property of the sponsor. The investigator agrees to use this information for purposes of trial execution through finalization and will not use it for other purposes without the written consent of the sponsor.

The information developed in this trial will be used by the sponsor in connection with the continued development of dasiglucagon and thus may be disclosed as required to other clinical investigators or government regulatory agencies.

The information generated by this trial is the property of the sponsor. Publication or other public presentation of dasiglucagon data resulting from this trial requires prior review and written approval of the sponsor. Abstracts, manuscripts, and presentation materials should be provided to the sponsor for review and approval at least 30 days prior to the relevant submission deadline.

It is agreed that the results of the trial will not be submitted for presentation, abstract, poster exhibition or publication by the investigator until the sponsor has reviewed and commented on such a presentation or manuscript for publication.

15. FINAL CLINICAL TRIAL REPORT

The sponsor will retain ownership of the data.

The final CTR will be prepared and reviewed in cooperation with the signatory investigator. The coordinating investigator will be appointed by the sponsor to review and sign the CTR on behalf of all participating investigators. This report will include a summary of the trial results based on a statistical evaluation and clinical assessment of the protocol-defined endpoints.

The final CTR may be submitted to the regulatory authorities.

16. ETHICAL AND LEGAL CONSIDERATIONS

16.1. Declaration of Helsinki and Good Clinical Practice

This trial will be conducted in compliance with the November 2016 ICH Guidance for Industry E6(R2) GCP (including archiving of essential trial documents), the 2013 version of the Declaration of Helsinki, and the applicable regulations of the country(ies) in which the trial is conducted.

See Appendix B for regulation and guidelines.

16.2. Patient Information and Informed Consent

According to the Declaration of Helsinki and ICH GCP, patients' parent(s)/guardian must provide their written informed consent prior to enrollment in a clinical trial and before any protocol-specified procedures are performed. Patients' parent(s)/guardian must declare their consent by personally signing and dating the ICF. The written ICF will embody the elements of informed consent as described in the Declaration of Helsinki and will also comply with local regulations.

Each patient's parent(s)/guardian should be made aware by the investigator of the nature of the trial (objectives, methods, and potential hazards and benefits) and the procedures involved using the information on the ICF. Information should be given in both oral and written form whenever possible and deemed appropriate by the IRB/IEC. Patients' relatives, or, if necessary, legal representatives must be given ample opportunity to inquire about details of the trial.

Patient information and the ICF must be in a language fully comprehensible to the prospective patient's parent(s)/guardian. The written information must be provided to the patient's parent(s)/guardian to give him or her sufficient time to understand the information and to prepare questions before being asked for his or her consent. The investigator must confirm that the text was understood by the patient's parent(s)/guardian. The patient's parent(s)/guardian will then sign and date the IRB/IEC-approved consent form indicating that he or she has given his or her consent for his or her child to participate in the trial. The signature confirms that the consent is based on information that has been understood. The form will also be signed by the investigator obtaining the consent and annotated with the trial patient number. Each signed patient parent(s)/guardian ICF must be kept on file by the investigator for possible inspection by regulatory authorities, the sponsor, and/or the sponsor's designee. Collection of informed consent has to be documented on the eCRF.

Furthermore, the patient's parent(s)/guardian will be informed that if he or she wishes to drop-out or withdraw his or her child (see Section 9.2.3) at any time during the trial, this will not have any negative consequences. Patients may be withdrawn by the investigator if any change

related to safety or ethics precludes further participation in the trial. Patients' parent(s)/guardian will be asked to agree to a final assessment in the event of an early termination of the trial.

If information becomes available that may be relevant to the patient's willingness to continue participating in the trial, the investigator must inform the patients' parent(s)/guardian in a timely manner, and a revised written informed consent must be obtained.

Patients' parent(s)/guardian will be informed that data from their children's case may be stored in a computer without inclusion of their name and that such data will not be revealed to any unauthorized third party. Data will be reviewed by the monitor, an independent auditor, and possibly by representatives of regulatory authorities and/or IRBs/IECs. The terms of the local data protection legislation will be applied as appropriate.

16.3. Approval by Institutional Review Board and Independent Ethics Committee

For Investigational New Drug studies, the minimum standards of conduct and requirements for informed consent are defined in the FDA regulations.

A valid IRB/IEC must review and approve this protocol before trial initiation. Written notification of approval is to be provided by the investigator to the sponsor's monitor and project manager before shipment of investigational drug supplies, and will include the date of the committee's approval and the chairperson's signature. This written approval must consist of a completed sponsor form, IRB/IEC Approval Form, or written documentation from the IRB/IEC containing the same information.

Until written approval by the IRB/IEC has been received by the investigator, no patient may undergo any procedure not part of routine care for the patient's condition.

Protocol amendments must also be reviewed and approved by the IRB/IEC. Written approval from the IRB/IEC, or a designee, must be received by the sponsor before implementation. This written approval will consist of a completed IRB Approval Form or written documentation from the IRB/IEC containing the same information.

16.4. Finance and Insurance

Details on finance and insurance will be provided in a separate agreement between the investigator and the sponsor.

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18. ATTACHMENTS

18.1. Schedule of Events

Table 2 Schedule of Events

Period	Screening	Part 1 (2x48-hr period: double-blind, randomized, placebo-controlled)				Part 2 (21-day Open-label Active)						Follow-up[a]
Visit day		1[b]	2	3	4	5	6	11[c]	18[c]	25	Telephone Call[d]	53
Time window	Day -28 to -1	0	0	0	0	0	0	±2	±2	±3		±3
Visit #	1	2		3		4		5	6	7		8
General assessments												
Informed consent	X											
Inclusion/exclusion criteria	X	X										
Randomization exclusion criteria		X										
Demography	X											
Body weight and length[e]	X[e]	X	X	X	X	X	X	X	X	X[e]		
Medical history (including current illness[f])	X											
Concomitant medication	X	X	X	X	X	X	X	X	X	X		X
Safety Assessment												
Electrocardiogram	X	X	X	X	X	X	X	X	X	X		X
Echocardiography	X[g]											X
Vital signs	X	X[h]	X[h]	X[h]	X[h]	X[h]	X[h]	X	X	X		X
Adverse events		X	X	X	X	X	X	X	X	X	X	X
Local tolerabilty		X	X	X	X	X	X	X	X	X		
Physical examination and neurological examination	X	X	X	X	X	X	X	X	X	X		X
Fluid balance assessment[i]		Continuous (when receiving IV Glucose)										
Laboratory												
Clinical laboratory tests[j]	X						X			X		X
Anti-drug antibodies		X[k]								X		X[k]

Period	**Screening**	**Part 1 (2x48-hr period: double-blind, randomized, placebo-controlled)**				**Part 2 (21-day Open-label Active)**						**Follow-up[a]**
Visit day		**1[b]**	**2**	**3**	**4**	**5**	**6**	**11[c]**	**18[c]**	**25**	**Telephone Call[d]**	**53**
Time window	**Day -28 to -1**	**0**	**0**	**0**	**0**	**0**	**0**	**±2**	**±2**	**±3**		**±3**
Visit #	**1**	**2**		**3**		**4**		**5**	**6**	**7**		**8**
Pharmacokinetics/drug exposure						X[l]	X			X		
Efficacy												
Continuous glucose monitoring	X (for 24 hours prior to randomization)	Continuous										
Self-monitoring plasma glucose		X										
IV GIR adjustment		Continuous (until weaned off)										
Trial Materials and Reminders												
Randomization		X										
Dispense patient diary								Upon discharge[m]				
Diary recording								Continuous after discharge from hospital				
Dispensing of trial product		X		X		X						
Drug accountability				X		X		X	X	X		

Abbreviations: GIR = glucose infusion rate; IV = intravenous; SOC = standard of care; SpO_2 = blood oxygen saturation level

Note: Unscheduled visits can occur at any time if the investigator deems it necessary for patient safety.

a The Follow-up Visit will only be performed for patients who will not enter the extension trial.
b After screening, eligible patients will complete a minimum 24-hour run-in period to confirm the IV glucose randomization requirement (≥10 mg/kg/min). All non-nutritional carbohydrates and carbohydrate fortification of feeds are prohibited during this 24-hour run-in period.
c Visits 5 (Day 11) and 6 (Day 18) can be converted to phone visits at the investigator's discretion.
d Patients who are discharged from the hospital before Day 25 will be contacted by the investigator by telephone the day after discharge. The investigator will ask the parent(s)/guardian if they have any questions about the trial procedures and if their child has experienced any AEs.
e Body length will be measured at screening and at the End of Treatment Visit.
f For CHI diagnosis: Data on biochemical parameters, genotyping results, and information about PET CT should be captured when available.
g An echocardiogram performed within 1 month of screening can be used.
h Vital signs should be measured at 6 ± 1, 12 ± 2, and 24 ± 4 hours after initiation of the trial drug and every 8 ± 2 hours hereafter.
i Fluid balance assessments are to be performed and documented every 8 hours as long as the patient is receiving IV glucose.
j Clinical laboratory tests include hematology and biochemistry.
k Blood test for anti-drug antibodies to be performed prior to dosing. Any anti-dasiglucagon antibody-positive patient (treatment induced or treatment boosted) will be monitored at an additional Follow-up Visit preferably 16 weeks after last ADA-positive sample. Patients completing the trial before the ADA screening and confirmatory assays have been approved by the FDA and who do not continue treatment in the long-term extension trial will have this additional visit 16 weeks after the end of trial visit (visit 8).

l Sampling for drug exposure at Day 5 is only applicable for patients with a body weight of ≥4 kg. This sample should only be collected if it does not compromise the total amount of blood drawn according to Section 11.2.3.1.
m The diary should be dispensed if a patient is discharged from the hospital during Part 2. At subsequent visits, the parent(s)/guardian will return the completed diary and obtain a new one.

18.2. Investigator's Agreement

PROTOCOL NUMBER:	ZP4207-17103
PROTOCOL TITLE:	A Randomized Trial in 2 parts: Double-Blind, Placebo-Controlled, Crossover Part 1 and Open-label Part 2, Evaluating the Efficacy and Safety of Dasiglucagon for the Treatment of Children with Congenital Hyperinsulinism
FINAL PROTOCOL:	Version 13.0, 04-Jun-2021

The undersigned acknowledges possession of and has read the product information (e.g., IB) on the trial drug and have discussed these data with the trial monitor. Having considered fully all the available information, the undersigned considers that it is ethically justifiable to give the trial drug to selected patients in his or her care, according to the trial protocol.

- He or she agrees to use the trial material, including trial drug, only as specified in the protocol. He or she understands that changes cannot be made to the protocol without prior written approval of Zealand.
- He or she understands that any deviation from the protocol may lead to early termination of the trial.
- He or she agrees to report to Zealand within time any clinical AE or abnormal laboratory value that is serious, whether or not considered related to the administration of trial drug.
- He or she agrees to comply with Zealand and regulatory requirements for the monitoring and auditing of this trial.

In addition, he or she agrees that the trial will be carried out in accordance with the revised Declaration of Helsinki (2013) and the local laws and regulations relevant to the use of new therapeutic agents.

I, the undersigned, have carefully read this protocol and agree that it contains all the necessary information required to conduct the trial.

Principal Investigator:

Printed Name: _____

Signature: _____

Date: _____

Investigator's name and address (stamp)

APPENDICES

A. Address List
B. Regulations and Good Clinical Practice Guidelines

A. Address List

Name: [redacted]
Address: [redacted]
[redacted]
E-mail: [redacted]
Telephone: [redacted]

B. Regulations and Good Clinical Practice Guidelines

1. Regulations

Refer to the following United States Code of Federal Regulations (CFR):

- FDA Regulations 21 CFR, Parts 50.20 – 50.27
  Subpart B – Informed Consent of Human Subjects
- FDA Regulations 21 CFR, Parts 56.107 – 56.115
  Part 56 – Institutional Review Boards
  Subpart B – Organization and Personnel
  Subpart C – IRB Functions and Operations
  Subpart D – Records and Reports
- FDA Regulations 21 CFR, Parts 312.50 – 312.70
  Subpart D – Responsibilities of Sponsors and Investigators

Refer to the following European Directives [and applicable regulations/guidances]:

- European Directive 2001/20/EC and related guidance documents
- European Directive 2005/28/EC and related guidance documents

2. Good Clinical Practice Guidelines

ICH GCP guidelines can be found at the following URL:

http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E6/E6_R2__Step_4_2016_1109.pdf