PROTOCOL TITLE: A Two-Period, Open-label Trial Evaluating the Efficacy and Safety of Dasiglucagon for the Treatment of Children with Congenital Hyperinsulinism

NCT Number: NCT03777176

Document name	Date	Approval/submission status	Comments
Version 1.0 – Global	14-Dec-2017	Submitted in the US only	
Version 2.0 – Global	22-May-2018	Submitted in all countries except France Approved in the US	Updated based on FDA feedback. First version submitted outside the US
Version 3.0 – UK only	05-Jul-2018	Approved in the UK	Minor updates based on MHRA feedback
Version 4.0 – DE, FR, IL only	25-Jul-2018	Approved (AMG and EC) in DE. Approved in IL	Minor updates based on BfArM feedback
Version 5.0 – Global	14-Dec-2018	Approved in the US and UK First version submitted in FR in Jan-2019 Not submitted in Germany and Israel	This version includes the updates to the stat section regarding primary endpoint (and analysis) and interim analysis based on FDA feedback. The changes included in the local versions 3.0 and 4.0 have been included in version 5.0
Version 6.0 – DE only	25-Mar-2019	Submitted to MPG Germany	Device adapted protocol to fulfill the requirements from BfArM MPG. New section 19 added + additional device related safety reporting added in section 11.

Document name	Date	Approval/submission status	Comments
Version 7.0 – All countries except DE	03-Jun-2019	Submitted in all countries except Germany	Main changes: - Primary analysis changed (FDA requested) - Endpoints aligned to 103 - Clarifications to hypo reporting and electronic SMPG data - Clarified that SOC open label CGM is not allowed - Immunogenicity strategy updated
Version 8.0 – DE only	05-Jul-2019	Submitted in Germany	Local German protocol combining protocol versions 6.0 and 7.0.
Version 9.0 – All countries except DE	19-Sep-2019	Submitted in all countries except Germany	- Additional ECG and vital signs assessments (FDA request) - Immunogenicity strategy updated (FDA request)
Version 10.0 – DE only	11-Oct- 2019	Submitted in Germany	Local German protocol combining protocol versions 8.0 and 9.0.
Version 11.0 – All countries except DE	06-Mar-2020	Submitted in all countries except Germany	- Interim analyses taken out - Immunogenicity section updated
Version 12.0 – DE only	11-Mar-2020	Submitted in Germany	Local German protocol combining protocol version 10.0 and changes according to protocol version 11.0.
Version 13.0 – All countries except DE	12-Nov-2020	To be submitted in all countries except Germany	- Second key secondary efficacy endpoint moved to only be a secondary efficacy endpoint. - All endpoints related to intake of gastric carbohydrates will only described in the subgroup of patients who have a gastrostomy/NG tube at screening
Version 14.0 – DE only	12-Nov-2020	To be submitted in Germany	Local German protocol combining protocol versions 12.0 and 13.0

PROTOCOL

PRODUCT NAME/NUMBER:	Dasiglucagon
PROTOCOL NUMBER:	ZP4207-17109
IND NUMBER:	135869
EUDRACT NUMBER:	2017-004547-21
DEVELOPMENT PHASE:	Phase 3
PROTOCOL TITLE:	A Two-Period, Open-label 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 Final Version 13.0, 12-Nov-2020 (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

REVISION HISTORY

PROTOCOL TITLE:	A Two-Period, Open-label 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
	This updated protocol version 13.0 is applicable for all countries except Germany, and includes:
AMENDMENT No. 1	Final Version 2.0, 22-May-2018 (All countries)
AMENDMENT No. 2	Final Version 3.0, 05-Jul-2018 (United Kingdom)
AMENDMENT No. 3	Final Version 4.0, 25-Jul-2018 (France, Germany, and Israel)
AMENDMENT No. 4	Final Version 5.0, 14-Dec-2018 (All countries)
AMENDMENT No. 5	Final Version 6.0, 25-Mar-2019 (Germany)
AMENDMENT No. 6	Final Version 7.0, 03-Jun-2019 (All countries except Germany)
AMENDMENT No. 7	Final Version 8.0, 05-Jul-2019 (Germany)
AMENDMENT No. 8	Final Version 9.0, 19-Sep-2019 (All countries except Germany)
AMENDMENT No. 9	Final Version 10.0, 11-Oct-2019 (Germany)
AMENDMENT No. 10	Final Version 11.0, 06-Mar-2020 (All countries except Germany)
AMENDMENT No. 11	Final Version 12.0, 11-Mar-2020 (Germany)
AMENDMENT No. 12	Final Version 13.0, 12-Nov-2020 (All countries except Germany)

1. APPROVAL SIGNATURES

PROTOCOL NUMBER:	ZP4207-17109
PROTOCOL TITLE:	A Two-Period, Open-label Trial 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]
Clinical Trial Manager
Zealand Pharma A/S

Date

[redacted]
Medical Director
Zealand Pharma A/S

Date

[redacted]
Manager Biostatistics
Premier Research

Date

2. SYNOPSIS

PRODUCT NAME/NUMBER	Dasiglucagon
PROTOCOL NUMBER	ZP4207-17109
EUDRACT NUMBER	2017-004547-21
DEVELOPMENT PHASE	Phase 3
PROTOCOL TITLE	A Two-Period, Open-label Trial 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 administered as a subcutaneous (SC) infusion in reducing hypoglycemia in children with CHI. Secondary: • To evaluate the safety and tolerability of dasiglucagon administered as an SC infusion in children with CHI • To evaluate the efficacy of dasiglucagon in reducing glucose requirements • To investigate quality of life and resource utilization
TRIAL DESIGN	This is a 2-period, open-label trial to evaluate the efficacy and safety of dasiglucagon in children between the ages of 3 months and 12 years (both inclusive) with CHI. Patients are to experience frequent (≥3 events per week) episodes of hypoglycemia despite standard of care (SOC) medications to be eligible for this trial, and are to have previously undergone sub-total pancreatectomy for CHI or be treated with a non-surgical approach, having been evaluated as not eligible for pancreatic surgery. After screening and eligibility assessments (during which a 2-week baseline for plasma glucose [PG] will be established), patients will be randomly assigned in a 1:1 ratio to continue receiving SOC alone or SOC plus dasiglucagon for 4 weeks. In Treatment Period 2, all patients will receive SOC plus dasiglucagon for 4 weeks. At the beginning of Treatment Period 1 (Week 1), all patients will be hospitalized for 1-2 days. Patients assigned to SOC plus dasiglucagon treatment will have dasiglucagon infusion initiated and titrated, and will be trained in the use of the infusion pump, continuous glucose monitoring (CGM) sensor, and glucose meter, and supervised. Patients assigned to SOC alone will receive a similar degree of supervision alongside the training in the use of the CGM sensor and glucose meter. This period can be extended for both treatment groups if dasiglucagon titration has not been finalized or if training of the family/caregivers has not been completed satisfactorily. Patients originally assigned to receive SOC alone in Treatment Period 1 will also be hospitalized for the first 2 days of Treatment Period 2 (Week 5), and will have dasiglucagon infusion initiated and titrated, and will be trained in the use of the infusion pump. This period can be extended if dasiglucagon titration or pump training has not been completed. Regardless of whether dasiglucagon is initiated during Treatment Period 1 or Treatment Period 2, SOC CHI medications should subsequently be kept constant throughout the trial. Adjustments to gastric and oral feeds are permitted. Patients' parent(s)/guardian will be trained to perform PG assessments. Events of hypoglycemia will be documented by self-monitored PG (SMPG) measurements. The extent of hypoglycemia (episodes and percent time below the threshold) will be further quantified by CGM measurements during the 2 weeks before randomization (baseline), Weeks 2-4 in Treatment Period 1, and Weeks 6-8 of Treatment Period 2. The CGM results will be masked.

	Patients completing the trial will (pending investigator confirmation of continued positive benefit-risk balance) be offered to enter a long-term extension trial (Trial ZP4207-17106) to continue dasiglucagon treatment. Patients who do not continue in the long-term extension trial will have a Follow-up Visit performed 4 weeks after stopping dasiglucagon treatment.
PLANNED NUMBER OF PATIENTS	A sufficient number of patients will be screened to reach a total maximum of 32 randomized patients with CHI. Patients who withdraw prematurely will not be replaced.
TRIAL ENTRY CRITERIA	Eligible patients will be male or female between 3 months and 12 years of age (both inclusive) at screening with an established and documented diagnosis of CHI and who are experiencing ≥3 events of hypoglycemia per week (events of PG <70 mg/dL [<3.9 mmol/L]) according to investigator's evaluation. Patients are to have previously undergone sub-total pancreatectomy or being treated with a non-surgical approach, having been evaluated as not eligible for pancreatic surgery.
INVESTIGATIONAL PRODUCT	Dasiglucagon injection 4 mg/mL in a 3 mL vial containing 1 mL.
REFERENCE PRODUCT	None
TREATMENT REGIMENS	Dosing of dasiglucagon will approximate continuous infusion by delivering small doses at frequent intervals via an infusion pump. During the trial, PG assessments will be performed regularly (at least 3 times daily, preferably before main meals, and as instructed by the investigator); adjustments to the gastric dextrose infusion and/or trial drug will occur at the investigator's discretion. Dasiglucagon treatment will be initiated at 10 µg/hr (t=0). Every 2 hours (t=2, 4, 6, etc.), the dose will be increased by an additional 10 µg/hr until either: 1. The patient is weaned off entirely from gastric dextrose infusion and/or glucose-fortified feeds, or 2. Plasma glucose during the last 2 hours was consistently above 120 mg/dL (6.7 mmol/L), or 3. The maximum trial drug product infusion rate of 70 µg/hr is reached, or 4. Adverse events (AEs) emerge that are considered to be related to dasiglucagon (e.g., nausea and vomiting) and limit further dose escalation The dose of dasiglucagon should not be escalated beyond the treatment objectives of PG in the range of 70 to 120 mg/dL (3.9-6.7 mmol/L) while approaching a normal feeding regimen according to age. The 2-hour dose-adjustment interval will allow plasma drug levels to approach approximately steady-state before the dose is further increased. The maximum cumulative dose over the first 24 hours is 1.26 mg. After the first 24 hours, the dose of dasiglucagon can still be modified to optimize each patient's treatment and to reduce gastric dextrose infusions. The maximum dose of dasiglucagon will be 70 µg/hr, corresponding to a maximum of 1.68 mg per day.
PLANNED TRIAL SITES	Up to 14 sites in the United States, Europe, and Israel.

CRITERIA FOR EVALUATION	Primary efficacy endpoint: *Treatment Period 1* • Hypoglycemia event rate, defined as average weekly number of hypoglycemic events (PG <70 mg/dL or 3.9 mmol/L) during Weeks 2-4, as detected by SMPG Key secondary efficacy endpoints: *Treatment Period 1* • Increase in fasting tolerance (time from beginning of meal to the beginning of the first continuous 15-minute CGM reading <70 mg/dL [3.9 mmol/L]) • CGM percent time in range 70-180 mg/dL (3.9-10.0 mmol/L) during Weeks 2-4 • Clinically significant hypoglycemia event rates, defined as average weekly number of events <54 mg/dL (3.0 mmol/L), as detected by SMPG during Weeks 2-4
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 pre-specified 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. <u>Analysis Populations</u> Three analysis populations are defined for this trial: The Safety Set: defined as all patients administered any randomized treatment. This population will be used to provide descriptive summaries of safety data. Patients will be summarized by treatment period according to treatment received. The Full Analysis Set (FAS): defined as all patients in the Safety Set who have a valid baseline efficacy assessment. This population will be used to analyze efficacy data. Patients will be analyzed by treatment period according to planned treatment. The Per Protocol (PP) Analysis Set: defined as all patients in the FAS without any major protocol deviations. This population will be used to analyze primary and key secondary endpoints as a supportive analysis. Patients will be analyzed according to planned treatment for the first treatment period only. The analysis of efficacy will be separated into 3 categories: 1. Treatment Period 1, FAS 2. Treatment Period 1, PP (primary and key secondary endpoints only) 3. Treatment Period 2, FAS In Treatment Period 1 (categories 1 and 2), the 2 treatment arms will be compared by a testing procedure starting with the primary and subsequently continuing through the key secondary endpoints if the hypotheses are rejected. In Treatment Period 2 (category 3), only descriptive analyses will performed. Graphical presentations of selected analyses in the 2 treatment periods will be prepared, and will be discussed in the formal statistical analysis plan (SAP).

	Efficacy Analyses *Primary Endpoint (Treatment Period 1)* The primary efficacy endpoint is the hypoglycemia event rate during Weeks 2-4 of Treatment Period 1. A hypoglycemia event is defined as PG <70 mg/dL or 3.9 mmol/L, as detected by SMPG. Baseline is defined as the average weekly number of hypoglycemic events during the 2-week baseline period. Weeks 2-4 of Treatment Period 1 is defined as the average weekly number of hypoglycemic events across the last 3 weeks of the treatment period. The hypoglycemic event rate will be analyzed by using negative binomial regression, with treatment group as a fixed effect and baseline hypoglycemic rate as a covariate. The null hypothesis is that there is no difference in the incidence of average weekly number of hypoglycemic events between the 2 treatment groups, which will be tested at the significance level of $\alpha = 0.05$. The primary analysis will estimate the treatment effect based on the de facto (treatment policy) estimand. All available data in the form of actual measurements will therefore be included in the analysis, irrespective of adherence to treatment or use of subsequent therapy. As a sensitivity analysis, the primary endpoint will also be analyzed without imputation of missing data; however, this analysis will not be included in the fixed-sequence hierarchical testing strategy. Similarly, the primary endpoint may be analyzed excluding post-baseline data collected after trial drug discontinuation/completion. *Key Secondary Endpoints (Treatment Period 1)* The key secondary endpoint of clinically significant hypoglycemia (<54 mg/dL [3.0 mmol/L]) event rates will be analyzed using a negative binomial regression, with treatment group as a fixed effect and baseline hypoglycemic rate as a covariate. Percent time in range (i.e., the percent time between 70 mg/dL [3.9 mmol] and 180 mg/dL [10.0 mmol], inclusive, as measured by CGM, where percent time is calculated as [number of minutes in range/total number of minutes patient is wearing CGM] * 100%) will be analyzed by using an ANCOVA, with treatment group and region as fixed effects and baseline time in range as a covariate. Increase in fasting tolerance (i.e., change from baseline in time from meal to PG <70 mg/dL) will be analyzed similarly using an ANCOVA, with treatment group and region as fixed effects and baseline fasting tolerance as a covariate. For the remaining efficacy endpoints, continuous and categorical endpoints will be presented using summary statistics or frequencies, respectively; no inference will be performed. Safety Analyses 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 dasiglucagon 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, incidence of anti-drug antibodies [ADAs]), 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. A SAP will be prepared to provide further details on the methods for statistical analysis, including rules for handling multiplicity and missing data.
SAMPLE SIZE DETERMINATION	Patients will be randomized into the trial if they have at least 3 events of hypoglycemia on average per week, as recorded in the diary during the 2 weeks prior to randomization. It is assumed that patients continuing on standard of care will maintain a similar level through Treatment Period 1, with the number of hypoglycemia events (PG <70 mg/dL or 3.9 mmol/L) during Weeks 2-4, as detected by SMPG following a Poisson distribution

	with a mean of 9. The trial is powered to detect a treatment effect of 50%, hence, assuming that the number of hypoglycemia events reported for patients in the dasiglucagon group during Weeks 2-4 will follow a Poisson distribution with a mean of 4.5. At the final analysis, 32 patients will have 99% power testing at a 0.05 significance level. The overall alpha level is strongly controlled in this setting, remaining at or below 0.05.
TRIAL AND TREATMENT DURATION	The overall trial duration is expected to be 15 months. The sequence and maximum duration of the trial periods will be as follows: 1. Screening: at least 14 days up to 28 days 2. Treatment Period 1: 4 weeks 3. Treatment Period 2: 4 weeks 4. Follow-up Period: 4 weeks The maximum trial duration for each patient is approximately 16 weeks. The dasiglucagon treatment duration for each patient is 4 or 8 weeks.

3. TABLE OF CONTENTS

3.1. LIST OF TABLES

3.2. LIST OF FIGURES

4. LIST OF ABBREVIATIONS

ADA	anti-drug antibodies
AE	adverse event
ALT	alanine aminotransferase
ANCOVA	analysis of covariance
$AOC_{glucose}$	area over the glucose curve up to 70 mg/dL
AST	aspartate aminotransferase
AUC	area under the plasma concentration-time curve
$AUC_{0\text{-}inf}$	area under the plasma concentration-time curve from time zero to infinity
CGM	continuous glucose monitoring
CHI	congenital hyperinsulinism
C_{max}	maximum observed concentration
CRA	clinical research associate
CRO	contract research organization
CTR	clinical trial report
DMC	data monitoring committee
ECG	electrocardiogram
eCRF	electronic case report form
EDC	electronic data capture
eGFR	estimated glomerular filtration rate
ER	emergency room
FAS	full analysis set
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GI	gastrointestinal
H	hypothesis
HbA1c	glycated hemoglobin
HIV	human immunodeficiency virus
HNF4A-DM	hepatocyte nuclear factor 4 alpha diabetes mellitus
HNF4A-IGT	hepatocyte nuclear factor 4 alpha impaired glucose tolerance

IB	investigator's brochure
ICF	informed consent form
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IEC	independent ethics committee
IFU	instructions for use
IM	intramuscular(ly)
IRB	institutional review board
ISF	Investigator Site File
ISPE	International Society of Pharmaceutical Engineers
IV	intravenous(ly)
J2C	jump to control
MedDRA	Medical Dictionary for Regulatory Activities
MI	multiple imputation
MNAR	missing not at random
NG	nasogastric
PD	pharmacodynamics(s)
PedsQL™	Pediatric Quality of Life Inventory
PG	plasma glucose
PK	pharmacokinetic(s)
PP	Per protocol
PT	preferred term
QoL	quality of life
RBC	red blood cell
RSI	Reference Safety Information
SAE	serious adverse event
SAP	statistical analysis plan
SC	subcutaneous(ly)
SD	standard deviation
SEM	standard error of the mean
SMPG	self-monitored plasma glucose

SpO_2	blood oxygen saturation level
SOC	standard of care
SUSAR	Suspected unexpected serious adverse reaction
ULN	upper limit of normal
Zealand	Zealand Pharma A/S

5. INTRODUCTION

5.1. 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 genes have been described to cause CHI, which can be either focal (only a small area of the pancreas is affected) or diffuse (most of the pancreas is affected). The condition can persist into adulthood; however, the severity generally decreases with age due to the increased insulin requirements and/or increased insulin resistance, and CHI is thus primarily a pediatric disease with regard to medical treatment needs. 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 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.

5.2. 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 trial[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, indicating the serious and long-term consequences of the procedure.

First-line medical treatment 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 non-responders, 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]

Glucagon has been shown to be effective in the treatment of CHI. The glycogenolytic effect of glucagon and its ability to increase PG levels has been confirmed in children with CHI or neonatal hypoglycemia,[8,9] and administration of reconstituted glucagon (via intravenous [IV] infusion or as repeated subcutaneous [SC] injection) is often used in the initial phase during the establishment of CHI diagnosis and to stabilize patients with CHI before surgery or initiation of other medical treatments.[10] 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 complicated by the fact that currently available glucagon products are unstable and form fibrils within hours after reconstitution.[12] This fibril formation may lead to infusion set clotting, catheter obstruction, and dosing errors that may cause acute severe hypoglycemia. Catheter obstruction and occlusion because of glucagon fibril formation and aggregation 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 SC glucagon experienced catheter occlusion.[6] In a home-care setting, this fibril formation and associated risk of dosing errors carry the risk of hypoglycemic events, which is a major barrier for using currently marketed glucagon products for long-term treatment of patients with CHI.

With respect to long-term glucagon treatment in CHI, there are a few reports on home treatment with subcutaneously infused glucagon in children with CHI over extended periods (years) that suggest benefit in patient care with a potentially good safety profile as compared to diazoxide and octreotide.[6,11,13] While this attests to the potential clinical relevance of long-term glucagon treatment in CHI, the use of SC infusion of currently marketed glucagon is severely limited by the issues with fibril formation and solution instability of recombinant glucagon as described previously.

5.3. Dasiglucagon for the Treatment of Congenital Hyperinsulinism

5.3.1. Dasiglucagon

Dasiglucagon is a peptide analog of human glucagon that is 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, compatibility/in-use studies have been performed with dasiglucagon 4 mg/mL in the 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 was 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.

5.3.2. 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 normal and insulin-induced hypoglycemic animals, similar to that of glucagon. The effects of dasiglucagon and glucagon were investigated in an insulin-induced hypoglycemic rat model, which is considered particularly relevant to characterize the use of dasiglucagon for the treatment of CHI because it mimics the inappropriate insulin to PG levels in CHI and resultant hypoglycemia. The onset of PG increase with dasiglucagon was rapid and similar to that observed for glucagon, confirming comparable pharmacodynamics.

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.

5.3.3. 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 and 3 phase 2 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 intramuscular (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 trial.

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 compared to an active comparator, Lilly Glucagon™. 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 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, 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 $AUC_{0\text{-}inf}$. 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 after insulin-induced hypoglycemia achieved a PG level of at least 70 mg/dL (3.9 mmol/L) at all dose levels of dasiglucagon, 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 pre-specified dose comparisons. This was likely an effect of the higher total drug exposure ($AUC_{0\text{-}inf}$) of dasiglucagon mentioned previously.

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.

5.3.4. 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.

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]

5.3.5. 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 to control blood glucose. Long-term subcutaneous infusion of dasiglucagon through a pump may be an attractive alternative or addition to diazoxide and octreotide, as it may reduce the dependency on intensive nutritional support whilst maintaining euglycemia by harnessing physiological mechanisms for combating hypoglycemia. It is anticipated that reduced need for frequent tube feedings or continuous gastric infusion of nutrients, and increased fasting tolerance will be demonstrated, together with improvements in the quality of life of the patients and their families/caregivers. If long-term euglycemia is achieved with medical therapy, pancreatectomy for the treatment of diffuse CHI could eventually be avoided, or at least postponed beyond the neonatal or very young infant period. In one cohort of non-surgically 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.

5.3.6. Anticipated Risks of Dasiglucagon in the Treatment of CHI

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 have been observed in clinical trials with dasiglucagon, but not hypertension. On the contrary, several episodes of hypotension have been noted in the clinical program.

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 observed with many injectable peptides. In the phase 1 and 2 clinical trials, injection site reactions occurred with a low frequency in all treatment groups (dasiglucagon and marketed glucagons), irrespective of dose.

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 after 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,[14] sustained exposure to high levels of glucagon may lead to development of 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).

5.3.7. 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/parents 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 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 enabling convenient and reliable long-term treatment via a pump device in a home setting, which holds the potential to delay and ultimately avoid pancreatectomy and its related exo/endocrine complications, particularly the development of insulin-dependent diabetes.

Dasiglucagon may overall provide significant added benefit in the treatment of CHI relative to currently marketed glucagon products by enabling long-term reliable SC infusion to control PG. The proposed trial population is still experiencing hypoglycemia despite medical treatments being escalated to the highest therapeutically permissible or tolerated doses, or despite having undergone subtotal pancreatectomy. Therefore, these patients are dependent on continuous or very frequent delivery of carbohydrates, often through invasive routes (NG tube or gastrostomy). This limits their ability to lead normal lives, including participating in everyday activities, and therefore, impacts their development. For this trial population, the major and clinically relevant benefit is the expected reduction in number and volume of nutritional interventions while avoiding hypoglycemia. The reduced volume of nutritional interventions should limit the risk of volume overload, especially in patients treated with significant doses of diazoxide. Achievement of euglycemia could lead to reduction of other CHI medication, further limiting the potential for adverse events associated with those treatments. In addition, the need for pancreatectomy, or re-surgery in those who already underwent pancreatic surgery is reduced and potentially eliminated.

Overall, the benefit to risk ratio for patients entering the ZP4207-17109 trial is considered acceptable.

6. OBJECTIVES AND ENDPOINTS

6.1. Objectives

6.1.1. Primary Objective

To evaluate the efficacy of dasiglucagon administered as subcutaneous (SC) infusion in reducing hypoglycemia in children with CHI.

6.1.2. Secondary Objectives

Secondary objectives are:

- To evaluate the safety and tolerability of dasiglucagon administered as an SC infusion in children with CHI
- To evaluate the efficacy of dasiglucagon in reducing glucose requirements
- To investigate quality of life and resource utilization

6.2. Endpoints

6.2.1. Primary Endpoint

Treatment Period 1

- Hypoglycemia event rate, defined as average weekly number of hypoglycemic events (PG <70 mg/dL or 3.9 mmol/L) during Weeks 2-4, as detected by self-monitored PG (SMPG)

6.2.2. Key Secondary Efficacy Endpoints

Treatment Period 1

- Increase in fasting tolerance (time from beginning of meal to the beginning of the first continuous 15-minute continuous glucose monitoring [CGM] reading <70 mg/dL [3.9 mmol/L])
- CGM percent time in range 70-180 mg/dL (3.9-10.0 mmol/L) during Weeks 2-4
- Clinically significant hypoglycemia event rates, defined as average weekly number of events <54 mg/dL (3.0 mmol/L), as detected by SMPG during Weeks 2-4

6.2.3. Secondary Efficacy Endpoints

Treatment Period 1

- Total amount of gastric carbohydrates administered (via nasogastric [NG] tube or gastrostomy) per week to treat hypoglycemia during Weeks 2-4
- Rate of gastric carbohydrate administrations (NG tube or gastrostomy) per week to treat hypoglycemia during Weeks 2-4
- Extent of hypoglycemia (area over the glucose curve [$AOC_{glucose}$] below 70 mg/dL [3.9 mmol/L]) as measured by CGM during Weeks 2-4
- Extent of hypoglycemia (area over the glucose curve [$AOC_{glucose}$] below 54 mg/dL [3.0 mmol/L]) as measured by CGM during Weeks 2-4
- Amount of nightly (midnight to 6 am) gastric carbohydrates administered (NG tube or gastrostomy) per week during Weeks 2-4

- Total amount of gastric carbohydrates administered (NG tube or gastrostomy) per week during Weeks 2-4
- CGM percent time in hypoglycemia (<70 mg/dL or 3.9 mmol/L) during Weeks 2-4
- Rate of hypoglycemic episodes, defined as number of episodes <70 mg/dL (3.9 mmol/L) for 15 minutes or more per week, as measured by CGM during Weeks 2-4

Treatment Period 2

- CGM percent time in hypoglycemia (<70 mg/dL or 3.9 mmol/L) during Weeks 6-8
- Rate of weekly number of gastric carbohydrate administrations (NG tube or gastrostomy) per week to treat hypoglycemia during Weeks 6-8
- Rate of hypoglycemic events, defined as number of episodes (PG <70 mg/dL or 3.9 mmol/L) per week during Weeks 6-8, as detected by SMPG
- Rate of clinically significant hypoglycemia episodes, defined as number of episodes <54 mg/dL (3.0 mmol/L) for 15 minutes or more per week, as measured by CGM during Weeks 6-8

6.2.4. Other Efficacy Endpoints

- Number of IV glucose infusions to treat hypoglycemia per week during Weeks 2-4 of Treatment Period 1
- Emergency department visits for hypoglycemia
- Number and length of hospitalizations due to CHI or CHI-related events
- Number of out-patient visits to health care providers (family doctors, specialist, etc.) caused by CHI or CHI-related events
- Number of home visits by paramedics due to hypoglycemia
- Quality of life endpoints (PedsQL™ [Total Scale Score, Physical Health Summary Score, and Psychosocial Health Summary Score] and CHI-specific questionnaire)
- Rate of SMPG readings per week during Weeks 2-4 of Treatment Period 1

6.2.5. Safety Endpoints

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

7. TRIAL DESIGN

7.1. Overall Trial Design and Plan

This is a 2-period, open-label trial to evaluate the efficacy and safety of dasiglucagon in children between the ages of 3 months and 12 years (both inclusive) with CHI. To qualify for participation, patients must be experiencing ≥3 events of hypoglycemia per week despite standard of care (SOC) medications. The determination of hypoglycemia frequency at trial entry is according to investigator's evaluation. Furthermore, patients are to have previously undergone sub-total pancreatectomy for CHI or be treated with a non-surgical approach, having been evaluated as not eligible for pancreatic surgery.

The primary objective of this trial is to evaluate whether dasiglucagon can reduce the number and severity of hypoglycemic episodes in the investigated population.

After screening and eligibility assessments, patients will complete a 2-week CGM period to establish plasma glucose baseline. Thereafter, patients will be randomly assigned in a 1:1 ratio to continue receiving SOC alone, or SOC plus dasiglucagon for 4 weeks. In Treatment Period 2, all patients will receive SOC plus dasiglucagon for 4 weeks (see Figure 1).

At the beginning of Treatment Period 1 (Week 1), all patients will be hospitalized for 1-2 days. Patients assigned to SOC plus dasiglucagon treatment will have dasiglucagon infusion initiated and titrated, and will be trained and supervised in the use of the infusion pump, CGM sensor, and glucose meter. Patients assigned to SOC alone will receive a similar degree of supervision alongside the training in the use of the CGM sensor and glucose meter. This period can be extended for both treatment groups if dasiglucagon titration has not been finalized or if training of the family/caregivers has not been completed satisfactorily.

Patients originally assigned to receive SOC alone in Treatment Period 1 will also be hospitalized for the first 1 to 2 days of Treatment Period 2 (Week 5), and will have dasiglucagon infusion initiated and titrated, and will be trained in the use of the infusion pump. This period can be extended if dasiglucagon titration or pump training has not been completed. Regardless of whether dasiglucagon is initiated during Treatment Period 1 or 2, SOC CHI medications should subsequently be kept constant throughout the trial. Adjustments to gastric and oral feeds are permitted.

Patients' parent(s)/guardian will be trained to perform PG assessments. Events of hypoglycemia will be documented by SMPG measurements. The extent of hypoglycemia (episodes and percent time below the threshold) will be further quantified by CGM measurements during the 2 weeks before randomization (baseline), Weeks 2-4 in Treatment Period 1, and Weeks 6-8 of Treatment Period 2. The CGM results will be masked. During the period where the masked trial CGM is used, patients are not allowed to use another un-masked CGM.

Patients completing the trial will (pending investigator confirmation of continued positive benefit-risk balance) be offered to enter a long-term extension trial (Trial ZP4207-17106) to continue dasiglucagon treatment. Patients who do not continue in the long-term extension trial will have a Follow-up visit performed 4 weeks after stopping dasiglucagon treatment.

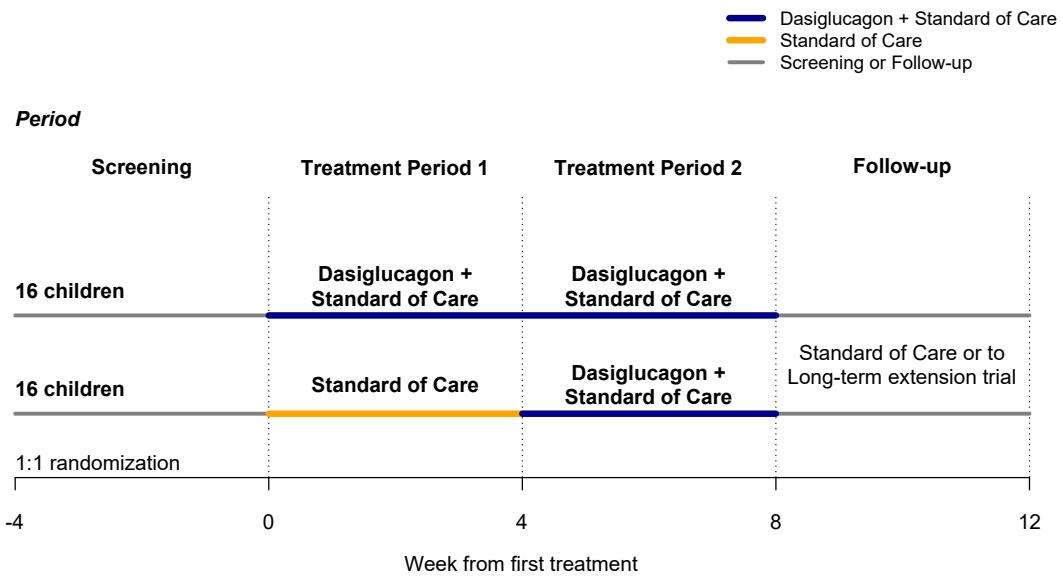

Figure 1 Trial Design

7.2. Discussion of Trial Design

Several sites across multiple geographical regions will be included to ensure results representative of the target CHI population.

A parallel design was chosen rather than a crossover design to avoid the risk of any carry-over effect and to limit the total treatment period for the patients included.

An open-label trial design was chosen because the added burden of a blinded trial design was not considered ethically justified.

7.3. Trial Sites

This trial will take place at up to 14 sites in the United States and Europe that are experienced in the treatment of CHI. A total of up to 32 patients with CHI will be randomized.

7.4. 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).

8. PATIENT POPULATION

8.1. Selection of Trial Population

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

8.2. Trial Entry Criteria

8.2.1. Inclusion Criteria

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

1. Established and documented diagnosis of CHI based on standard of care.
2. Male or female between 3 months and 12 years of age (both inclusive) at screening.
3. Has a negative serum pregnancy test at screening/baseline (only for girls of childbearing potential).
4. Sexually active female patients (and their partners) must continue to use acceptable contraception or refrain from sexual activity from screening until 30 days after the last dose of trial drug. Females must abstain from sexual activity that could result in pregnancy or agree to use acceptable methods of contraception. Abstinence can only be accepted if this is true abstinence in line with the preferred and usual lifestyle of the patient.

   Acceptable methods of contraception are:

   a) Hormonal contraceptives (e.g., oral contraceptive pill, depot, patch, intramuscular implant or injection, sponge, or vaginal ring), stabilized for at least 30 days if first use

   or

   b) Barrier method, e.g., (i) condom (male or female) and (ii) diaphragm with spermicide

   Germany*: Only highly effective methods of birth control are accepted (i.e., one that results in less than 1% per year failure rate when used consistently and correctly, such as implants, injectables, combined oral contraceptives, some intrauterine device), or sexual abstinence.*
5. Experiencing ≥3 events of hypoglycemia per week (PG <70 mg/dL [<3.9 mmol/L]) according to the investigator's evaluation.
6. Following receipt of oral and written information about the trial, the patient (depending on local IRB/IEC requirements) must provide assent and one or both parents* or guardians of the patient must provide signed informed consent before any trial-related activity is carried out.

   France, Germany, Israel*: The consent must correspond to the patient's presumed will where such a will can be ascertained.*
7. Previously undergone near-total pancreatectomy or being treated with a non-surgical approach, having been evaluated as not eligible for pancreatic surgery.
8. If somatostatin analogues or sirolimus are used, the therapy should be well established as judged by the investigator, especially when considering their biological half-life.

* If required by local regulations, both parents must give their permission unless one parent is deceased, unknown, incompetent, or not reasonably available, or when only one parent has legal responsibility for the care and custody of the child.

8.2.2. Exclusion Criteria

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

1. Previous administration of dasiglucagon (previously referred to as ZP4207).
2. Known or suspected allergy to the trial drug or related products.
3. Previous participation (randomization) in this trial.
4. Circulatory instability requiring supportive medication or presence of pheochromocytoma
   United Kingdom*: Presence of hypertension or hypotension, including circulatory instability requiring supportive medication or presence of pheochromocytoma.*
5. Requires exogenous insulin.
6. Body weight of <4 kg (8.8 lbs).
7. Documented HbA1c ≥7% subsequent to near-total pancreatectomy and within 6 months prior to screening.
8. Known or suspected presence of significant central nervous system disease/injury such that in the investigator's opinion will affect trial participation.
9. Use of systemic corticosteroids, e.g., hydrocortisone >20 mg/mg/m^2 body surface area or equivalent in the 5 days before screening.
10. Use of anti-inflammatory biological agents, or other immune-modulating agents in the 3 months prior to screening.
11. 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).
12. Any clinically significant abnormality identified on echocardiogram that in the opinion of the investigator would affect the patient's ability to participate in the trial.
13. 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.
14. Any recognized clotting or bleeding disorders.
15. Has participated in an interventional clinical trial (investigational or marketed product) within 3 months of screening or 5 half-lives of the drug under investigation (whichever comes first), or plans to participate in another clinical trial.
16. The use of prescription or non-prescription medications known to cause QT prolongation.

8.2.3. Randomization Exclusion Criteria

1. Use of glucagon within 24 hours before randomization.
2. Significant changes to CHI medications during screening.
3. An average of <3 events per week of hypoglycemia, as recorded in the diary during the 2 weeks prior to randomization.

The Randomization Visit may be rescheduled if randomization Exclusion Criterion 1 is met.

8.3. Premature Patient Withdrawal

Patients' parent(s)/guardian will be advised that they are free to withdraw their children 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. 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, the AE must be documented, reported, and followed as described in Section 11.2.

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.

8.4. 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 withdraws consent for trial treatment 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. At a minimum the patient will be asked to attend the Follow-up Visit 28 days (± 3 days) after discontinuation of trial treatment.

8.5. 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 who subsequently withdraw from the trial may not re-enter. The patient number for a withdrawn patient will not be reassigned to another patient.

9. TREATMENTS

9.1. Identification of Investigational Product

Dasiglucagon injection 4 mg/mL will be supplied by the sponsor in a 3 mL vial containing 1 mL.

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

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

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

9.1.1. Packaging and Labeling

The trial drug product will be packaged and labeled by the sponsor.

Dispensing unit configuration: 6 vials containing dasiglucagon, 4 mg/mL, packaged in an outer carton. The vial and carton will be packaged and labeled in local language indicating the content (open label).

Detailed information on all labels will be in the local language.

Storage conditions for the trial drug product will be described on the trial drug product label. The labels will supply no information about the patients. Each treatment unit (containing 6 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, International Council for Harmonisation (ICH) Good Clinical Practice (GCP) guidelines, local laws, and regulations.

9.2. Treatments Administered

In Treatment Period 1, patients will receive SOC only or SOC plus dasiglucagon for 4 weeks based on their treatment assignment. In Treatment Period 2, all patients will receive SOC plus dasiglucagon for 4 weeks.

Dosing of dasiglucagon will approximate continuous infusion by delivering small doses at frequent intervals via the infusion pump.

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

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

Plasma glucose assessments will be performed regularly (at least 3 times daily, preferably before main meals, and as instructed by the investigator) throughout the trial to guide adjustments to the gastric dextrose infusion and/or trial drug product upon the investigator's discretion.

Dasiglucagon treatment will be initiated at 10 µg/hr (t=0). Every 2 hours (t=2, 4, 6, etc.), the dose will be increased by an additional 10 µg/hr until either:

1. The patient is weaned off entirely from gastric dextrose infusion and/or glucose-fortified feeds, or
2. Plasma glucose during the last 2 hours was consistently above 120 mg/dL (6.7 mmol/L), or
3. The maximum trial drug product infusion rate of 70 µg/hr is reached, or
4. Adverse events emerge that are considered to be related to dasiglucagon (e.g., nausea and vomiting) and limit further dose escalation

The dose of dasiglucagon should not be escalated beyond reaching the treatment objectives of PG in the range of 70-120 mg/dL (3.9-6.7 mmol/L) while approaching a normal feeding regimen according to age.

The 2-hour dose-adjustment interval will allow plasma drug levels to approach approximately steady-state before the dose is further increased. The maximum dose of trial drug product that can be administered over the first 24 hours is shown in Table 1. The maximum cumulative dose over the first 24 hours is 1.26 mg.

After the first 24 hours, the dose of dasiglucagon can still be modified to optimize each patient's treatment and to reduce gastric dextrose infusions. The maximum dose of dasiglucagon will be 70 µg/hr, corresponding to a maximum of 1.68 mg per day.

Details on the administration instructions and guidelines for preparation and handling of the trial drug product are in the pharmacy manual.

Table 1 Initial 24-hour Maximum Dose of Dasiglucagon

Time (hours)	0	1	2	3	4	5	6	7	8	9	10	11
Dose (µg)	10	10	20	20	30	30	40	40	50	50	60	60
Cumulative dose (µg)	10	20	40	60	90	120	160	200	250	300	360	420

Time (hours)	12	13	14	15	16	17	18	19	20	21	22	23
Dose (µg)	70	70	70	70	70	70	70	70	70	70	70	70
Cumulative dose (µg)	490	560	630	700	770	840	910	980	1050	1120	1190	1260

Patients who complete this trial will be offered to enter a long-term extension trial (Trial ZP4207-17106) to continue dasiglucagon treatment (pending investigator confirmation of continued positive benefit-risk balance). Patients who do not continue in the long-term extension trial will have a Follow-up visit performed 4 weeks after stopping dasiglucagon treatment.

9.3. 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)/guardians on the use of the infusion pump.

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

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
PG monitoring	StatStrip Xpress2	Nova Biomedical, Waltham, MA, USA
CGM	Dexcom G4	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 is used as intended according to the CE mark, except for the age group and the disease.

The pump device will be packaged and labeled for use in investigational trials and will contain a unique device number.

9.4. Dispensing and Storage

Trial drug product supplied by the sponsor is to be dispensed exclusively for patients enrolled in this clinical trial according to the instructions of this protocol and the pharmacy manual. The investigator is responsible for dispensing the trial drug product according to the dosage scheme.

Trial drug product will be allocated using an interactive web response system (IWRS) that has been validated for the intended use under the International Society of Pharmaceutical Engineers (ISPE) GAMP guidelines, 21CFR Part 11 (FDA regulation for Electronic Records and Electronic Signatures), and the ICH Guidance E6 for Industry on Good Clinical Practice.

Dasiglucagon injection 4 mg/mL 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 refer to the pharmacy manual 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 product 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 product to any person except subinvestigators, designated trial personnel, and patients in this trial. The trial drug product may not be relabeled or reassigned for use by other patients. If any of the trial drug product 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.

9.5. Method of Assigning Patients to Treatment Groups

In Treatment Period 1, patients will be randomly assigned in a 1:1 ratio to receive either SOC only or SOC plus dasiglucagon for 4 weeks using a block randomization scheme stratified by region US/non-US). In Treatment Period 2, all patients will receive SOC plus dasiglucagon for 4 weeks. The stratification by region addresses the difference in practice of treatment for CHI, especially the prominent difference in frequency of sub-total pancreatectomy for diffuse CHI between US/non-US.

9.6. Blinding and Unblinding Treatment Assignment

This is an open-label trial. During parts of Screening, Treatment Period 1, and Treatment Period 2, patients will have CGM performed but the results will be masked.

9.7. 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.[15]

The predicted plasma concentration is expected to give a low PD response at the lowest dose level and be above maximum effect at the highest dose level. The maximum expected plasma concentrations of dasiglucagon is in the range of what is achieved following a rescue dose to adults. The doses of dasiglucagon will be titrated to meet the needs of the individual patient. The titration will stop when no additional PD effects are observed as the infusion rate is increased. The infusion rate of dasiglucagon will be monitored and adjusted to meet the needs of the individual patient throughout the trial period.

Since the dasiglucagon dose is titrated individually based on the desired PD response, it does not need to be related to any measure of the patient's size.

The titration interval of 2 hours was chosen based on the PK data of dasiglucagon showing that an approximate steady-state is expected after 2 hours.

9.8. Selection of Timing of Dose for Each Patient

Dosing details are presented in Section 9.2.

9.9. Dose Adjustment Criteria

Dose adjustment criteria are presented in Section 9.2.

9.10. Treatment Compliance

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

9.11. 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.

9.11.1. Permitted Therapies

Concomitant CHI treatments (e.g., somatostatin analogs) that were initiated prior to trial entry are permitted throughout the trial. Somatostatin analogs may also be added throughout the trial at the investigator's discretion if the maximum dose level of dasiglucagon (70 µg/hr) has been reached or if further titration is not possible due to undesirable side effects.

Other CHI-specific treatments either prior to trial initiation or added during the trial need to be discussed with the medical monitor.

9.11.2. Prohibited Therapies

The following therapies are prohibited during the trial:

- Systemic corticosteroids, e.g., hydrocortisone >20 mg/mg/m^2 body surface area or equivalent from 5 days before screening and onwards
- Anti-inflammatory biological agents, or other immune-modulating agents in the 3 months prior to screening (except for sirolimus/mTOR inhibitors, as discussed in Section 9.11.1)
- Exogenous insulin
- Use of paracetamol/acetaminophen is strongly discouraged for the duration of trial because it interferes with CGM accuracy. Parent(s)/guardians should contact the trial site before dosing their child with paracetamol/acetaminophen. Both the site staff and the parent(s)/guardians should explore other options for treating fever and mild pain before deciding that paracetamol/acetaminophen is needed
- Other investigational agent
- 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 SOC
- Prescription or non-prescription medications known to cause QT prolongation

Continuation in the trial after the patient has received excluded therapies will be at the investigator's discretion after consultation with the medical monitor.

9.12. Patients May be Discharged for Home Treatment

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

- Appropriate training of patient's parent(s)/guardians 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 the 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 the hospital 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.

9.13. Treatment After End of Trial

After completing both treatment periods, patients may be eligible to continue in a long-term extension trial (Trial ZP4207-17106) (see Section 9.2). Patients who do not continue in the long-term extension trial will not be offered further treatment with dasiglucagon.

10. TRIAL PROCEDURES

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

France, Germany, Israel*: The consent must correspond to the patient's presumed will where such a will can be ascertained. Depending on local IRB/IEC requirements the patient should also provide assent before any trial-related procedures are initiated.*

For the timing of assessments and procedures throughout the trial, refer to the Schedule of Events (Section 17.1). Throughout the trial, trial personnel should make every reasonable effort to follow the timing of assessments and procedures in Section 17.1 for each patient. If a patient misses a trial visit for any reason, it should be rescheduled as soon as possible.

10.1. Trial Duration

The overall trial duration is expected to be 15 months.

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

1. Screening: at least 14 days up to 28 days
2. Treatment Period 1: 4 weeks
3. Treatment Period 2: 4 weeks
4. Follow-up Period: 4 weeks

The maximum trial duration for each patient is approximately 16 weeks. The dasiglucagon treatment duration for each patient is 4 or 8 weeks.

10.2. Assessments

Quality of life should be the first assessments performed at each visit according to the Schedule of Events (Section 17.1).

10.2.1. Efficacy

10.2.1.1. Plasma Glucose Monitoring

During the trial, SMPG assessments (StatStrip Xpress2) will be performed regularly (at least 3 times daily, preferably before meals, as instructed by the investigator) to evaluate efficacy.

At each visit, the investigator will ensure that SMPG data are downloaded from the patient's devices. The investigator will check for patient compliance in number of daily SMPG measurements and that hypoglycemic episodes are recorded in the diary. The procedure for download of SMPG data will be described in the pharmacy manual.

10.2.1.2. Continuous Glucose Monitoring

Additionally, CGM will be used (Dexcom G4) in a blinded manner to evaluate efficacy in terms of hypoglycemic episodes. The CGM will be supplied for use throughout the trial, and continuous glucose monitoring is required for the 2 weeks up to randomization, during Week 2-4 of Treatment Period 1, and during Week 6-8 of Treatment Period 2. Short pauses of 1-3 days due to skin irritation or discomfort are allowed after consultation with the investigator.

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

Dexcom G4 CGMs configured and labeled for use in this trial will be provided. Each CGM device should be calibrated and used according to the manufacturer's instructions.

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

10.2.1.3. Quality of Life

Quality of life (Appendix B) will be assessed using the PedsQL and additional CHI disease-specific QoL questions (parent-reported versions) according to the Schedule of Events (Section 17.1).

The PedsQL Measurement Model is a modular approach to measuring health-related quality of life in healthy children and adolescents and those with acute and chronic health conditions. The PedsQL Measurement Model integrates seamlessly both generic core scales and disease-specific modules into one measurement system.

The 23-item PedsQL Generic Core Scales was designed to measure the core dimensions of health as delineated by the World Health Organization, as well as role (school) functioning. The 4 Multidimensional Scales and 3 Summary Scores are:

Scales	**Summary Scores**
• Physical Functioning (8 items)	• Total Scale Score (23 items)
• Emotional Functioning (5 items)	• Physical Health Summary Score (8 items)
• Social Functioning (5 items)	• Psychosocial Health Summary Score (15 items)
• School Functioning (5 items)	

The CHI disease-specific questions have been developed by the patient association, Congenital Hyperinsulinism International, and taken from the patient-reported registry, the HI Global Registry. The HI Global Registry questions are grouped mostly under general quality of life; however, some questions relate specifically to diet and feeding, surgical management, glucose monitoring, and child development. The HI Global Registry is governed by a Global Steering Committee, including key global clinical experts.

10.2.1.4. Other Assessments

Resource Utilization

- Emergency department visits for hypoglycemia
- Number and length of hospitalizations due to CHI or CHI-related events
- Number of outpatient visits to health care providers (family doctors, specialist, etc.) caused by CHI or CHI-related events
- Number of home visits by paramedics due to hypoglycemia

Diary

The patient's parent(s)/guardian will be provided with a paper diary at all visits except at Visit 7 and the Follow-up Visit. The investigator will instruct the patient's parent(s)/guardian on how to complete the diary. The diary should be completed throughout the trial until the end of treatment visit (Visit 7). The following information should be recorded in the diary:

- Type and volume of fluid administered through NG tube/gastrostomy (if applicable)
- Hypoglycemic events, including related SMPG measurements
- Concomitant medications
- AEs
- Hospitalizations, visits to health care providers or ER, and visit 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 should be transcribed to the eCRF on an ongoing basis.

10.2.1.5. Fasting Tolerance Test/Safety Fast

During the screening period and at the end of Treatment Period 1, all patients will undergo a fasting tolerance test. This test must be performed when the CGM is active, and not the first day of CGM sensor, e.g., the day before randomization. This fasting tolerance test will be initiated according to the site's normal clinical practice and the anticipated duration of the test will be at investigator's discretion.

The test will commence after the patient's normal meal (t=0 will be the beginning of the meal). The meals have to be the same for both tests for the same individual. The frequency of PG sampling using the PG meter will be conducted according to site's normal practice and the individual patient's needs. This test is planned to last for 12 hours but will be stopped when PG is ≤60 mg/dL (3.3 mmol/L) and then ketones, insulin, and free fatty acids will be measured. The child will then be fed. If this value has not been reached at 12 hours, the test should be extended until it is reached.

The investigator will record the type and size of the meal, the date, start time, all PG measurements obtained, ketone measurement, and the end time of the test in a dedicated eCRF page.

The duration of fasting tolerance will be measured from the beginning of the normal meal until the beginning of the first continuous 15-minute CGM reading of <70 mg/dL (3.9 mmol/L).

10.2.2. Pharmacokinetics/Drug Exposure

Blood samples will be collected twice during the trial to measure for dasiglucagon levels at steady-state (Schedule of Events; Section 17.1).

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

10.2.3. Safety

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

10.2.3.1. Laboratory Safety Assessments

Trial procedures require a maximum total of 12 mL blood in a 30-day period. Where this exceeds the 2.5 mL/kg maximum,[16,17] 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.

Laboratory Tests to be Performed

Samples for hematology and chemistry will be collected at the time points specified in the Schedule of Events (Section 17.1).

Hematology:	hemoglobin, hematocrit, red blood cell (RBC) count, RBC indices, mean corpuscular hemoglobin, mean corpuscular volume, 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, sodium, potassium, chloride, eGFR, urea, insulin, ketones (measured at the local laboratory or with the PG meter), free fatty acids

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 5 (Week 5, prior to dosing), Visit 7 (Week 9) and at the Follow-up Visit (Visit 8, Week 12) (Section 17.1) 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.

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 an additional ADA Follow-up Visit (not included in the Schedule of Events) approximately 16 weeks after the End of Trial visit (Visit 8).

Upon ADA assay approval, the ADA samples will be analyzed in batches during the trial. The ADA samples will be analyzed at a special laboratory.

The clinical ADA assays for dasiglucagon have been validated in accordance with existing guidelines and recommendations.[18,19,20]

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

- Cross-reactivity against endogenous glucagon (cross-reactivity Yes/No)
- Establishment of anti-dasiglucagon binding titer
- Dasiglucagon in-vitro neutralizing potential of the antibodies
- Glucagon in-vitro neutralizing potential of the antibodies (only if positive for cross-reactivity)
- Neutralizing antibody titers, in case of a positive result in the in-vitro neutralizing antibody assays

The in-vitro neutralizing effects of antibodies will be measured using an assay based on glucagon receptor-transfected human embryonic kidney cells. The assays were validated for both dasiglucagon and recombinant glucagon neutralizing antibodies. The cell-based neutralizing antibody analyses will be performed by a special laboratory.

Anti-dasiglucagon antibody-positive patients (treatment induced or treatment boosted [titer increase above 4 fold]) will be monitored until the ADA level returns to pre-dose level.

Any residual serum samples will be stored until approval of market authorization by the health authorities.

In addition to the above, the neutralizing potential in samples from ADA-positive patients will be evaluated in relation to PK/PD data.

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 laboratory 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 significant during or after termination of the treatment have to be reported as AEs and followed, as described in Section 11.3.

10.2.3.2. Clinical Examinations

Vital Signs

Vital signs, including blood pressure, heart rate, respiratory rate, and SpO_2 will be measured according to the Schedule of Events (Section 17.1).

Twelve-lead Electrocardiogram

A standard 12-lead ECG will be performed while the child is in a sleeping or calm state according to the time points specified in the Schedule of Events (Section 17.1). If it is not practical or possible, then a 2-lead may be used.[21] If arrhythmia is detected on a 2-lead ECG, this should be followed by a 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, or 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 17.1).

Physical Examination and Neurological Examination

A complete physical examination of body systems (excluding breast and genitourinary examinations) according to standard of care and a neurological examination (including cranial nerves, muscle strength and tone, reflexes, coordination, sensory function, and gait, all as applicable for the patient's age) will be performed according to the Schedule of Events (Section 17.1).

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 take (e.g., no action, interruption of infusion). The likely cause of the reaction will also be collected (e.g., insertion site, drug, or 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 11.1.8), together with a photograph or series of photographs of the lesion(s) uploaded to a central repository.

10.2.3.3. Reporting of Hypoglycemia Events

All hypoglycemic episodes are to be reported via the dedicated hypoglycemia eCRF form. Hypoglycemic episodes that fulfill the definition of an SAE should furthermore be recorded as an SAE. The following information should be collected:

- Date, start time
- PG start 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 (time when PG exceeded a threshold of 70 mg/dL [3.9 mmol/L] or 60 minutes after the start of the event) and PG value, if available
- 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 even if normoglycemia (>70 mg/dL) is not reached within this time. A new episode of hypoglycemia is to be reported when the next PG value below 70 mg/dL (3.9 mmol/L) is measured.

10.2.3.4. Reporting of Technical Complaints

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

- Dasiglucagon 4 mg/mL vial containing 1 mL
- Accu-Chek Spirit pump
- Accu-Chek Spirit 3.15 mL Cartridge system, Accu-Chek FlexLink 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 system

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 11.2; 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 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 sponsor QA, refer to Appendix A for contact details.

Collection, Storage, and Shipment of Technical Complaint Item(s)

The investigator must collect and store the item(s) that is the subject of the technical complaint 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.

10.2.3.5. Adverse Events

The definitions and management of and special considerations for AEs are provided in Section 11.

11. ADVERSE EVENTS AND PREGNANCIES

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.

11.1. Definitions

11.1.1. Adverse Events

An AE is any untoward medical occurrence in a clinical trial patient administered a medicinal (investigational or non-investigational) product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease 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)
- Pre-planned procedure, unless the condition for which the procedure was planned has worsened from the first trial-related activity after the patient has signed the informed consent

11.1.2. 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.

11.1.3. 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.

11.1.4. 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 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.

11.1.5. 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 emergency room or home treatment of allergic bronchospasm or convulsion

11.1.6. 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

11.1.7. Non-serious Adverse Events

A non-serious AE is any AE that does not fulfill the definition of an SAE.

11.1.8. Adverse Events of Special Interest

For this trial, the following events are to be regarded as AEs of special interest (AESI events or 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

11.1.9. 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.

11.2. Collection, Recording, and Reporting of Adverse Events

All AEs, whether serious or non-serious, will be reported from the time a signed and dated ICF is obtained until the end of the 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 calendar 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 EDC. 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's 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 dedicated AESI eCRF page(s). Reporting requirements for serious and non-serious AEs as described previously also apply for serious and non-serious AESIs.

Other important events (Section 11.1.6) 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.

11.2.1. Contact Information

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

11.3. Follow-up of Adverse Events

The investigator must record follow-up information in the eCRF. 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 non-serious AEs which subsequently become SAEs.

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

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

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.

11.4. Pregnancy

Parent(s)/guardian of female patients who are of childbearing potential must be instructed to notify the investigator immediately if their child becomes pregnant or if they suspect she is pregnant during the trial. All initial reports of pregnancy in female patients must be reported by trial site personnel using the appropriate pregnancy form in EDC within 24 hours of their knowledge of the event. Abnormal pregnancy outcomes (e.g., spontaneous abortion, fetal death, stillbirth, congenital anomalies, or ectopic pregnancy) are considered SAEs and must be reported using the SAE form. If a patient becomes pregnant during the trial, treatment must be discontinued.

The investigator must follow the pregnancy until its outcome is known and the newborn infant is 1 month of age. The investigator must report information about the pregnancy, pregnancy outcome, and health of the newborn infant(s), as well as AEs in connection with the pregnancy, and AEs in the fetus and newborn infant.

11.5. 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' parents. 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 parents 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.

11.6. 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 additionally on an ad hoc basis as needed.

11.7. 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.

12. STATISTICS

12.1. Statistical Hypotheses

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

The hypothesis relating to the primary endpoint is:

H_{11}: Hypoglycemia event rate $_{\text{dasiglucagon}}$ = hypoglycemia event rate $_{\text{SOC only}}$

The hypotheses relating to the key secondary endpoints are:

H_{12}: Increase in fasting tolerance $_{\text{dasiglucagon}}$ = increase in fasting tolerance $_{\text{SOC only}}$

H_{13}: CGM percent time in range $_{\text{dasiglucagon}}$ = CGM percent time in range $_{\text{SOC only}}$

H_{14}: Clinically significant hypoglycemia event rate $_{\text{dasiglucagon}}$ = clinically significant hypoglycemia event rate $_{\text{SOC only}}$

A fixed-sequence statistical strategy will test the primary (Section 6.2.1) and 3 key secondary endpoints of Treatment Period 1 (Section 6.2.2) in a pre-defined order, all at the same significance level ($\alpha = 0.05$ to maintain an overall Type I error rate of at maximum $\alpha = 0.05$; see statistical analysis plan [SAP] for more details), moving to a second endpoint only after a success on the previous endpoint.

The test hierarchy is:

Treatment Period 1

H_{11}: Hypoglycemia event rate (measured by SMPG) during Weeks 2-4 of the treatment period (primary endpoint)

H_{12}: Increase in fasting tolerance (i.e., time from meal to PG <70 mg/dL) from baseline to Weeks 2-4 of the treatment period (key secondary endpoint)

H_{13}: CGM percent time in range 70-180 mg/dL (3.9-10.0 mmol/L) during Weeks 2-4 of the treatment period (key secondary endpoint)

H_{14}: Clinically significant hypoglycemia event rates, defined as average weekly number of events <54 mg/dL (3.0 mmol/L) (as detected by SMPG) during Weeks 2-4 of the treatment period (key secondary endpoint)

12.2. Sample Size Determination

Patients will be randomized into the trial if they have at least 3 events of hypoglycemia on average per week, as recorded in the diary during the 2 weeks prior to randomization. It is assumed that patients continuing on standard of care will maintain a similar level through Treatment Period 1, with the number of hypoglycemia events (PG <70 mg/dL or 3.9 mmol/L) during Weeks 2-4, as detected by SMPG following a Poisson distribution with a mean of 9. The trial is powered to detect a treatment effect of 50%, hence, assuming that the number of hypoglycemia events reported for patients in the dasiglucagon group during Weeks 2-4 will follow a Poisson distribution with a mean of 4.5. At the final analysis, 32 patients will have 99% power testing at a 0.05 significance level. The overall alpha level is strongly controlled in this setting, remaining at or below 0.05.

12.3. Analysis Populations

Three analysis populations are defined for this trial:

- The Safety Set: defined as all patients administered any randomized treatment. This population will be used to provide descriptive summaries of safety data. Patients will be summarized by treatment period according to treatment received
- The Full Analysis Set (FAS): defined as all patients in the Safety Set who have a valid baseline efficacy assessment. This population will be used to analyze efficacy data. Patients will be analyzed by treatment period according to planned treatment
- The Per Protocol (PP) Analysis Set: defined as all patients in the FAS without any major protocol deviations. This population will be used to analyze primary and key secondary endpoints as a supportive analysis. Patients will be analyzed according to planned treatment for the first treatment period only

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

12.4. Statistical Analyses

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

Unless otherwise indicated, all testing of statistical significance will be 2-sided with a significance level of $\alpha = 0.05$ to maintain an overall Type I error rate of $\alpha = 0.05$; see SAP for more details.

For analyses 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.

Continuous endpoints will be summarized with number (n), mean, SD, median, minimum, and maximum. In addition, change from baseline values will be calculated at each time point and summarized descriptively. For categorical endpoints, descriptive summaries will include counts and percentages.

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.

12.4.1. Trial Patients and Demographics

12.4.1.1. Disposition and Withdrawals

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

12.4.1.2. Protocol Deviations

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

12.4.1.3. Demographics and Other Baseline Characteristics

Demographic and baseline characteristics (including age, sex, race, ethnicity, weight, and length/height) 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 group and the number and percentage of patients taking each medication, classified using World Health Organization Drug Dictionary Anatomical Therapeutic Chemical classes and preferred term (PT).

12.4.2. Duration of Exposure and Compliance

Trial drug administration 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 group.

12.4.3. Efficacy Analyses

The efficacy analysis will be separated into 3 categories:

1. Treatment Period 1, FAS
2. Treatment Period 1, PP (primary and key secondary endpoints only)
3. Treatment Period 2, FAS

12.4.3.1. Primary Analysis

Primary Endpoint (Treatment Period 1)

The primary efficacy endpoint is the hypoglycemia event rate during Weeks 2-4 of Treatment Period 1. A hypoglycemia event is defined as PG <70 mg/dL or 3.9 mmol/L, as detected by SMPG. The analysis will be based on the hypoglycemia events reported in the eCRF. Baseline is defined as the average weekly number of hypoglycemic events during the 2-week baseline period. Weeks 2-4 of Treatment Period 1 is defined as the average weekly number of hypoglycemic events across the last 3 weeks of the treatment period. The hypothesis:

H_{11}: Hypoglycemia event rate $_{\text{dasiglucagon}}$ = hypoglycemia event rate $_{\text{SOC only}}$

will be analyzed by using negative binomial regression, with treatment group as a fixed effect and baseline hypoglycemic rate as a covariate. The log-transformed number of days in Treatment Period 1 will be used as an offset variable. The null hypothesis is that there is no difference in the incidence of average weekly number of hypoglycemic events between the 2 treatment groups, which will be tested at the significance level of α=0.05.

The primary analysis will estimate the treatment effect based on the de facto (treatment policy) estimand. All available data in the form of actual measurements will therefore be included in the analysis, irrespective of adherence to treatment or use of subsequent therapy. Missing data will be imputed using multiple imputation (MI) methodology as described in Section 12.4.3.4.

As a sensitivity analysis, the primary endpoint will also be analyzed without imputation of missing data; however, this analysis will not be included in the fixed-sequence hierarchical testing strategy. Similarly, the primary endpoint may be analyzed excluding post-baseline data collected after trial drug discontinuation/completion.

12.4.3.2. Key Secondary Analyses

Treatment Period 1

The key secondary endpoint of clinically significant hypoglycemia (<54 mg/dL [3.0 mmol/L]) event rates will be analyzed using a negative binomial regression, with treatment group as a fixed effect and baseline hypoglycemic rate as a covariate. Percent time in range (i.e., the percent time between 70 mg/dL [3.9 mmol] and 180 mg/dL [10.0 mmol], inclusive, as measured by CGM, where percent time is calculated as [number of minutes in range/total number of minutes patient is wearing CGM] * 100%) will be analyzed by using an ANCOVA, with treatment group and region as fixed effects and baseline time in range as a covariate. Increase in fasting tolerance (i.e., change from baseline in time from meal to PG <70 mg/dL) will be analyzed similarly, using an ANCOVA, with treatment group and region as fixed effects and baseline fasting tolerance as a covariate. Baseline is defined as the mean weekly value during the 2-week baseline period; Weeks 2-4 of Treatment Period 1 is defined as the mean weekly value across the last 3 weeks of the treatment period.

Missing data will be imputed in a similar manner to the primary endpoint. Analyses will be repeated without imputation of missing data; as with the primary endpoint, these analyses will not be included in the fixed-sequence hierarchical testing strategy.

12.4.3.3. Secondary and Other Efficacy Analyses

For the remaining efficacy endpoints, continuous and categorical endpoints will be presented using summary statistics or frequencies, respectively; no inference will be performed. Missing data will not be imputed.

The secondary efficacy endpoints related to the use of gastric carbohydrates will be evaluated descriptively only in the subgroup of patients with gastrostomy or NG tube at baseline.

Resource Utilization

The number and percentage of patients with admissions/emergency department visits for glycemia, hospitalizations due to CHI, visits to health care providers (family doctor, specialists, etc.) caused by CHI or CHI-related events, and need for home visits by parameters will be summarized. Additionally, number and length (in days) of hospitalizations due to CHI, number of visits to health care providers (family doctor, specialists, etc.) caused by CHI or CHI-related events, and number of home visits by paramedics will be summarized.

Quality of Life

Quality of life will be assessed by the PedsQL and a CHI disease-specific questionnaire (Appendix B). For each item of the PedsQL instrument (parent), a 5-point response scale is used (0 = never, 1 = almost never, 2 = sometimes, 3 = often, 4 = almost always). Items are reverse-scored and linearly transformed to a 0-100 scale (0 → 100, 1 → 75, 2 → 50, 3 → 25, 4 → 0) so that higher scores indicate better health-related QoL (less negative impact). Scale scores are computed as the sum of the items divided by the number of items answered (this accounts for missing data). If more than 50% of the items in the scale are missing, the scale score is not computed. Change from baseline for PedsQL for each of the scales (physical functioning, emotional functioning, social functioning, and school functioning) and summary scores (total scale score, physical health summary score, and psychosocial health summary score) will be summarized.

Answers to each question on the CHI disease-specific questionnaire will be summarized using frequencies at each relevant visit.

12.4.3.4. Imputation of Missing Data

For analysis of primary and key secondary endpoints, a jump-to-control (J2C) MI method, with SAS procedures PROC MI and PROC MIANALYZE, will be performed, including demographics and baseline characteristics in a first MI analysis. Due to the expectation of rapid metabolism and excretion of trial drug, it is expected that efficacy would be similar to the control group in cases of premature treatment discontinuation; thus, the J2C method is appropriate to handle missing values in this situation (considered missing not at random [MNAR]). Any missing values occurring before the patient discontinues/completes treatment will be imputed based on the data from the same treatment group (considered missing at random [MAR]).

The analysis will have 3 broad components: i) the MI process for the SOC only data; ii) the MI process for the dasiglucagon + SOC patients' data; and iii) the analysis model that will be used to draw inference regarding the primary causal estimand, along with the method for combining the results across the multiply imputed datasets. The seed to be used in the analysis is 12255070. The algorithm will use 20 burn-in iterations before each imputation, and 1000 imputed datasets will be created for the analysis for each of steps i and ii.

To accommodate both monotone and non-monotone missingness patterns, imputed values will be generated by a pattern mixture imputation model under the MNAR assumption using the fully conditional specification (FCS) method. The primary analysis will be performed using a negative binomial regression model from which hypoglycemia incidence density ratios (IDR) and 95% confidence intervals are obtained, along with the model parameter estimates, standard errors, and *p* values; analyses of the key secondary endpoints will be performed using the appropriate modeling. The final step in the imputation will involve using PROC MIANALYZE to derive valid univariate inference for these parameters. Multivariate inference based on the Wald test will be derived to test for the significance of these parameters.

Complete details for conducting this analysis will be outlined in the SAP.

12.4.4. Safety and Tolerability Analyses

Safety analyses will be conducted using data from the Safety Population (as defined in Section 12.3). 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 non-missing assessment before first dose in each treatment period.

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

12.4.4.1. Adverse Events

Adverse events will be coded using the latest 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 will be displayed by system organ class, PT, and treatment group. The incidence of AEs will also be presented by severity and by relationship to the trial drug. Serious AEs and AEs resulting in discontinuation of trial drug will be summarized separately in a similar manner. Patient listings of AEs, SAEs, and AEs causing discontinuation of trial drug will be produced.

12.4.4.2. 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.

12.4.4.3. 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 below, within, or above normal ranges will be tabulated showing change from baseline (shift tables) for each parameter.

12.4.4.4. 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.

12.4.4.5. Physical Examination Findings

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

12.4.4.6. Local Tolerability

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

12.4.5. Interim Analysis

No interim analysis is planned.

13. TRIAL CONDUCT

The accuracy and reliability of data is 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.

13.1. Sponsor and Investigator Responsibilities

13.1.1. Sponsor Responsibilities

The sponsor is obligated to conduct the trial in accordance with strict ethical principles (Section 15). The sponsor reserves the right to terminate participation of a trial site at any time (Section 13.7), and/or to discontinue the trial (Section 13.6 for US studies and Section 13.6.2 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.

13.1.2. Investigator Responsibilities

By signing the Investigator's Agreement (Section 17.2), 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.

13.2. 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.

13.3. 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.

13.4. 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.

13.4.1. Investigator's Regulatory Documents

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

13.4.2. Case Report Forms

By signing the Investigator's Agreement (Section 17.2), 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.

13.4.3. 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.

13.5. Data Quality Control

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

13.5.1. 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 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 17.2), 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.

13.5.2. 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.

13.5.3. 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.

13.6. Trial Termination

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

13.6.1. 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.

13.6.2. 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.

13.7. 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

13.7.1. 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.

13.7.2. 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.

13.8. 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.

13.9. 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. Data from individual trial sites must not be published separately.

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.

14. 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 results from the neutralizing antibody assay may be included or reported separately pending availability of the results.

The final CTR may be submitted to the regulatory authorities.

15. ETHICAL AND LEGAL CONSIDERATIONS

15.1. 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 C for regulation and guidelines.

15.2. 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.

France, Germany, Israel*: The consent must correspond to the patient's presumed will where such a will can be ascertained.*

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.

France, Germany, Israel*: Additionally, the patient will be informed about the nature, significance, risks, and implications of the trial with age-appropriate information, and his or her assent will be obtained in accordance with local regulations, as applicable.*

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 its 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 withdraw his or her child (see Section 8.3 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.

15.3. 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 PM 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.

15.4. Finance and Insurance

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

16. REFERENCES

1. Arnoux J-B, Verkarre V, Saint-Martin C, et al. Congenital hyperinsulinism: current trends in diagnosis and therapy. Orphanet J Rare Dis. 2011;6:63:1-14.
2. De Leon DD, Stanley CA. Congenital hypoglycemia disorders: New aspects of etiology, diagnosis, treatment and outcomes. Pediatr Diabetes. 2016;1-7.
3. Lord K, Radcliffe J, Gallagher PR, et al. High risk of diabetes and neurobehavioral deficits in individuals with surgically treated hyperinsulinism. J Clin Endocrinol Metab. 2015;100(11):4133-4139.
4. Stanley CA. Perspective on the genetics and diagnosis of congenital hyperinsulinism disorders. J Clin Endocrinol Metab. 2016;101(3):815-826.
5. Mazor-Aronovitch K, Gillis D, Lobel D, et al. Long-term neurodevelopmental outcome in conservatively treated congenital hyperinsulinism. Eur J Endocrinol. 2007;157(4):491-497.
6. Welters A, Lerch C, Kummer S, et al. Long-term medical treatment in congenital hyperinsulinism: a descriptive analysis in a large cohort of patients from different clinical centers. Orphanet J Rare Dis. 2015;10;150:1-10.
7. Arya VB, Senniappan S, Demirbilek H, et al. Pancreatic endocrine and exocrine function in children following near-total pancreatectomy for diffuse congenital hyperinsulinism. PLoS ONE. 2014;9(5):e98054. https://doi.org/10.1371/journal.pone.0098054.
8. Cederblad F, Ewald U, Gustafsson J. Effect of glucagon on glucose production, lipolysis and gluconeogenesis in familial hyperinsulinism. Horm. Res. 1998;50:94-98.
9. Miralles RE, Lodha A, Perlman M, Moore AM. Experience with intravenous glucagon infusions as a treatment for resistant neonatal hypoglycemia. Arch Pediatr Adolesc Med. 2002;156(10):999-1004.
10. Lord K, Dzato E, Snider KE, Gallagher PR, De Leon DD. Clinical presentation and management of children with diffuse and focal hyperinsulinism: a review of 223 cases. J Clin Endocrinol Metab. 2013;98(11):E1786-E1789.
11. Mohnike K, Blankenstein O, Pfuetzner A, et al. Long-term non-surgical therapy of severe persistent congenital hyperinsulinism with glucagon. Horm Res. 2008;70:59-64.
12. Pedersen JS, Dikov D, Flink JL, Jhuler HA, Christiansen G, Otzen DE. The changing face of glucagon fibrillation: structural polymorphism and conformational imprinting. J Mol Biol. 2006;355(3):501-523.
13. Rose SR, Chrousos G, Cornblath M, Sidbury J. Management of postoperative nesidioblastosis with zinc protamine glucagon and oral starch. J Pediatr. 1986;108(1):97-100.
14. Chastain MA. The glucagonoma syndrome: A review of its features and discussion of new perspectives. Am J Med Sci. 2001;321(5):306–320.
15. Zealand Pharma report. Population PK modeling and simulation of dasiglucagon in pediatric patients with type I diabetes mellitus. 2017.
16. European Medicines Agency. ICH Topic E11: Clinical Investigation of Medicinal Products in the Paediatric Population. January 2001.
17. Howie, S. Blood sample volumes in child health research: review of safe limits. Bull World Health Organ 2011; 89:46–53.

18. Food and Drug Administration (FDA). Guidance for Industry on Bioanalytical Method Validation. Food and Drug Administration. 2001.
19. Viswanathan CT, Bansal S, Booth B, et al. Quantitative bioanalytical methods validation and implementation: best practices for chromatographic and ligand binding assays. Pharm Res. 2007;24(10):1962-73.
20. Shankar G, Devanarayan V, Amaravadi L, et al. Recommendations for the validation of immunoassays used for detection of host antibodies against biotechnology products. J Pharm Biomed Anal. 2008;48(5):1267-81.
21. Food and Drug Administration (FDA). Guidance Industry. E14 Clinical Evaluation of QT/QTc Interval Prolongation and Proarrhythmic Potential for Non-antiarrhythmic Drugs. Food and Drug Administration. 2005.

17. ATTACHMENTS

17.1. Schedule of Events

Table 2 Schedule of Events

Trial Period	Screening	*Treatment Period 1*				*Treatment Period 2*				Follow-up
Week		**1[b]**	**Telephone Call[c]**	**2**	**4**	**5[d]**	**Telephone Call[c]**	**6**	**9**	**12**
Day		**1**		**8**	**22**	**29**		**36**	**57**	**85**
Time window (days)	**Day -28 to -14[a]**	**±2**		**±2**	**±2**	**±2**		**±2**	**±2**	**±5**
Visit #	**1**	**2**		**3**	**4**	**5**		**6**	**7[e]**	**8**
General assessments										
Informed consent/assent	X									
Inclusion/exclusion criteria	X	X								
Randomization criteria		X								
Demography	X									
Body weight and length/height[f]	X	X				X			X	X
Medical history (including current illness)	X									
Concomitant medication	X	X		X	X	X		X	X	X
Safety Assessment										
Electrocardiogram	X	X[r]		X		X[r]		X	X	X
Echocardiography	X[g]								X	X
Vital signs[h]	X	X[s]		X	X	X[s]		X	X	X
Serum Pregnancy test[i]	X								X	X
Adverse events		X	X	X	X	X	X	X	X	X
Local tolerability		X		X	X	X		X	X	
Physical examination and neurological examination	X	X		X	X	X		X	X	X
Laboratory Assessments										
Clinical laboratory test[j]	X			X		X		X	X	X
Antibodies[k]		X[l]				X			X	X[k]
Pharmacokinetics/drug exposure								X	X	

Trial Period	**Screening**	***Treatment Period 1***				***Treatment Period 2***				**Follow-up**
Week		**1[b]**	**Telephone Call[c]**	**2**	**4**	**5[d]**	**Telephone Call[c]**	**6**	**9**	**12**
Day		**1**		**8**	**22**	**29**		**36**	**57**	**85**
Time window (days)	**Day -28 to -14[a]**	**±2**		**±2**	**±2**	**±2**		**±2**	**±2**	**±5**
Visit #	**1**	**2**		**3**	**4**	**5**		**6**	**7[e]**	**8**
Efficacy										
Continuous glucose monitoring	X (for at least 14 days prior to randomization)	Continuous[m]								
Self-monitored plasma glucose	X (for at least 14 days prior to randomization)	X (at least 3 times daily)								
Fasting tolerance test[n]	X (during active CGM period)					X[o]				
Trial Materials and reminders										
Randomization		X								
Dispense patient diary	X[p]	X		X	X	X		X		
Diary review		X		X	X	X		X	X	
QoL questionnaires[q]		X				X			X	X
Dispensing of trial product		X		X	X	X		X		

Abbreviations: CGM = continuous glucose monitoring; PG = plasma glucose; QoL = quality of life; SMPG = self-monitoring of plasma glucose; SOC = standard of care; SpO_2 = blood oxygen saturation level; W = week

Note: An unscheduled visit can occur at any time if the investigator deems it necessary for patient safety.

a Screening must occur within a minimum of 14 days to allow for solid baseline assessment.

b At the beginning of Treatment Period 1 (Week 1), all patients will be hospitalized for 1-2 days. Patients assigned to SOC plus dasiglucagon treatment will be initiated and titrated on dasiglucagon, trained in the use of the infusion pump, and supervised. Patients are assigned to SOC alone will receive a similar degree of supervision alongside the training in the use of the infusion pump. This period can be extended for both treatment groups if dasiglucagon titration has not been finalized or if training of the family/caregivers has not been completed satisfactorily.

c Patients 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 whether their child has experienced any AEs.

d In Treatment Period 2, patients who were assigned to SOC treatment only in Treatment Period 1 will be hospitalized for the first 1-2 days of Week 5.

e Visit 7 can be used as the first visit for Trial ZP4207 17106 (long-term extension trial) if the patient is continuing in long-term extension trial.

f Length/height will be measured at Screening only.

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

h Vital signs include blood pressure, heart rate, respiratory rate, and SpO_2.

i A serum pregnancy test will be performed for girls of childbearing potential.

j Clinical laboratory tests include hematology and biochemistry.

k Any anti-dasiglucagon antibody-positive patient (treatment induced or treatment boosted) will be monitored at an additional ollow-up Visit, preferably 16 weeks after the 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 The sample for ADAs should be taken prior to dosing of dasiglucagon in Treatment Period 1.
m Continuous glucose monitoring is required during Weeks 2-4 of Treatment Period 1 and Weeks 6-8 of Treatment Period 2.
n The fasting tolerance test will be stopped when PG is ≤60 mg/dL (3.3 mmol/L) and then ketones, insulin, and free fatty acids will be measured.
o The fasting tolerance test at Visit 5 should take place before initiation of dosing in Treatment Period 2 but while the patient is still on CGM.
p At screening, dispense diary and instruct patients' parent(s)/guardian in its use. At all other visits, the parent(s)/guardian will return the completed diary and obtain a new one.
q The PedsQL (parent-reported versions) and CHI disease-specific questionnaires should be the first assessments performed at each visit.
r ECG should be performed at the start of the visit and at 24 ± 4 hours after initiation of trial drug for patients initiating treatment.
s Vital signs should be measured at the start of the visit and at 6 ± 1, 12 ± 2, and 24 ± 4 hours after initiation of the trial drug for patients initiating treatment.

17.2. Investigator's Agreement

PROTOCOL NUMBER:	ZP4207-17109
PROTOCOL TITLE:	A Two-Period, Open-label Trial Evaluating the Efficacy and Safety of Dasiglucagon for the Treatment of Children with Congenital Hyperinsulinism
FINAL PROTOCOL:	Version 13.0, 12-Nov-2020

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 the sponsor
- 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 the sponsor 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 the sponsor 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)

18. APPENDICES

A. Contact Information
B. Quality of Life Questionnaires
C. Regulations and Good Clinical Practice Guidelines

A. Contact Information

Pharmacovigilance

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

Technical Complaints

Name: Zealand Pharma A/S Quality Assurance
E-mail: [redacted]

B. Quality of Life Questionnaires

- Infants 1-12 Months: "PedsQL™ Pediatric Quality of Life Inventory Infant Scales – Version 1 - Parent Report for Infants (ages 1-12 months)"
- Infants 13-24 Months: "PedsQL™ Pediatric Quality of Life Inventory Infant Scales – Version 1 - Parent Report for Infants (ages 13-24 months)"
- Parent Report for Teens 13-18 Years: "PedsQL™ Pediatric Quality of Life Inventory – Version 4 - Parent Report for Teens (ages 13-18 yrs)"
- Parent Report for Children 8-12 Years: "PedsQL™ Pediatric Quality of Life Inventory – Version 4 - Parent Report for Children (ages 8-12 yrs)"
- Parent Report for Children 5-7 Years: "PedsQL™ Pediatric Quality of Life Inventory – Version 4 - Parent Report for Young Children (ages 5-7 yrs)"
- Parent Report for Toddlers 2-4 Years: "PedsQL™ Pediatric Quality of Life Inventory – Version 4 - Parent Report for Toddlers (ages 2-4 yrs)"
- CHI Disease-Specific Questionnaire Developed by the Patient Association, Congenital Hyperinsulinism International, and Taken from the Patient-Reported Registry, the HI Global Registry

C. 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_Guideline.pdf

PROTOCOL

PRODUCT NAME/NUMBER:	Dasiglucagon
PROTOCOL NUMBER:	ZP4207-17109
IND NUMBER:	135869
EUDRACT NUMBER:	2017-004547-21
DEVELOPMENT PHASE:	Phase 3
PROTOCOL TITLE:	A Two-Period, Open-label 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 Final Version 14.0, 12-Nov-2020 (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 Two-Period, Open-label 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
	This updated protocol version 12.0 is applicable for Germany only, and includes:
AMENDMENT No. 1	Final Version 2.0, 22-May-2018 (All countries)
AMENDMENT No. 2	Final Version 3.0, 05-Jul-2018 (United Kingdom)
AMENDMENT No. 3	Final Version 4.0, 25-Jul-2018 (France, Germany, and Israel)
AMENDMENT No. 4	Final Version 5.0, 14-Dec-2018 (All countries)
AMENDMENT No. 5	Final Version 6.0, 25-Mar-2019 (Germany)
AMENDMENT No. 6	Final Version 7.0, 03-Jun-2019 (All countries except Germany)
AMENDMENT No. 7	Final Version 8.0, 05-Jul-2019 (Germany)
AMENDMENT No. 8	Final Version 9.0, 19-Sep-2019 (All countries except Germany)
AMENDMENT No. 9	Final Version 10.0, 11-Oct-2019 (Germany)
AMENDMENT No. 10	Final Version 11.0, 06-Mar-2020 (All countries except Germany)
AMENDMENT No. 11	Final Version 12.0, 11-Mar-2020 (Germany)
AMENDMENT No. 12	Final Version 13.0, 12-Nov-2020 (All countries except Germany)
AMENDMENT No. 13	Final Version 14.0, 12-Nov-2020 (Germany)

2. APPROVAL SIGNATURES

PROTOCOL NUMBER:	ZP4207-17109
PROTOCOL TITLE:	A Two-Period, Open-label Trial 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]
Clinical Trial Manager
Zealand Pharma A/S

Date

[redacted]
Medical Director
Zealand Pharma A/S

Date

[redacted]
Manager Biostatistics
Premier Research

Date

3. SYNOPSIS

PRODUCT NAME/NUMBER	Dasiglucagon
PROTOCOL NUMBER	ZP4207-17109
EUDRACT NUMBER	2017-004547-21
DEVELOPMENT PHASE	Phase 3
PROTOCOL TITLE	A Two-Period, Open-label Trial 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 administered as a subcutaneous (SC) infusion in reducing hypoglycemia in children with CHI. Secondary: • To evaluate the safety and tolerability of dasiglucagon administered as an SC infusion in children with CHI • To evaluate the efficacy of dasiglucagon in reducing glucose requirements • To investigate quality of life and resource utilization
TRIAL DESIGN	This is a 2-period, open-label trial to evaluate the efficacy and safety of dasiglucagon in children between the ages of 3 months and 12 years (both inclusive) with CHI. Patients are to experience frequent (≥3 events per week) episodes of hypoglycemia despite standard of care (SOC) medications to be eligible for this trial, and are to have previously undergone sub-total pancreatectomy for CHI or be treated with a non-surgical approach, having been evaluated as not eligible for pancreatic surgery. After screening and eligibility assessments (during which a 2-week baseline for plasma glucose [PG] will be established), patients will be randomly assigned in a 1:1 ratio to continue receiving SOC alone or SOC plus dasiglucagon for 4 weeks. In Treatment Period 2, all patients will receive SOC plus dasiglucagon for 4 weeks. At the beginning of Treatment Period 1 (Week 1), all patients will be hospitalized for 1-2 days. Patients assigned to SOC plus dasiglucagon treatment will have dasiglucagon infusion initiated and titrated, and will be trained in the use of the infusion pump, continuous glucose monitoring (CGM) sensor, and glucose meter, and supervised. Patients assigned to SOC alone will receive a similar degree of supervision alongside the training in the use of the CGM sensor and glucose meter. This period can be extended for both treatment groups if dasiglucagon titration has not been finalized or if training of the family/caregivers has not been completed satisfactorily. Patients originally assigned to receive SOC alone in Treatment Period 1 will also be hospitalized for the first 2 days of Treatment Period 2 (Week 5), and will have dasiglucagon infusion initiated and titrated, and will be trained in the use of the infusion pump. This period can be extended if dasiglucagon titration or pump training has not been completed. Regardless of whether dasiglucagon is initiated during Treatment Period 1 or Treatment Period 2, SOC CHI medications should subsequently be kept constant throughout the trial. Adjustments to gastric and oral feeds are permitted. Patients' parent(s)/guardian will be trained to perform PG assessments. Events of hypoglycemia will be documented by self-monitored PG (SMPG) measurements. The extent of hypoglycemia (episodes and percent time below the threshold) will be further quantified by CGM measurements during the 2 weeks before randomization (baseline), Weeks 2-4 in Treatment Period 1, and Weeks 6-8 of Treatment Period 2. The CGM results will be masked.

	Patients completing the trial will (pending investigator confirmation of continued positive benefit-risk balance) be offered to enter a long-term extension trial (Trial ZP4207-17106) to continue dasiglucagon treatment. Patients who do not continue in the long-term extension trial will have a Follow-up visit performed 4 weeks after stopping dasiglucagon treatment.
PLANNED NUMBER OF PATIENTS	A sufficient number of patients will be screened to reach a total maximum of 32 randomized patients with CHI. Patients who withdraw prematurely will not be replaced.
TRIAL ENTRY CRITERIA	Eligible patients will be male or female between 3 months and 12 years of age (both inclusive) at screening with an established and documented diagnosis of CHI and who are experiencing ≥3 events of hypoglycemia per week (events of PG <70 mg/dL [<3.9 mmol/L]) according to investigator's evaluation. Patients are to have previously undergone sub-total pancreatectomy or being treated with a non-surgical approach, having been evaluated as not eligible for pancreatic surgery.
INVESTIGATIONAL PRODUCT	Dasiglucagon injection 4 mg/mL in a 3 mL vial containing 1 mL.
REFERENCE PRODUCT	None
TREATMENT REGIMENS	Dosing of dasiglucagon will approximate continuous infusion by delivering small doses at frequent intervals via an infusion pump. During the trial, PG assessments will be performed regularly (at least 3 times daily, preferably before main meals, and as instructed by the investigator); adjustments to the gastric dextrose infusion and/or trial drug will occur at the investigator's discretion. Dasiglucagon treatment will be initiated at 10 µg/hr (t=0). Every 2 hours (t=2, 4, 6, etc.), the dose will be increased by an additional 10 µg/hr until either: 1) The patient is weaned off entirely from gastric dextrose infusion and/or glucose-fortified feeds, or 2) Plasma glucose during the last 2 hours was consistently above 120 mg/dL (6.7 mmol/L), or 3) The maximum trial drug product infusion rate of 70 µg/hr is reached, or 4) Adverse events (AEs) emerge that are considered to be related to dasiglucagon (e.g., nausea and vomiting) and limit further dose escalation The dose of dasiglucagon should not be escalated beyond the treatment objectives of PG in the range of 70 to 120 mg/dL (3.9-6.7 mmol/L) while approaching a normal feeding regimen according to age. The 2-hour dose-adjustment interval will allow plasma drug levels to approach approximately steady-state before the dose is further increased. The maximum cumulative dose over the first 24 hours is 1.26 mg. After the first 24 hours, the dose of dasiglucagon can still be modified to optimize each patient's treatment and to reduce gastric dextrose infusions. The maximum dose of dasiglucagon will be 70 µg/hr, corresponding to a maximum of 1.68 mg per day.
PLANNED TRIAL SITES	Up to 14 sites in the United States, Europe, and Israel.

CRITERIA FOR EVALUATION	Primary efficacy endpoint: *Treatment Period 1* • Hypoglycemia event rate, defined as average weekly number of hypoglycemic events (PG <70 mg/dL or 3.9 mmol/L) during Weeks 2-4, as detected by SMPG. Key secondary efficacy endpoints: *Treatment Period 1* • Increase in fasting tolerance (time from beginning of meal to the beginning of the first continuous 15-minute CGM reading <70 mg/dL [3.9 mmol/L]) • CGM percent time in range 70-180 mg/dL (3.9-10.0 mmol/L) during Weeks 2-4 • Clinically significant hypoglycemia event rates, defined as average weekly number of events <54 mg/dL (3.0 mmol/L), as detected by SMPG during Weeks 2-4
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 pre-specified 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. <u>Analysis Populations</u> Three analysis populations are defined for this trial: The Safety Set: defined as all patients administered any randomized treatment. This population will be used to provide descriptive summaries of safety data. Patients will be summarized by treatment period according to treatment received. The Full Analysis Set (FAS): defined as all patients in the Safety Set who have a valid baseline efficacy assessment. This population will be used to analyze efficacy data. Patients will be analyzed by treatment period according to planned treatment. The Per Protocol (PP) Analysis Set: defined as all patients in the FAS without any major protocol deviations. This population will be used to analyze primary and key secondary endpoints as a supportive analysis. Patients will be analyzed according to planned treatment for the first treatment period only. The analysis of efficacy will be separated into 3 categories: 1. Treatment Period 1, FAS 2. Treatment Period 1, PP (primary and key secondary endpoints only) 3. Treatment Period 2, FAS In Treatment Period 1 (categories 1 and 2), the 2 treatment arms will be compared by a testing procedure starting with the primary and subsequently continuing through the key secondary endpoints if the hypotheses are rejected. In Treatment Period 2 (category 3), only descriptive analyses will performed. Graphical presentations of selected analyses in the 2 treatment periods will be prepared, and will be discussed in the formal statistical analysis plan (SAP).

	Efficacy Analyses *Primary Endpoint (Treatment Period 1)* The primary efficacy endpoint is the hypoglycemia event rate during Weeks 2-4 of Treatment Period 1. A hypoglycemia event is defined as PG <70 mg/dL or 3.9 mmol/L, as detected by SMPG. Baseline is defined as the average weekly number of hypoglycemic events during the 2-week baseline period. Weeks 2-4 of Treatment Period 1 is defined as the average weekly number of hypoglycemic events across the last 3 weeks of the treatment period. The hypoglycemic event rate will be analyzed by using negative binomial regression, with treatment group as a fixed effect and baseline hypoglycemic rate as a covariate. The null hypothesis is that there is no difference in the incidence of average weekly number of hypoglycemic events between the 2 treatment groups, which will be tested at the significance level of $\alpha = 0.05$. The primary analysis will estimate the treatment effect based on the de facto (treatment policy) estimand. All available data in the form of actual measurements will therefore be included in the analysis, irrespective of adherence to treatment or use of subsequent therapy. As a sensitivity analysis, the primary endpoint will also be analyzed without imputation of missing data; however, this analysis will not be included in the fixed-sequence hierarchical testing strategy. Similarly, the primary endpoint may be analyzed excluding post-baseline data collected after trial drug discontinuation/completion. *Key Secondary Endpoints (Treatment Period 1)* The key secondary endpoint of clinically significant hypoglycemia (<54 mg/dL [3.0 mmol/L]) event rates will be analyzed using a negative binomial regression, with treatment group as a fixed effect and baseline hypoglycemic rate as a covariate. Percent time in range (i.e., the percent time between 70 mg/dL [3.9 mmol] and 180 mg/dL [10.0 mmol], inclusive, as measured by CGM, where percent time is calculated as [number of minutes in range/total number of minutes patient is wearing CGM] * 100%) will be analyzed by using an ANCOVA, with treatment group and region as fixed effects and baseline time in range as a covariate. Increase in fasting tolerance (i.e., change from baseline in time from meal to PG <70 mg/dL) will be analyzed similarly using an ANCOVA, with treatment group and region as fixed effects and baseline fasting tolerance as a covariate. For the remaining efficacy endpoints, continuous and categorical endpoints will be presented using summary statistics or frequencies, respectively; no inference will be performed. Safety Analyses 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 dasiglucagon 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, incidence of anti-drug antibodies [ADAs]), 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. A SAP will be prepared to provide further details on the methods for statistical analysis, including rules for handling multiplicity and missing data.
SAMPLE SIZE DETERMINATION	Patients will be randomized into the trial if they have at least 3 events of hypoglycemia on average per week, as recorded in the diary during the 2 weeks prior to randomization. It is assumed that patients continuing on standard of care will maintain a similar level through Treatment Period 1, with the number of hypoglycemia events (PG <70 mg/dL or

	3.9 mmol/L) during Weeks 2-4, as detected by SMPG following a Poisson distribution with a mean of 9. The trial is powered to detect a treatment effect of 50%, hence, assuming that the number of hypoglycemia events reported for patients in the dasiglucagon group during Weeks 2-4 will follow a Poisson distribution with a mean of 4.5. At the final analysis, 32 patients will have 99% power testing at a 0.05 significance level. The overall alpha level is strongly controlled in this setting, remaining at or below 0.05.
TRIAL AND TREATMENT DURATION	The overall trial duration is expected to be 15 months. The sequence and maximum duration of the trial periods will be as follows: 1. Screening: at least 14 days up to 28 days 2. Treatment Period 1: 4 weeks 3. Treatment Period 2: 4 weeks 4. Follow-up Period: 4 weeks The maximum trial duration for each patient is approximately 16 weeks. The dasiglucagon treatment duration for each patient is 4 or 8 weeks.

4. TABLE OF CONTENTS

4.1. LIST OF TABLES

4.2. LIST OF FIGURES

5. LIST OF ABBREVIATIONS

ADA	anti-drug antibodies
ADE	adverse device effect
AE	adverse event
ALT	alanine aminotransferase
ANCOVA	analysis of covariance
$AOC_{glucose}$	area over the glucose curve up to 70 mg/dL
AST	aspartate aminotransferase
AUC	area under the plasma concentration-time 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
C_{max}	maximum observed concentration
CRA	clinical research associate
CRO	contract research organization
CTR	clinical trial report
DMC	data monitoring committee
ECG	electrocardiogram
eCRF	electronic case report form
EDC	electronic data capture
eGFR	estimated glomerular filtration rate
ER	emergency room
FAS	full analysis set
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GI	gastrointestinal
H	hypothesis
HbA1c	glycated hemoglobin
HIV	human immunodeficiency virus
HNF4A-DM	hepatocyte nuclear factor 4 alpha diabetes mellitus
HNF4A-IGT	hepatocyte nuclear factor 4 alpha impaired glucose tolerance

IB	investigator's brochure
ICF	informed consent form
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IEC	independent ethics committee
IFU	instructions for use
IM	intramuscular(ly)
IRB	institutional review board
ISF	Investigator Site File
ISPE	International Society of Pharmaceutical Engineers
IV	intravenous(ly)
J2C	jump to control
MedDRA	Medical Dictionary for Regulatory Activities
MI	multiple imputation
MNAR	missing not at random
NG	nasogastric
PD	pharmacodynamics(s)
PedsQL™	Pediatric Quality of Life Inventory
PG	plasma glucose
PK	pharmacokinetic(s)
PP	Per protocol
PT	preferred term
QoL	quality of life
RBC	red blood cell
RSI	Reference Safety Information
SADE	serious adverse device effect
SAE	serious adverse event
SAP	statistical analysis plan
SC	subcutaneous(ly)
SD	standard deviation
SEM	standard error of the mean
SMPG	self-monitored plasma glucose

SpO_2	blood oxygen saturation level
SOC	standard of care
SUSAR	Suspected unexpected serious adverse reaction
ULN	upper limit of normal
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 genes have been described to cause CHI, which can be either focal (only a small area of the pancreas is affected) or diffuse (most of the pancreas is affected). The condition can persist into adulthood; however, the severity generally decreases with age due to the increased insulin requirements and/or increased insulin resistance, and CHI is thus primarily a pediatric disease with regard to medical treatment needs. 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 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 trial[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, indicating the serious and long-term consequences of the procedure.

First-line medical treatment 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 non-responders, 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]

Glucagon has been shown to be effective in the treatment of CHI. The glycogenolytic effect of glucagon and its ability to increase PG levels has been confirmed in children with CHI or neonatal hypoglycemia,[8,9] and administration of reconstituted glucagon (via intravenous [IV] infusion or as repeated subcutaneous [SC] injection) is often used in the initial phase during the establishment of CHI diagnosis and to stabilize patients with CHI before surgery or initiation of other medical treatments.[10] 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 complicated by the fact that currently available glucagon products are unstable and form fibrils within hours after reconstitution.[12] This fibril formation may lead to infusion set clotting, catheter obstruction, and dosing errors that may cause acute severe hypoglycemia. Catheter obstruction and occlusion because of glucagon fibril formation and aggregation 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 SC glucagon experienced catheter occlusion.[6] In a home-care setting, this fibril formation and associated risk of dosing errors carry the risk of hypoglycemic events, which is a major barrier for using currently marketed glucagon products for long-term treatment of patients with CHI.

With respect to long-term glucagon treatment in CHI, there are a few reports on home treatment with subcutaneously infused glucagon in children with CHI over extended periods (years) that suggest benefit in patient care with a potentially good safety profile as compared to diazoxide and octreotide.[6,11,13] While this attests to the potential clinical relevance of long-term glucagon treatment in CHI, the use of SC infusion of currently marketed glucagon is severely limited by the issues with fibril formation and solution instability of recombinant glucagon as described previously.

6.3. Dasiglucagon for the Treatment of Congenital Hyperinsulinism

6.3.1. Dasiglucagon

Dasiglucagon is a peptide analog of human glucagon that is 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, compatibility/in-use studies have been performed with dasiglucagon 4 mg/mL in the 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 was 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 normal and insulin-induced hypoglycemic animals, similar to that of glucagon. The effects of dasiglucagon and glucagon were investigated in an insulin-induced hypoglycemic rat model, which is considered particularly relevant to characterize the use of dasiglucagon for the treatment of CHI because it mimics the inappropriate insulin to PG levels in CHI and resultant hypoglycemia. The onset of PG increase with dasiglucagon was rapid and similar to that observed for glucagon, confirming comparable pharmacodynamics.

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 and 3 phase 2 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 intramuscular (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 trial.

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 compared to an active comparator, Lilly Glucagon™. 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 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, 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 $AUC_{0\text{-}inf}$. 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 after insulin-induced hypoglycemia achieved a PG level of at least 70 mg/dL (3.9 mmol/L) at all dose levels of dasiglucagon, 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 pre-specified dose comparisons. This was likely an effect of the higher total drug exposure ($AUC_{0\text{-}inf}$) of dasiglucagon mentioned previously.

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.

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 to control blood glucose. Long-term subcutaneous infusion of dasiglucagon through a pump may be an attractive alternative or addition to diazoxide and octreotide, as it may reduce the dependency on intensive nutritional support whilst maintaining euglycemia by harnessing physiological mechanisms for combating hypoglycemia. It is anticipated that reduced need for frequent tube feedings or continuous gastric infusion of nutrients, and increased fasting tolerance will be demonstrated, together with improvements in the quality of life of the patients and their families/caregivers. If long-term euglycemia is achieved with medical therapy, pancreatectomy for the treatment of diffuse CHI could eventually be avoided, or at least postponed beyond the neonatal or very young infant period. In one cohort of non-surgically 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.

6.3.6. Anticipated Risks of Dasiglucagon in the Treatment of CHI

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 have been observed in clinical trials with dasiglucagon, but not hypertension. On the contrary, several episodes of hypotension have been noted in the clinical program.

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 observed with many injectable peptides. In the phase 1 and 2 clinical trials, injection site reactions occurred with a low frequency in all treatment groups (dasiglucagon and marketed glucagons), irrespective of dose.

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 after 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,[14] sustained exposure to high levels of glucagon may lead to development of 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/parents 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 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 enabling convenient and reliable long-term treatment via a pump device in a home setting, which holds the potential to delay and ultimately avoid pancreatectomy and its related exo/endocrine complications, particularly the development of insulin-dependent diabetes.

Dasiglucagon may overall provide significant added benefit in the treatment of CHI relative to currently marketed glucagon products by enabling long-term reliable SC infusion to control PG. The proposed trial population is still experiencing hypoglycemia despite medical treatments being escalated to the highest therapeutically permissible or tolerated doses, or despite having undergone subtotal pancreatectomy. Therefore, these patients are dependent on continuous or very frequent delivery of carbohydrates, often through invasive routes (NG tube or gastrostomy). This limits their ability to lead normal lives, including participating in everyday activities, and therefore, impacts their development. For this trial population, the major and clinically relevant benefit is the expected reduction in number and volume of nutritional interventions while avoiding hypoglycemia. The reduced volume of nutritional interventions should limit the risk of volume overload, especially in patients treated with significant doses of diazoxide. Achievement of euglycemia could lead to reduction of other CHI medication, further limiting the potential for adverse events associated with those treatments. In addition, the need for pancreatectomy, or re-surgery in those who already underwent pancreatic surgery is reduced and potentially eliminated.

Overall, the benefit to risk ratio for patients entering the ZP4207-17109 trial is considered acceptable.

7. OBJECTIVES AND ENDPOINTS

7.1. Objectives

7.1.1. Primary Objective

To evaluate the efficacy of dasiglucagon administered as subcutaneous (SC) infusion in reducing hypoglycemia in children with CHI.

7.1.2. Secondary Objectives

Secondary objectives are:

- To evaluate the safety and tolerability of dasiglucagon administered as an SC infusion in children with CHI
- To evaluate the efficacy of dasiglucagon in reducing glucose requirements
- To investigate quality of life and resource utilization

7.2. Endpoints

7.2.1. Primary Endpoint

Treatment Period 1

- Hypoglycemia event rate, defined as average weekly number of hypoglycemic events (PG <70 mg/dL or 3.9 mmol/L) during Weeks 2-4, as detected by self-monitored PG (SMPG).

7.2.2. Key Secondary Efficacy Endpoints

Treatment Period 1

- Increase in fasting tolerance (time from beginning of meal to the beginning of the first continuous 15-minute continuous glucose monitoring [CGM] reading <70 mg/dL [3.9 mmol/L]).
- CGM percent time in range 70-180 mg/dL (3.9-10.0 mmol/L) during Weeks 2-4.
- Clinically significant hypoglycemia event rates, defined as average weekly number of events <54 mg/dL (3.0 mmol/L), as detected by SMPG during Weeks 2-4.

7.2.3. Secondary Efficacy Endpoints

Treatment Period 1

- Total amount of gastric carbohydrates administered (via nasogastric [NG] tube or gastrostomy) per week to treat hypoglycemia during Weeks 2-4.
- Rate of gastric carbohydrate administrations (NG tube or gastrostomy) per week to treat hypoglycemia during Weeks 2-4.
- Extent of hypoglycemia (area over the glucose curve [$AOC_{glucose}$] below 70 mg/dL [3.9 mmol/L]) as measured by CGM during Weeks 2-4.
- Extent of hypoglycemia (area over the glucose curve [$AOC_{glucose}$] below 54 mg/dL [3.0 mmol/L]) as measured by CGM during Weeks 2-4.
- Amount of nightly (midnight to 6 am) gastric carbohydrates administered (NG tube or gastrostomy) per week during Weeks 2-4.

- Total amount of gastric carbohydrates administered (NG tube or gastrostomy) per week during Weeks 2-4.
- CGM percent time in hypoglycemia (<70 mg/dL or 3.9 mmol/L) during Weeks 2-4.
- Rate of hypoglycemic episodes, defined as number of episodes <70 mg/dL (3.9 mmol/L) for 15 minutes or more per week, as measured by CGM during Weeks 2-4.

Treatment Period 2

- CGM percent time in hypoglycemia (<70 mg/dL or 3.9 mmol/L) during Weeks 6-8.
- Rate of weekly number of gastric carbohydrate administrations (NG tube or gastrostomy) per week to treat hypoglycemia during Weeks 6-8.
- Rate of hypoglycemic events, defined as number of episodes (PG <70 mg/dL or 3.9 mmol/L) per week during Weeks 6-8, as detected by SMPG.
- Rate of clinically significant hypoglycemia episodes, defined as number of episodes <54 mg/dL (3.0 mmol/L) for 15 minutes or more per week, as measured by CGM during Weeks 6-8.

7.2.4. Other Efficacy Endpoints

- Number of IV glucose infusions to treat hypoglycemia per week during Weeks 2-4 of Treatment Period 1.
- Emergency department visits for hypoglycemia.
- Number and length of hospitalizations due to CHI or CHI-related events.
- Number of out-patient visits to health care providers (family doctors, specialist, etc.) caused by CHI or CHI-related events.
- Number of home visits by paramedics due to hypoglycemia.
- Quality of life endpoints (PedsQL™ [Total Scale Score, Physical Health Summary Score, and Psychosocial Health Summary Score] and CHI-specific questionnaire).
- Rate of SMPG readings per week during Weeks 2-4 of Treatment Period 1.

7.2.5. Safety Endpoints

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

8. TRIAL DESIGN

8.1. Overall Trial Design and Plan

This is a 2-period, open-label trial to evaluate the efficacy and safety of dasiglucagon in children between the ages of 3 months and 12 years (both inclusive) with CHI. To qualify for participation, patients must be experiencing ≥3 events of hypoglycemia per week despite standard of care (SOC) medications. The determination of hypoglycemia frequency at trial entry is according to investigator's evaluation. Furthermore, patients are to have previously undergone sub-total pancreatectomy for CHI or be treated with a non-surgical approach, having been evaluated as not eligible for pancreatic surgery.

The primary objective of this trial is to evaluate whether dasiglucagon can reduce the number and severity of hypoglycemic episodes in the investigated population.

After screening and eligibility assessments, patients will complete a 2-week CGM period to establish plasma glucose baseline. Thereafter, patients will be randomly assigned in a 1:1 ratio to continue receiving SOC alone, or SOC plus dasiglucagon for 4 weeks. In Treatment Period 2, all patients will receive SOC plus dasiglucagon for 4 weeks (see Figure 1).

At the beginning of Treatment Period 1 (Week 1), all patients will be hospitalized for 1-2 days. Patients assigned to SOC plus dasiglucagon treatment will have dasiglucagon infusion initiated and titrated, and will be trained and supervised in the use of the infusion pump, CGM sensor, and glucose meter. Patients assigned to SOC alone will receive a similar degree of supervision alongside the training in the use of the CGM sensor and glucose meter. This period can be extended for both treatment groups if dasiglucagon titration has not been finalized or if training of the family/caregivers has not been completed satisfactorily.

Patients originally assigned to receive SOC alone in Treatment Period 1 will also be hospitalized for the first 1 to 2 days of Treatment Period 2 (Week 5), and will have dasiglucagon infusion initiated and titrated, and will be trained in the use of the infusion pump. This period can be extended if dasiglucagon titration or pump training has not been completed. Regardless of whether dasiglucagon is initiated during Treatment Period 1 or 2, SOC CHI medications should subsequently be kept constant throughout the trial. Adjustments to gastric and oral feeds are permitted.

Patients' parent(s)/guardian will be trained to perform PG assessments. Events of hypoglycemia will be documented by SMPG measurements. The extent of hypoglycemia (episodes and percent time below the threshold) will be further quantified by CGM measurements during the 2 weeks before randomization (baseline), Weeks 2-4 in Treatment Period 1, and Weeks 6-8 of Treatment Period 2. The CGM results will be masked. During the period where the masked trial CGM is used, patients are not allowed to use another un-masked CGM.

Patients completing the trial will (pending investigator confirmation of continued positive benefit-risk balance) be offered to enter a long-term extension trial (Trial ZP4207-17106) to continue dasiglucagon treatment. Patients who do not continue in the long-term extension trial will have a Follow-up visit performed 4 weeks after stopping dasiglucagon treatment.

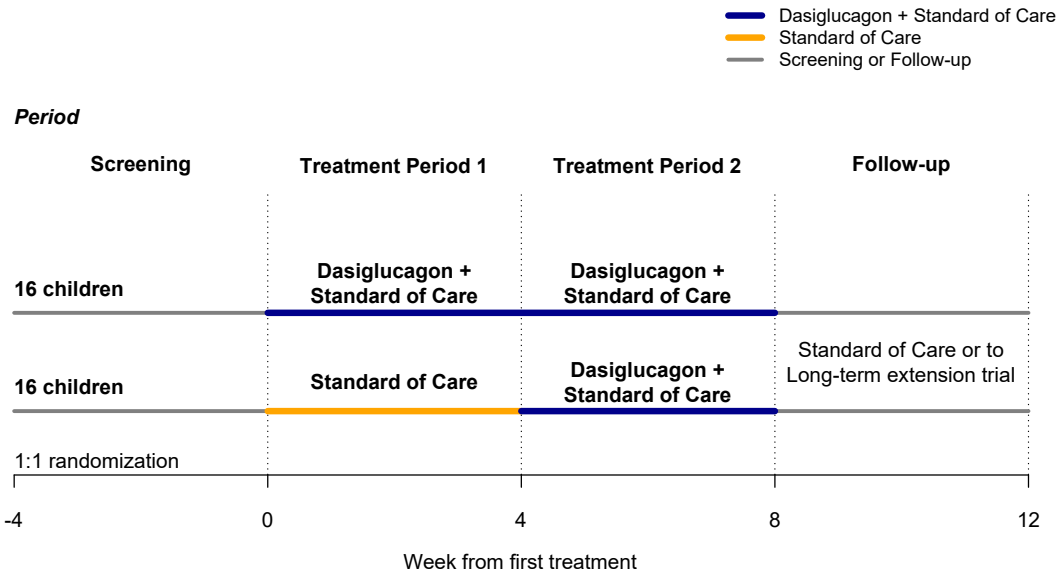

Figure 1 Trial Design

8.2. Discussion of Trial Design

Several sites across multiple geographical regions will be included to ensure results representative of the target CHI population.

A parallel design was chosen rather than a crossover design to avoid the risk of any carry-over effect and to limit the total treatment period for the patients included.

An open-label trial design was chosen because the added burden of a blinded trial design was not considered ethically justified.

8.3. Trial Sites

This trial will take place at up to 14 sites in the United States and Europe that are experienced in the treatment of CHI. A total of up to 32 patients with CHI will be randomized.

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. Established and documented diagnosis of CHI based on standard of care.
2. Male or female between 3 months and 12 years of age (both inclusive) at screening
3. Has a negative serum pregnancy test at screening/baseline (only for girls of childbearing potential)
4. Sexually active female patients (and their partners) must continue to use acceptable contraception or refrain from sexual activity from screening until 30 days after the last dose of trial drug. Females must abstain from sexual activity that could result in pregnancy or agree to use acceptable methods of contraception. Abstinence can only be accepted if this is true abstinence in line with the preferred and usual lifestyle of the patient.

   Acceptable methods of contraception are:

   a) Hormonal contraceptives (e.g., oral contraceptive pill, depot, patch, intramuscular implant or injection, sponge, or vaginal ring), stabilized for at least 30 days if first use

   or

   b) Barrier method, e.g., (i) condom (male or female) and (ii) diaphragm with spermicide

   Germany*: Only highly effective methods of birth control are accepted (i.e., one that results in less than 1% per year failure rate when used consistently and correctly, such as implants, injectables, combined oral contraceptives, some intrauterine device), or sexual abstinence.*
5. Experiencing ≥3 events of hypoglycemia per week (PG <70 mg/dL [<3.9 mmol/L]) according to the investigator's evaluation
6. Following receipt of oral and written information about the trial, the patient (depending on local IRB/IEC requirements) must provide assent and one or both parents* or guardians of the patient must provide signed informed consent before any trial-related activity is carried out

   France, Germany, Israel*: The consent must correspond to the patient's presumed will where such a will can be ascertained.*
7. Previously undergone near-total pancreatectomy or being treated with a non-surgical approach, having been evaluated as not eligible for pancreatic surgery
8. If somatostatin analogues or sirolimus are used, the therapy should be well established as judged by the investigator, especially when considering their biological half-life

* If required by local regulations, both parents must give their permission unless one parent is deceased, unknown, incompetent, or not reasonably available, or when only one parent has legal responsibility for the care and custody of the child.

9.2.2. Exclusion Criteria

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

1. Previous administration of dasiglucagon (previously referred to as ZP4207)
2. Known or suspected allergy to the trial drug or related products
3. Previous participation (randomization) in this trial
4. Circulatory instability requiring supportive medication or presence of pheochromocytoma
   United Kingdom*: Presence of hypertension or hypotension, including circulatory instability requiring supportive medication or presence of pheochromocytoma*
5. Requires exogenous insulin
6. Body weight of <4 kg (8.8 lbs.)
7. Documented HbA1c ≥7% subsequent to near-total pancreatectomy and within 6 months prior to screening
8. Known or suspected presence of significant central nervous system disease/injury such that in the investigator's opinion will affect trial participation
9. Use of systemic corticosteroids, e.g., hydrocortisone >20 mg/mg/m^2 body surface area or equivalent in the 5 days before screening
10. Use of anti-inflammatory biological agents, or other immune-modulating agents in the 3 months prior to screening
11. 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)
12. Any clinically significant abnormality identified on echocardiogram that in the opinion of the investigator would affect the patient's ability to participate in the trial
13. 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
14. Any recognized clotting or bleeding disorders.
15. Has participated in an interventional clinical trial (investigational or marketed product) within 3 months of screening or 5 half-lives of the drug under investigation (whichever comes first), or plans to participate in another clinical trial.
16. The use of prescription or non-prescription medications known to cause QT prolongation.

9.2.3. Randomization Exclusion Criteria

1. Use of glucagon within 24 hours before randomization
2. Significant changes to CHI medications during screening
3. An average of <3 events per week of hypoglycemia, as recorded in the diary during the 2 weeks prior to randomization

The Randomization Visit may be rescheduled if randomization Exclusion Criterion 1 is met.

9.3. Premature Patient Withdrawal

Patients' parent(s)/guardian will be advised that they are free to withdraw their children 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. 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, the AE must be documented, reported, and followed as described in Section 12.2.

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 withdraws consent for trial treatment 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. At a minimum the patient will be asked to attend the Follow-up Visit 28 days (± 3 days) after discontinuation of trial treatment.

9.5. 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 who subsequently withdraw 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.

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

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

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

10.1.1. Packaging and Labeling

The trial drug product will be packaged and labeled by the sponsor.

Dispensing unit configuration: 6 vials containing dasiglucagon, 4 mg/mL, packaged in an outer carton. The vial and carton will be packaged and labeled in local language indicating the content (open label).

Detailed information on all labels will be in the local language.

Storage conditions for the trial drug product will be described on the trial drug product label. The labels will supply no information about the patients. Each treatment unit (containing 6 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, International Council for Harmonisation (ICH) Good Clinical Practice (GCP) guidelines, local laws, and regulations.

10.2. Treatments Administered

In Treatment Period 1, patients will receive SOC only or SOC plus dasiglucagon for 4 weeks based on their treatment assignment. In Treatment Period 2, all patients will receive SOC plus dasiglucagon for 4 weeks.

Dosing of dasiglucagon will approximate continuous infusion by delivering small doses at frequent intervals via the infusion pump.

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

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

Plasma glucose assessments will be performed regularly (at least 3 times daily, preferably before main meals, and as instructed by the investigator) throughout the trial to guide adjustments to the gastric dextrose infusion and/or trial drug product upon the investigator's discretion.

Dasiglucagon treatment will be initiated at 10 µg/hr (t=0). Every 2 hours (t=2, 4, 6, etc.), the dose will be increased by an additional 10 µg/hr until either:

1) The patient is weaned off entirely from gastric dextrose infusion and/or glucose-fortified feeds, or
2) Plasma glucose during the last 2 hours was consistently above 120 mg/dL (6.7 mmol/L), or
3) The maximum trial drug product infusion rate of 70 µg/hr is reached, or
4) Adverse events emerge that are considered to be related to dasiglucagon (e.g., nausea and vomiting) and limit further dose escalation

The dose of dasiglucagon should not be escalated beyond reaching the treatment objectives of PG in the range of 70-120 mg/dL (3.9-6.7 mmol/L) while approaching a normal feeding regimen according to age.

The 2-hour dose-adjustment interval will allow plasma drug levels to approach approximately steady-state before the dose is further increased. The maximum dose of trial drug product that can be administered over the first 24 hours is shown in Table 1. The maximum cumulative dose over the first 24 hours is 1.26 mg.

After the first 24 hours, the dose of dasiglucagon can still be modified to optimize each patient's treatment and to reduce gastric dextrose infusions. The maximum dose of dasiglucagon will be 70 µg/hr, corresponding to a maximum of 1.68 mg per day.

Details on the administration instructions and guidelines for preparation and handling of the trial drug product are in the pharmacy manual.

Table 1 Initial 24-hour Maximum Dose of Dasiglucagon

Time (hours)	0	1	2	3	4	5	6	7	8	9	10	11
Dose (µg)	10	10	20	20	30	30	40	40	50	50	60	60
Cumulative dose (µg)	10	20	40	60	90	120	160	200	250	300	360	420

Time (hours)	12	13	14	15	16	17	18	19	20	21	22	23
Dose (µg)	70	70	70	70	70	70	70	70	70	70	70	70
Cumulative dose (µg)	490	560	630	700	770	840	910	980	1050	1120	1190	1260

Patients who complete this trial will be offered to enter a long-term extension trial (Trial ZP4207-17106) to continue dasiglucagon treatment (pending investigator confirmation of continued positive benefit-risk balance). Patients who do not continue in the long-term extension trial will have a Follow-up visit performed 4 weeks after stopping 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)/guardians on the use of the infusion pump, the PG meter and CGM.

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

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
PG monitoring	StatStrip Xpress2	Nova Biomedical, Waltham, MA, USA
CGM	Dexcom G4	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 is used as intended according to the CE mark, except for the age group and the disease.

The pump device will be packaged and labeled for use in investigational trials and will contain a unique device number. For more information on the devices please refer to Section 20.

10.4. Dispensing and Storage

Trial drug product supplied by the sponsor is to be dispensed exclusively for patients enrolled in this clinical trial according to the instructions of this protocol and the pharmacy manual. The investigator is responsible for dispensing the trial drug product according to the dosage scheme.

Trial drug product will be allocated using an interactive web response system (IWRS) that has been validated for the intended use under the International Society of Pharmaceutical Engineers (ISPE) GAMP guidelines, 21CFR Part 11 (FDA regulation for Electronic Records and Electronic Signatures), and the ICH Guidance E6 for Industry on Good Clinical Practice.

Dasiglucagon injection 4 mg/mL 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 refer to the pharmacy manual 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 product 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 product to any person except subinvestigators, designated trial personnel, and patients in this trial. The trial drug product may not be relabeled or reassigned for use by other patients. If any of the trial drug product 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

In Treatment Period 1, patients will be randomly assigned in a 1:1 ratio to receive either SOC only or SOC plus dasiglucagon for 4 weeks using a block randomization scheme stratified by region US/non-US). In Treatment Period 2, all patients will receive SOC plus dasiglucagon for 4 weeks. The stratification by region addresses the difference in practice of treatment for CHI, especially the prominent difference in frequency of sub-total pancreatectomy for diffuse CHI between US/non-US.

10.6. Blinding and Unblinding Treatment Assignment

This is an open-label trial. During parts of Screening, Treatment Period 1, and Treatment Period 2, patients will have CGM performed but the results will be masked.

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.[15]

The predicted plasma concentration is expected to give a low PD response at the lowest dose level and be above maximum effect at the highest dose level. The maximum expected plasma concentrations of dasiglucagon is in the range of what is achieved following a rescue dose to adults. The doses of dasiglucagon will be titrated to meet the needs of the individual patient. The titration will stop when no additional PD effects are observed as the infusion rate is increased. The infusion rate of dasiglucagon will be monitored and adjusted to meet the needs of the individual patient throughout the trial period.

Since the dasiglucagon dose is titrated individually based on the desired PD response, it does not need to be related to any measure of the patient's size.

The titration interval of 2 hours was chosen based on the PK data of dasiglucagon showing that an approximate steady-state is expected after 2 hours.

10.8. Selection of Timing of Dose for Each Patient

Dosing details are presented 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 by trial personnel and drug accountability will be performed as detailed in the pharmacy manual.

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

Concomitant CHI treatments (e.g., somatostatin analogs) that were initiated prior to trial entry are permitted throughout the trial. Somatostatin analogs may also be added throughout the trial at the investigator's discretion if the maximum dose level of dasiglucagon (70 µg/hr) has been reached or if further titration is not possible due to undesirable side effects.

Other CHI-specific treatments either prior to trial initiation or added during the trial need to be discussed with the medical monitor.

10.11.2. Prohibited Therapies

The following therapies are prohibited during the trial:

- Systemic corticosteroids, e.g., hydrocortisone >20 mg/mg/m^2 body surface area or equivalent from 5 days before screening and onwards
- Anti-inflammatory biological agents, or other immune-modulating agents in the 3 months prior to screening (except for sirolimus/mTOR inhibitors, as discussed in Section 10.11.1)
- Exogenous insulin
- Use of paracetamol/acetaminophen is strongly discouraged for the duration of trial because it interferes with CGM accuracy. Parent(s)/guardians should contact the trial site before dosing their child with paracetamol/acetaminophen. Both the site staff and the parent(s)/guardians should explore other options for treating fever and mild pain before deciding that paracetamol/acetaminophen is needed.
- Other investigational agent
- 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 SOC
- Prescription or non-prescription medications known to cause QT prolongation

Continuation in the trial after the patient has received excluded therapies will be at the investigator's discretion after consultation with the medical monitor.

10.12. Patients May be Discharged for Home Treatment

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

- Appropriate training of patient's parent(s)/guardians 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 the 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 the hospital 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

After completing both treatment periods, patients may be eligible to continue in a long-term extension trial (Trial ZP4207-17106) (see Section 10.2). Patients who do not continue in the long-term extension trial will not be offered further treatment with dasiglucagon.

11. TRIAL PROCEDURES

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

France, Germany, Israel*: The consent must correspond to the patient's presumed will where such a will can be ascertained. Depending on local IRB/IEC requirements the patient should also provide assent before any trial-related procedures are initiated.*

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 overall trial duration is expected to be 15 months.

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

1. Screening: at least 14 days up to 28 days
2. Treatment Period 1: 4 weeks
3. Treatment Period 2: 4 weeks
4. Follow-up Period: 4 weeks

The maximum trial duration for each patient is approximately 16 weeks. The dasiglucagon treatment duration for each patient is 4 or 8 weeks.

11.2. Assessments

Quality of life should be the first assessments performed at each visit according to the Schedule of Events (Section 18.1).

11.2.1. Efficacy

11.2.1.1. Plasma Glucose Monitoring

During the trial, SMPG assessments (StatStrip Xpress2) will be performed regularly (at least 3 times daily, preferably before meals, as instructed by the investigator) to evaluate efficacy.

At each visit, the investigator will ensure that SMPG data are downloaded from the patient's devices. The investigator will check for patient compliance in number of daily SMPG measurements and that hypoglycemic episodes are recorded in the diary. The procedure for download of SMPG data will be described in the pharmacy manual.

11.2.1.2. Continuous Glucose Monitoring

Additionally, CGM will be used (Dexcom G4) in a blinded manner to evaluate efficacy in terms of hypoglycemic episodes. The CGM will be supplied for use throughout the trial, and continuous glucose monitoring is required for the 2 weeks up to randomization, during Week 2-4 of Treatment Period 1, and during Week 6-8 of Treatment Period 2. Short pauses of 1-3 days due to skin irritation or discomfort are allowed after consultation with the investigator.

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

Dexcom G4 CGMs configured and labeled for use in this trial will be provided. Each CGM device should be calibrated and used according to the manufacturer's instructions.

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

11.2.1.3. Quality of Life

Quality of life (Appendix B) will be assessed using the PedsQL and additional CHI disease-specific QoL questions (parent-reported versions) according to the Schedule of Events (Section 18.1).

The PedsQL Measurement Model is a modular approach to measuring health-related quality of life in healthy children and adolescents and those with acute and chronic health conditions. The PedsQL Measurement Model integrates seamlessly both generic core scales and disease-specific modules into one measurement system.

The 23-item PedsQL Generic Core Scales was designed to measure the core dimensions of health as delineated by the World Health Organization, as well as role (school) functioning. The 4 Multidimensional Scales and 3 Summary Scores are:

Scales	**Summary Scores**
• Physical Functioning (8 items)	• Total Scale Score (23 items)
• Emotional Functioning (5 items)	• Physical Health Summary Score (8 items)
• Social Functioning (5 items)	• Psychosocial Health Summary Score (15 items)
• School Functioning (5 items)	

The CHI disease-specific questions have been developed by the patient association, Congenital Hyperinsulinism International, and taken from the patient-reported registry, the HI Global Registry. The HI Global Registry questions are grouped mostly under general quality of life; however, some questions relate specifically to diet and feeding, surgical management, glucose monitoring, and child development. The HI Global Registry is governed by a Global Steering Committee, including key global clinical experts.

11.2.1.4. Other Assessments

Resource Utilization

- Emergency department visits for hypoglycemia
- Number and length of hospitalizations due to CHI or CHI-related events
- Number of outpatient visits to health care providers (family doctors, specialist, etc.) caused by CHI or CHI-related events
- Number of home visits by paramedics due to hypoglycemia

Diary

The patient's parent(s)/guardian will be provided with a paper diary at all visits except at Visit 7 and the Follow-up Visit. The investigator will instruct the patient's parent(s)/guardian on how to complete the diary. The diary should be completed throughout the trial until the end of treatment visit (Visit 7). The following information should be recorded in the diary:

- Type and volume of fluid administered through NG tube/gastrostomy (if applicable)
- Hypoglycemic events, including related SMPG measurements
- Concomitant medications
- AEs
- Hospitalizations, visits to health care providers or ER, and visit 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 should be transcribed to the eCRF on an ongoing basis.

11.2.1.5. Fasting Tolerance Test/Safety Fast

During the screening period and at the end of Treatment Period 1, all patients will undergo a fasting tolerance test. This test must be performed when the CGM is active, and not the first day of CGM sensor, e.g., the day before randomization. This fasting tolerance test will be initiated according to the site's normal clinical practice and the anticipated duration of the test will be at investigator's discretion.

The test will commence after the patient's normal meal (t=0 will be the beginning of the meal). The meals have to be the same for both tests for the same individual. The frequency of PG sampling using the PG meter will be conducted according to site's normal practice and the individual patient's needs. This test is planned to last for 12 hours but will be stopped when PG is ≤60 mg/dL (3.3 mmol/L) and then ketones, insulin, and free fatty acids will be measured. The child will then be fed. If this value has not been reached at 12 hours, the test should be extended until it is reached.

The investigator will record the type and size of the meal, the date, start time, all PG measurements obtained, ketone measurement, and the end time of the test in a dedicated eCRF page.

The duration of fasting tolerance will be measured from the beginning of the normal meal until the beginning of the first continuous 15-minute CGM reading of <70 mg/dL (3.9 mmol/L).

11.2.2. Pharmacokinetics/Drug Exposure

Blood samples will be collected twice during the trial to measure for dasiglucagon levels at steady-state (Schedule of Events; Section 18.1).

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; electrocardiograms (ECGs), echocardiography, and local tolerability issues.

11.2.3.1. Laboratory Safety Assessments

Trial procedures require a maximum total of 12 mL blood in a 30-day period. Where this exceeds the 2.5 mL/kg maximum,[16,17] 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.

Laboratory Tests to be Performed

Samples for hematology and chemistry 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 volume, 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, sodium, potassium, chloride, eGFR, urea, insulin, ketones (measured at the local laboratory or with the PG meter), free fatty acids

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 5 (Week 5, prior to dosing), Visit 7 (Week 9) and at the Follow-up Visit (Visit 8, Week 12) (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.

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 an additional ADA Follow-up Visit (not included in the Schedule of Events) approximately 16 weeks after the End of Trial visit (Visit 8).

Upon ADA assay approval, the ADA samples will be analyzed in batches during the trial. The ADA samples will be analyzed at a special laboratory.

The clinical ADA assays for dasiglucagon have been validated in accordance with existing guidelines and recommendations.[18,19,20]

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

- Cross-reactivity against endogenous glucagon (cross-reactivity Yes/No)
- Establishment of anti-dasiglucagon binding titer.
- Dasiglucagon in-vitro neutralizing potential of the antibodies.
- Glucagon in-vitro neutralizing potential of the antibodies (only if positive for cross-reactivity).
- Neutralizing antibody titers, in case of a positive result in the in-vitro neutralizing antibody assays.

The in-vitro neutralizing effects of antibodies will be measured using an assay based on glucagon receptor-transfected human embryonic kidney cells. The assays were also validated for both dasiglucagon and recombinant glucagon neutralizing antibodies. The cell-based neutralizing antibody analyses will be performed by a special laboratory.

Anti-dasiglucagon antibody-positive patients (treatment induced or treatment boosted [titer increase above 4 fold]) will be monitored until the ADA level returns to pre-dose level.

Any residual serum samples will be stored until approval of market authorization by health authorities.

In addition to the above, the neutralizing potential in samples from ADA-positive patients will be evaluated in relation to PK/PD data.

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 laboratory 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 significant 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 according to the Schedule of Events (Section 18.1).

Twelve-lead Electrocardiogram

A standard 12-lead ECG will be performed while the child is in a sleeping or calm state according to the time points specified in the Schedule of Events (Section 18.1). If it is not practical or possible, then a 2-lead may be used.[21] If arrhythmia is detected on a 2-lead ECG, this should be followed by a 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, or 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 (excluding breast and genitourinary examinations) according to standard of care and a neurological examination (including cranial nerves, muscle strength and tone, reflexes, coordination, sensory function, and gait, all as applicable for the patient's age) 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 take (e.g., no action, interruption of infusion). The likely cause of the reaction will also be collected (e.g., insertion site, drug, or 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.

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 an SAE should furthermore be recorded as an SAE. The following information should be collected:

- Date, start time
- PG start 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 (time when PG exceeded a threshold of 70 mg/dL [3.9 mmol/L] or 60 minutes after the start of the event) and PG value, if available
- 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 even if normoglycemia (>70 mg/dL) is not reached within this time. A new episode of hypoglycemia is to be reported when the next PG value below 70 mg/dL (3.9 mmol/L) is measured.

11.2.3.4. Reporting of Technical Complaints

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

- Dasiglucagon 4 mg/mL 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 system

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 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 sponsor QA, refer to Appendix A for contact details.

Collection, Storage, and Shipment of Technical Complaint Item(s)

The investigator must collect and store the item(s) that is the subject of the technical complaint 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 AND PREGNANCIES

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 non-investigational) product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease 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).
- Pre-planned procedure, unless the condition for which the procedure was planned has worsened from the first trial-related activity after the patient has signed the informed consent.

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 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 emergency room 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. Non-serious Adverse Events

A non-serious 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 AEs of special interest (AESI events or 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 non-serious, 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 calendar 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 EDC. 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's 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 dedicated AESI eCRF page(s). Reporting requirements for serious and non-serious AEs as described previously also apply for serious and non-serious 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 in the eCRF. 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 non-serious AEs which subsequently become SAEs.

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

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

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. Pregnancy

Parent(s)/guardian of female patients who are of childbearing potential must be instructed to notify the investigator immediately if their child becomes pregnant or if they suspect she is pregnant during the trial. All initial reports of pregnancy in female patients must be reported by trial site personnel using the appropriate pregnancy form in EDC within 24 hours of their knowledge of the event. Abnormal pregnancy outcomes (e.g., spontaneous abortion, fetal death, stillbirth, congenital anomalies, or ectopic pregnancy) are considered SAEs and must be reported

using the SAE form. If a patient becomes pregnant during the trial, treatment must be discontinued.

The investigator must follow the pregnancy until its outcome is known and the newborn infant is 1 month of age. The investigator must report information about the pregnancy, pregnancy outcome, and health of the newborn infant(s), as well as AEs in connection with the pregnancy, and AEs in the fetus and newborn infant.

12.5. 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' parents. 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 parents 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.

12.6. 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 additionally on an ad hoc basis as needed.

12.7. 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, which are presented in the form H_{ij}, where i = treatment period (i = 1) and j = hypothesis number (j = 1, 2, 3, 4).

The hypothesis relating to the primary endpoint is:

H_{11}: Hypoglycemia event rate $_{dasiglucagon}$ = hypoglycemia event rate $_{SOC\ only}$

The hypotheses relating to the key secondary endpoints are:

H_{12}: Increase in fasting tolerance $_{dasiglucagon}$ = increase in fasting tolerance $_{SOC\ only}$

H_{13}: CGM percent time in range $_{dasiglucagon}$ = CGM percent time in range $_{SOC\ only}$

H_{14}: Clinically significant hypoglycemia event rate $_{dasiglucagon}$ = clinically significant hypoglycemia event rate $_{SOC\ only}$

A fixed-sequence statistical strategy will test the primary (Section 7.2.1) and 3 key secondary endpoints of Treatment Period 1 (Section 7.2.2) in a pre-defined order, all at the same significance level ($\alpha = 0.05$ for the final analysis to maintain an overall Type I error rate of at maximum $\alpha = 0.05$; see statistical analysis plan [SAP] for more details), moving to a second endpoint only after a success on the previous endpoint.

The test hierarchy is:

Treatment Period 1

H_{11}: Hypoglycemia event rate (measured by SMPG) during Weeks 2-4 of the treatment period (primary endpoint)

H_{12}: Increase in fasting tolerance (i.e., time from meal to PG <70 mg/dL) from baseline to Weeks 2-4 of the treatment period (key secondary endpoint)

H_{13}: CGM percent time in range 70-180 mg/dL (3.9-10.0 mmol/L) during Weeks 2-4 of the treatment period (key secondary endpoint)

H_{14}: Clinically significant hypoglycemia event rates, defined as average weekly number of events <54 mg/dL (3.0 mmol/L) (as detected by SMPG) during Weeks 2-4 of the treatment period (key secondary endpoint)

13.2. Sample Size Determination

Patients will be randomized into the trial if they have at least 3 events of hypoglycemia on average per week, as recorded in the diary during the 2 weeks prior to randomization. It is assumed that patients continuing on standard of care will maintain a similar level through Treatment Period 1, with the number of hypoglycemia events (PG <70 mg/dL or 3.9 mmol/L) during Weeks 2-4, as detected by SMPG following a Poisson distribution with a mean of 9. The trial is powered to detect a treatment effect of 50%, hence, assuming that the number of hypoglycemia events reported for patients in the dasiglucagon group during Weeks 2-4 will follow a Poisson distribution with a mean of 4.5. At the final analysis, 32 patients will have 99% power testing at a 0.05 significance level. The overall alpha level is strongly controlled in this setting, remaining at or below 0.05.

13.3. Analysis Populations

Three analysis populations are defined for this trial:

- The Safety Set: defined as all patients administered any randomized treatment. This population will be used to provide descriptive summaries of safety data. Patients will be summarized by treatment period according to treatment received.
- The Full Analysis Set (FAS): defined as all patients in the Safety Set who have a valid baseline efficacy assessment. This population will be used to analyze efficacy data. Patients will be analyzed by treatment period according to planned treatment.
- The Per Protocol (PP) Analysis Set: defined as all patients in the FAS without any major protocol deviations. This population will be used to analyze primary and key secondary endpoints as a supportive analysis. Patients will be analyzed according to planned treatment for the first treatment period only.

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

13.4. Statistical Analyses

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

Unless otherwise indicated, all testing of statistical significance will be 2-sided with a significance level of $\alpha = 0.05$ to maintain an overall Type I error rate of $\alpha = 0.05$; see SAP for more details.

For analyses 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.

Continuous endpoints will be summarized with number (n), mean, SD, median, minimum, and maximum. In addition, change from baseline values will be calculated at each time point and summarized descriptively. For categorical endpoints, descriptive summaries will include counts and percentages.

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 group. 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/height) 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 group and 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 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 group.

13.4.3. Efficacy Analyses

The efficacy analysis will be separated into 3 categories:

1. Treatment Period 1, FAS
2. Treatment Period 1, PP (primary and key secondary endpoints only)
3. Treatment Period 2, FAS

13.4.3.1. Primary Analysis

Primary Endpoint (Treatment Period 1)

The primary efficacy endpoint is the hypoglycemia event rate during Weeks 2-4 of Treatment Period 1. A hypoglycemia event is defined as PG <70 mg/dL or 3.9 mmol/L, as detected by SMPG. The analysis will be based on the hypoglycemia events reported in the eCRF. Baseline is defined as the average weekly number of hypoglycemic events during the 2-week baseline period. Weeks 2-4 of Treatment Period 1 is defined as the average weekly number of hypoglycemic events across the last 3 weeks of the treatment period. The hypothesis:

$$H_{11}: \text{Hypoglycemia event rate}_{\text{dasiglucagon}} = \text{hypoglycemia event rate}_{\text{SOC only}}$$

will be analyzed by using negative binomial regression, with treatment group as a fixed effect and baseline hypoglycemic rate as a covariate. The log-transformed number of days in Treatment Period 1 will be used as an offset variable. The null hypothesis is that there is no difference in the incidence of average weekly number of hypoglycemic events between the 2 treatment groups, which will be tested at the significance level of α=0.05.

The primary analysis will estimate the treatment effect based on the de facto (treatment policy) estimand. All available data in the form of actual measurements will therefore be included in the analysis, irrespective of adherence to treatment or use of subsequent therapy. Missing data will be imputed using multiple imputation (MI) methodology as described in Section 13.4.3.4.

As a sensitivity analysis, the primary endpoint will also be analyzed without imputation of missing data; however, this analysis will not be included in the fixed-sequence hierarchical

testing strategy. Similarly, the primary endpoint may be analyzed excluding post-baseline data collected after trial drug discontinuation/completion.

13.4.3.2. Key Secondary Analyses

Treatment Period 1

The key secondary endpoint of clinically significant hypoglycemia (<54 mg/dL [3.0 mmol/L]) event rates will be analyzed using a negative binomial regression, with treatment group as a fixed effect and baseline hypoglycemic rate as a covariate. Percent time in range (i.e., the percent time between 70 mg/dL [3.9 mmol] and 180 mg/dL [10.0 mmol], inclusive, as measured by CGM, where percent time is calculated as [number of minutes in range/total number of minutes patient is wearing CGM] * 100%) will be analyzed by using an ANCOVA, with treatment group and region as fixed effects and baseline time in range as a covariate. Increase in fasting tolerance (i.e., change from baseline in time from meal to PG <70 mg/dL) will be analyzed similarly, using an ANCOVA, with treatment group and region as fixed effects and baseline fasting tolerance as a covariate. Baseline is defined as the mean weekly value during the 2-week baseline period; Weeks 2-4 of Treatment Period 1 is defined as the mean weekly value across the last 3 weeks of the treatment period.

Missing data will be imputed in a similar manner to the primary endpoint. Analyses will be repeated without imputation of missing data; as with the primary endpoint, these analyses will not be included in the fixed-sequence hierarchical testing strategy.

13.4.3.3. Secondary and Other Efficacy Analyses

For the remaining efficacy endpoints, continuous and categorical endpoints will be presented using summary statistics or frequencies, respectively; no inference will be performed. Missing data will not be imputed.

The secondary efficacy endpoints related to the use of gastric carbohydrates will be evaluated descriptively only in the subgroup of patients with gastrostomy or NG tube at baseline.

Resource Utilization

The number and percentage of patients with admissions/emergency department visits for glycemia, hospitalizations due to CHI, visits to health care providers (family doctor, specialists, etc.) caused by CHI or CHI-related events, and need for home visits by parameters will be summarized. Additionally, number and length (in days) of hospitalizations due to CHI, number of visits to health care providers (family doctor, specialists, etc.) caused by CHI or CHI-related events, and number of home visits by paramedics will be summarized.

Quality of Life

Quality of life will be assessed by the PedsQL and a CHI disease-specific questionnaire (Appendix B). For each item of the PedsQL instrument (parent), a 5-point response scale is used (0 = never, 1 = almost never, 2 = sometimes, 3 = often, 4 = almost always). Items are reverse-scored and linearly transformed to a 0-100 scale (0 → 100, 1 → 75, 2 → 50, 3 → 25, 4 → 0) so that higher scores indicate better health-related QoL (less negative impact). Scale scores are computed as the sum of the items divided by the number of items answered (this accounts for missing data). If more than 50% of the items in the scale are missing, the scale score is not computed. Change from baseline for PedsQL for each of the scales (physical functioning,

emotional functioning, social functioning, and school functioning) and summary scores (total scale score, physical health summary score, and psychosocial health summary score) will be summarized.

Answers to each question on the CHI disease-specific questionnaire will be summarized using frequencies at each relevant visit.

13.4.3.4. Imputation of Missing Data

For analysis of primary and key secondary endpoints, a jump-to-control (J2C) MI method, with SAS procedures PROC MI and PROC MIANALYZE, will be performed, including demographics and baseline characteristics in a first MI analysis. Due to the expectation of rapid metabolism and excretion of trial drug, it is expected that efficacy would be similar to the control group in cases of premature treatment discontinuation; thus, the J2C method is appropriate to handle missing values in this situation (considered missing not at random [MNAR]). Any missing values occurring before the patient discontinues/completes treatment will be imputed based on the data from the same treatment group (considered missing at random [MAR]).

The analysis will have 3 broad components: i) the MI process for the SOC-only data; ii) the MI process for the dasiglucagon + SOC patients' data; and iii) the analysis model that will be used to draw inference regarding the primary causal estimand, along with the method for combining the results across the multiply imputed datasets. The seed to be used in the analysis is 12255070. The algorithm will use 20 burn-in iterations before each imputation, and 1000 imputed datasets will be created for the analysis for each of steps i and ii.

To accommodate both monotone and non-monotone missingness patterns, imputed values will be generated by a pattern mixture imputation model under the MNAR assumption using the fully conditional specification (FCS) method. The primary analysis will be performed using a negative binomial regression model from which hypoglycemia incidence density ratios (IDR) and 95% confidence intervals are obtained, along with the model parameter estimates, standard errors, and *p* values; analyses of the key secondary endpoints will be performed using the appropriate modeling. The final step in the imputation will involve using PROC MIANALYZE to derive valid univariate inference for these parameters. Multivariate inference based on the Wald test will be derived to test for the significance of these parameters.

Complete details for conducting this analysis will be outlined in the SAP.

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 non-missing assessment before first dose in each treatment period.

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

13.4.4.1. Adverse Events

Adverse events will be coded using the latest 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 will be displayed by system organ class, PT, and treatment group. The incidence of AEs will also be presented by severity and by relationship to the trial drug. Serious AEs and AEs resulting in discontinuation of trial drug will be summarized separately in a similar manner. Patient listings of AEs, SAEs, and AEs causing discontinuation of trial drug 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 below, within, or above normal ranges 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. Physical Examination Findings

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

13.4.4.6. Local Tolerability

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

13.4.4.7. Interim Analysis

No interim analysis is planned.

14. TRIAL CONDUCT

The accuracy and reliability of data is 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 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. Data from individual trial sites must not be published separately.

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 results from the neutralizing antibody assay may be included or reported separately pending availability of the results.

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 C 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.

France, Germany, Israel*: The consent must correspond to the patient's presumed will where such a will can be ascertained.*

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.

France, Germany, Israel*: Additionally, the patient will be informed about the nature, significance, risks, and implications of the trial with age-appropriate information, and his or her assent will be obtained in accordance with local regulations, as applicable.*

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 its 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 withdraw his or her child (see Section 9.3 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 PM 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.

17. REFERENCES

1. Arnoux J-B, Verkarre V, Saint-Martin C, et al. Congenital hyperinsulinism: current trends in diagnosis and therapy. Orphanet J Rare Dis. 2011;6:63:1-14.
2. De Leon DD, Stanley CA. Congenital hypoglycemia disorders: New aspects of etiology, diagnosis, treatment and outcomes. Pediatr Diabetes. 2016;1-7.
3. Lord K, Radcliffe J, Gallagher PR, et al. High risk of diabetes and neurobehavioral deficits in individuals with surgically treated hyperinsulinism. J Clin Endocrinol Metab. 2015;100(11):4133-4139.
4. Stanley CA. Perspective on the genetics and diagnosis of congenital hyperinsulinism disorders. J Clin Endocrinol Metab. 2016;101(3):815-826.
5. Mazor-Aronovitch K, Gillis D, Lobel D, et al. Long-term neurodevelopmental outcome in conservatively treated congenital hyperinsulinism. Eur J Endocrinol. 2007;157(4):491-497.
6. Welters A, Lerch C, Kummer S, et al. Long-term medical treatment in congenital hyperinsulinism: a descriptive analysis in a large cohort of patients from different clinical centers. Orphanet J Rare Dis. 2015;10;150:1-10.
7. Arya VB, Senniappan S, Demirbilek H, et al. Pancreatic endocrine and exocrine function in children following near-total pancreatectomy for diffuse congenital hyperinsulinism. PLoS ONE. 2014;9(5):e98054. https://doi.org/10.1371/journal.pone.0098054.
8. Cederblad F, Ewald U, Gustafsson J. Effect of glucagon on glucose production, lipolysis and gluconeogenesis in familial hyperinsulinism. Horm. Res. 1998;50:94-98.
9. Miralles RE, Lodha A, Perlman M, Moore AM. Experience with intravenous glucagon infusions as a treatment for resistant neonatal hypoglycemia. Arch Pediatr Adolesc Med. 2002;156(10):999-1004.
10. Lord K, Dzato E, Snider KE, Gallagher PR, De Leon DD. Clinical presentation and management of children with diffuse and focal hyperinsulinism: a review of 223 cases. J Clin Endocrinol Metab. 2013;98(11):E1786-E1789.
11. Mohnike K, Blankenstein O, Pfuetzner A, et al. Long-term non-surgical therapy of severe persistent congenital hyperinsulinism with glucagon. Horm Res. 2008;70:59-64.
12. Pedersen JS, Dikov D, Flink JL, Jhuler HA, Christiansen G, Otzen DE. The changing face of glucagon fibrillation: structural polymorphism and conformational imprinting. J Mol Biol. 2006;355(3):501-523.
13. Rose SR, Chrousos G, Cornblath M, Sidbury J. Management of postoperative nesidioblastosis with zinc protamine glucagon and oral starch. J Pediatr. 1986;108(1):97-100.
14. Chastain MA. The glucagonoma syndrome: A review of its features and discussion of new perspectives. Am J Med Sci. 2001;321(5):306–320.
15. Zealand Pharma report. Population PK modeling and simulation of dasiglucagon in pediatric patients with type I diabetes mellitus. 2017.
16. European Medicines Agency. ICH Topic E11: Clinical Investigation of Medicinal Products in the Paediatric Population. January 2001.
17. Howie, S. Blood sample volumes in child health research: review of safe limits. Bull World Health Organ 2011; 89:46–53.

18. Food and Drug Administration (FDA). Guidance for Industry on Bioanalytical Method Validation. Food and Drug Administration. 2001.
19. Viswanathan CT, Bansal S, Booth B, et al. Quantitative bioanalytical methods validation and implementation: best practices for chromatographic and ligand binding assays. Pharm Res. 2007;24(10):1962-73.
20. Shankar G, Devanarayan V, Amaravadi L, et al. Recommendations for the validation of immunoassays used for detection of host antibodies against biotechnology products. J Pharm Biomed Anal. 2008;48(5):1267-81.
21. Food and Drug Administration (FDA). Guidance Industry. E14 Clinical Evaluation of QT/QTc Interval Prolongation and Proarrhythmic Potential for Non-antiarrhythmic Drugs. Food and Drug Administration. 2005.
22. McKinlay CJD, Chase JG, Dickson J, et al. Continuous glucose monitoring in neonates: a review. Matern Health Neonatol Perinatol. 2017;3:18.
23. Rabbone I, Barbetti F, Marigliano M, et al. Successful treatment of young infants presenting neonatal diabetes mellitus with continuous subcutaneous insulin infusion before genetic diagnosis. Acta Diabetol. 2016 Aug;53(4):559-65.
24. Beardsall K, Vanhaesebrouck S, Ogilvy-Staurt AK, et al. Validation of the continuous glucose monitoring sensor in preterm infants. Arch Dis Child Fetal Neonatal Ed. 2013;98(2):F136-40.
25. Baumeister FA, et al. Glucose monitoring with long-term subcutaneous microdialysis in neonates. Pediatrics. 2001;108:1187-1192.
26. Kerr D, Hoogma RP, Buhr A, et al. Multicenter User Evaluation of ACCU-Chek® Combo, an Integrated System for Continuous Subcutaneous Insulin Infusion. J Diabetes Sci Technol. 2010;4(6):1400-7.
27. Heinemann L, Fleming GA, Petrie JR, et al. Insulin pump risks and benefits: a clinical appraisal of pump safety standards, adverse event reporting, and research needs: a joint statement of the European Association for the Study of Diabetes and the American Diabetes Association Diabetes Technology Working Group. Diabetes Care. 2015;38(4):716-22.
28. 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

Trial Period	**Screening**	***Treatment Period 1***				***Treatment Period 2***				**Follow-up**
Week		**1[b]**	**Telephone Call[c]**	**2**	**4**	**5[d]**	**Telephone Call[c]**	**6**	**9**	**12**
Day		**1**		**8**	**22**	**29**		**36**	**57**	**85**
Time window (days)	**Day -28 to -14[a]**	**±2**		**±2**	**±2**	**±2**		**±2**	**±2**	**±5**
Visit #	**1**	**2**		**3**	**4**	**5**		**6**	**7[e]**	**8**
General assessments										
Informed consent/assent	X									
Inclusion/exclusion criteria	X	X								
Randomization criteria		X								
Demography	X									
Body weight and length/height[f]	X	X				X			X	X
Medical history (including current illness)	X									
Concomitant medication	X	X		X	X	X		X	X	X
Safety Assessment										
Electrocardiogram	X	X[r]		X		X[r]		X	X	X
Echocardiography	X[g]								X	X
Vital signs[h]	X	X[s]		X	X	X[s]		X	X	X
Serum Pregnancy test[i]	X								X	X
Adverse events		X	X	X	X	X	X	X	X	X
Local tolerability		X		X	X	X		X	X	
Physical examination and neurological examination	X	X		X	X	X		X	X	X

Trial Period	**Screening**	***Treatment Period 1***				***Treatment Period 2***				**Follow-up**
Week		**1[b]**	**Telephone Call[c]**	**2**	**4**	**5[d]**	**Telephone Call[c]**	**6**	**9**	**12**
Day		**1**		**8**	**22**	**29**		**36**	**57**	**85**
Time window (days)	**Day -28 to -14[a]**	**±2**		**±2**	**±2**	**±2**		**±2**	**±2**	**±5**
Visit #	**1**	**2**		**3**	**4**	**5**		**6**	**7[e]**	**8**
Laboratory Assessments										
Clinical laboratory test[j]	X			X		X		X	X	X
Antibodies[k]		X[l]				X			X	X[k]
Pharmacokinetics/drug exposure								X	X	
Efficacy										
Continuous glucose monitoring	X (for at least 14 days prior to randomization)	Continuous[m]								
Self-monitored plasma glucose	X (for at least 14 days prior to randomization)	X (at least 3 times daily)								
Fasting tolerance test[n]	X (during active CGM period)					X[o]				
Trial Materials and reminders										
Randomization		X								
Dispense patient diary	X[p]	X		X	X	X		X		
Diary review		X		X	X	X		X	X	
QoL questionnaires[q]		X				X			X	X
Dispensing of trial product		X		X	X	X		X		

Abbreviations: CGM = continuous glucose monitoring; PG = plasma glucose; QoL = quality of life; SMPG = self-monitoring of plasma glucose; SOC = standard of care; SpO_2 = blood oxygen saturation level; W = week

Note: An unscheduled visit can occur at any time if the investigator deems it necessary for patient safety.

a Screening must occur within a minimum of 14 days to allow for solid baseline assessment.

b At the beginning of Treatment Period 1 (Week 1), all patients will be hospitalized for 1-2 days. Patients assigned to SOC plus dasiglucagon treatment will be initiated and titrated on dasiglucagon, trained in the use of the infusion pump, and supervised. Patients are assigned to SOC alone will receive a similar degree of supervision alongside the training in the use of the infusion pump. This period can be extended for both treatment groups if dasiglucagon titration has not been finalized or if training of the family/caregivers has not been completed satisfactorily.

c Patients 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 whether their child has experienced any AEs.

d In Treatment Period 2, patients who were assigned to SOC treatment only in Treatment Period 1 will be hospitalized for the first 1-2 days of Week 5.

e Visit 7 can be used as the first visit for Trial ZP4207 17106 (long-term extension trial) if the patient is continuing in long-term extension trial.

f Length/height will be measured at Screening only.

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

h Vital signs include blood pressure, heart rate, respiratory rate, and SpO_2.

i A serum pregnancy test will be performed for girls of childbearing potential.

j Clinical laboratory tests include hematology and biochemistry.

k Any anti-dasiglucagon antibody-positive patient (treatment induced or treatment boosted) will be monitored at an additional follow-up visit, preferably 16 weeks after the 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 The sample for ADAs should be taken prior to dosing of dasiglucagon in Treatment Period 1.

m Continuous glucose monitoring is required during Weeks 2-4 of Treatment Period 1 and Weeks 6-8 of Treatment Period 2.

n The fasting tolerance test will be stopped when PG is ≤60 mg/dL (3.3 mmol/L) and then ketones, insulin, and free fatty acids will be measured.

o The fasting tolerance test at Visit 5 should take place before initiation of dosing in Treatment Period 2 but while the patient is still on CGM.

p At screening, dispense diary and instruct patients' parent(s)/guardian in its use. At all other visits, the parent(s)/guardian will return the completed diary and obtain a new one.

q The PedsQL (parent-reported versions) and CHI disease-specific questionnaires should be the first assessments performed at each visit.

r ECG should be performed at the start of the visit and at 24 ± 4 hours after initiation of trial drug for patients initiating treatment.

s Vital signs should be measured at the start of the visit and at 6 ± 1, 12 ± 2, and 24 ± 4 hours after initiation of the trial drug for patients initiating treatment.

18.2. Investigator's Agreement

PROTOCOL NUMBER:	ZP4207-17109
PROTOCOL TITLE:	A Two-Period, Open-label Trial Evaluating the Efficacy and Safety of Dasiglucagon for the Treatment of Children with Congenital Hyperinsulinism
FINAL PROTOCOL:	Version 14.0 (Germany), 12-Nov-2020

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 the sponsor.
- 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 the sponsor 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 the sponsor 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. Contact Information
B. Quality of Life Questionnaires
C. Regulations and Good Clinical Practice Guidelines

20. ADDENDUM

20.1. Administrative Information

The Coordinating Investigator for Germany is:

[redacted]

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 ZP4207-17109 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 l00 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-Check 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 x 64.0 x 22.9 mm and a weight of 78.5g) 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 Platinum Professional G4 Continuous Glucose Monitoring System

In this trial, the Dexcom Platinum Professional Continuous Glucose Monitoring system G4 from Dexcom will be used. The Dexcom G4 is indicated for detecting trends and tracking patterns in patients (aged 2 and older) with diabetes. The system is intended for single-patient use in the home setting and also in healthcare facilities. It is indicated for use as an adjunct device to complement, not replace, information obtained from standard home glucose-monitoring devices. The G4 Platinum 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 G4 results should be based on the trends and patterns noted in several sequential readings over time.

The 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 7 days. The sensor is a sterile device inserted by the user into the abdominal subcutaneous tissue 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 G4 Platinum transmitter component is a miniature radio transmitter operating at an internationally accepted radiofrequency. 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 all the radiofrequency circuitry necessary to transmit the sensor signal to the receiver. 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 7-day sessions by a single user over the lifetime of the battery encased in the device.
- **Receiver** – The G4 receiver is a small hand-held device that contains an antenna and the associated electrical circuitry to receive the wireless sensor signal from the transmitter. The receiver contains a rechargeable battery. In typical use, the receiver may last for up to 3 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. Calibrations are performed twice daily by the patient's parent(s) /guardian(s) using measurements from a standard blood glucose meter device and manually entered into the receiver. In this trial the StatStrip Xpress2 meter GLU/KET from Nova Biomedical will be used to calibrate the CGM.

Other accessories include the following:

- Receiver USB charging / download cable
- Alternating current (AC) power adapter - MT21255
- User's guide
- Site training checklist
- Receiver shield pack containing receiver shield, square seals, triangle seal, and acoustic spacer

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/SBT and Receiver: Class IIa, Rule 10 according to Annex IX of the MDD 93/42/EEC and 2007/47/EC
- Dexcom G4 Platinum 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 October 2012.

The system components (applicator, transmitter housing, insertion needle and sensor are sterilized via electron beam radiation using the $(VD)_{max}$[22] method. Transmitters and receivers are not sterile products.

The trial sites will be supplied with the Dexcom Platinum CGM system G4 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 G4 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 same Dexcom Platinum G4 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 (3 months or older 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.[23] 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 with 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 device will still indicate when calibration is needed (as if the device was in unblinded mode). 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.

20.2.1.5. Risk Evaluation for Off-label Use of Devices

20.2.1.5.1. 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 G4 devices 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 5.3.5. 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.

20.2.1.5.2. 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 G4 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.

20.2.1.5.3. 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.

20.2.1.5.4. 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 G4 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 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 standard of care (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 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, 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 standard of care 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[24,25]
- 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 Platinum Profession G4

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.[26]

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 3 months and 12 years (both inclusive) 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:** With the planned sample size, the overall alpha level is strongly controlled, remaining at or below 0.05. Due to the short period of trial conduct, 4 weeks for Treatment Period 1 and another 4 weeks for Treatment Period 2, no formal drop-out rate was anticipated.
- **Pass/fail criteria:** No pass/fail criteria were defined since no trial endpoints are device related
- **Criteria for termination of trial on statistical grounds:** Please refer to Section 13.4.4.7 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.

A. Contact Information

Pharmacovigilance

Name: [redacted]

Address: [redacted]

[redacted]

E-mail: [redacted]

Telephone: [redacted]

Technical Complaints

Name: Zealand Pharma A/S Quality Assurance

Email: [redacted]

B. Quality of Life Questionnaires

- Infants 1-12 Months: "PedsQL™ Pediatric Quality of Life Inventory Infant Scales – Version 1 - Parent Report for Infants (ages 1-12 months)"
- Infants 13-24 Months: "PedsQL™ Pediatric Quality of Life Inventory Infant Scales – Version 1 - Parent Report for Infants (ages 13-24 months)"
- Parent Report for Teens 13-18 Years: "PedsQL™ Pediatric Quality of Life Inventory – Version 4 - Parent Report for Teens (ages 13-18 yrs)"
- Parent Report for Children 8-12 Years: "PedsQL™ Pediatric Quality of Life Inventory – Version 4 - Parent Report for Children (ages 8-12 yrs)"
- Parent Report for Children 5-7 Years: "PedsQL™ Pediatric Quality of Life Inventory – Version 4 - Parent Report for Young Children (ages 5-7 yrs)"
- Parent Report for Toddlers 2-4 Years: "PedsQL™ Pediatric Quality of Life Inventory – Version 4 - Parent Report for Toddlers (ages 2-4 yrs)"
- CHI Disease-Specific Questionnaire Developed by the Patient Association, Congenital Hyperinsulinism International, and Taken from the Patient-Reported Registry, the HI Global Registry

C. 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_Guideline.pdf