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

NCT Number: NCT04172441

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Version	Date	Author	Description
Final 1.0	18JUN2020	[redacted]	Initial Version
Final 2.0	11JUN2021	[redacted]	Updates to be consistent with ZP4207-17109 trial and to consider Protocol Amendment.
Final 3.0	08-APR-2022	[redacted]	Add tipping point analysis and multiple imputation for primary endpoint Clarify definition of Complete Weaning off GIR Clarify analysis of primary endpoint regarding data in between periods Add sensitivity analysis on CGM percent time in hypoglycemia for patients using Dexcom G4 with paracetamol Add sensitivity analysis on primary endpoint due to different GIR values at start of each cross-over period for few patients

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1. Overview

This statistical analysis plan (SAP) describes the planned analysis and reporting for Zealand protocol number ZP4207-17103 (A Randomized Trial in 2 parts: Double-Blind, Placebo-Controlled, Crossover Part 1 and Open-label Part 2, Evaluating the Efficacy and Safety of Dasiglucagon for the Treatment of Children with Congenital Hyperinsulinism), dated 04-Jun-2021 final version 14.0 (Germany) and 04-Jun-2021 final version 13.0 (all except Germany). Reference materials for this statistical plan include the protocol and the accompanying sample data collection documents. Operational aspects related to collection and timing of planned clinical assessments are not repeated in this SAP unless relevant to the planned analysis.

The structure and content of this SAP provides sufficient detail to meet the requirements identified by the Food and Drug Administration (FDA), European Medicines Agency (EMA), and International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use: Guidance on Statistical Principles in Clinical Trials[1]. All work planned and reported for this SAP will follow internationally accepted guidelines, published by the American Statistical Association[2] and the Royal Statistical Society for statistical practice[4].

The planned analyses identified in this SAP may be included in clinical study reports (CSRs), regulatory submissions, or future manuscripts. Also, post-hoc exploratory analyses not necessarily identified in this SAP may be performed to further examine study data. Any post-hoc or unplanned, exploratory analysis performed will be clearly identified as such in the final CSR.

The statistical plan described hereafter is an *a priori* plan. It will be submitted to file prior to any unblinded inferential or descriptive analysis of data pertaining to Zealand's study ZP4207-17103.

2. Study Objectives and Endpoints

2.1. Study Objectives

2.1.1. Primary Objective

The primary objective is to evaluate the efficacy of dasiglucagon in reducing glucose requirements in children with persistent congenital hyperinsulinism (CHI) requiring continuous IV glucose administration to prevent/manage hypoglycemia.

2.1.2. Secondary Objectives

The secondary objectives are to evaluate the safety and tolerability of dasiglucagon administered as an SC infusion in patients with CHI.

2.2. Study Endpoints

2.2.1. Safety Endpoints

The safety endpoints of this study will be analyzed for each part of this study and will include the following:

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

2.2.2. Efficacy Endpoints

Primary Efficacy Endpoint

The primary efficacy endpoint of this study is the evaluation of the weighted Mean IV glucose infusion rate (GIR) in the last 12 hours of each treatment period during Part 1 (Day 1 to 4) (dasiglucagon or placebo administration).

Key Secondary Efficacy Endpoint(s)

The key secondary efficacy endpoints of this study study evaluated during Part 1 (Day 1 to 4, for each 48-hour treatment period) includes the following:

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

Secondary Efficacy Endpoint(s)

The secondary efficacy endpoints of this study evaluated during Part 1 (Day 1 to 4, for each 48-hour treatment period) includes the following:

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

The secondary efficacy endpoints of this study evaluated during Part 2 (Day 5 to 25, assessed from the start of treatment in part 2) includes the following:

- Time to complete weaning off IV GIR will be described and a Kaplan-Meier curve will be produced
- Rate of SMPG-detected Hypoglycemia episode, defined as number of hypoglycemic events (PG <70 mg/dL or 3.9 mmol/L)

- Rate of SMPG-detected Clinically significant hypoglycemia episode, defined as number of events <54 mg/dL (3.0 mmol/L)
- Time to actual hospital discharge will be described and a Kaplan-Meier curve will be produced
- Time to pancreatic surgery (sub-total or total pancreatectomy) will be described and a Kaplan-Meier curve will be produced
- Total amount (g) of carbohydrates administered (regardless of route) per day, together with amounts (g) of carbohydrates administered per day:
    - via IV glucose infusion or bolus (not as part of parenteral nutrition),
    - as part of total parenteral nutrition (TPN) (if applicable),
    - via oral route, and
    - via NG tube or gastrostomy
- CGM percent time in range 70-180 mg/dL (3.9-10.0 mmol/L)
- CGM percent time in hypoglycemia (<70 mg/dL or 3.9 mmol/L)
- CGM percent time in clinically significant hypoglycemia (<54 mg/dL or 3.0 mmol/L).
- Rate of CGM-detected hypoglycemia episodes, defined as number of episodes <70 mg/dL (3.9 mmol/L) for 15 min or more
- Rate of CGM-detected clinically significant hypoglycemia episodes, defined as number of episodes <54 mg/dL (3.0 mmol/L) for 15 min or more
- Extent of hypoglycemia (area over the glucose curve [$AOC_{glucose}$] below 70 mg/dL [3.9 mmol/L]) as measured by CGM
- Extent of clinically significant hypoglycemia ($AOC_{glucose}$ below 54 mg/dL [3.0 mmol/L]) as measured by CGM
- CGM percent time in hyperglycemia (>180 mg/dL (10.0 mmol/L).

2.2.3. Pharmacokinetic Variable(s)

For all sujects, blood samples will be collected twice during the trial to measure for dasiglucagon levels at steady-state on Day 6 and Day 25. PK Sampling for drug exposure at Visit 5 is only applicable for patients with a body weight of ≥4 kg.

3. Overall Study Design and Plan

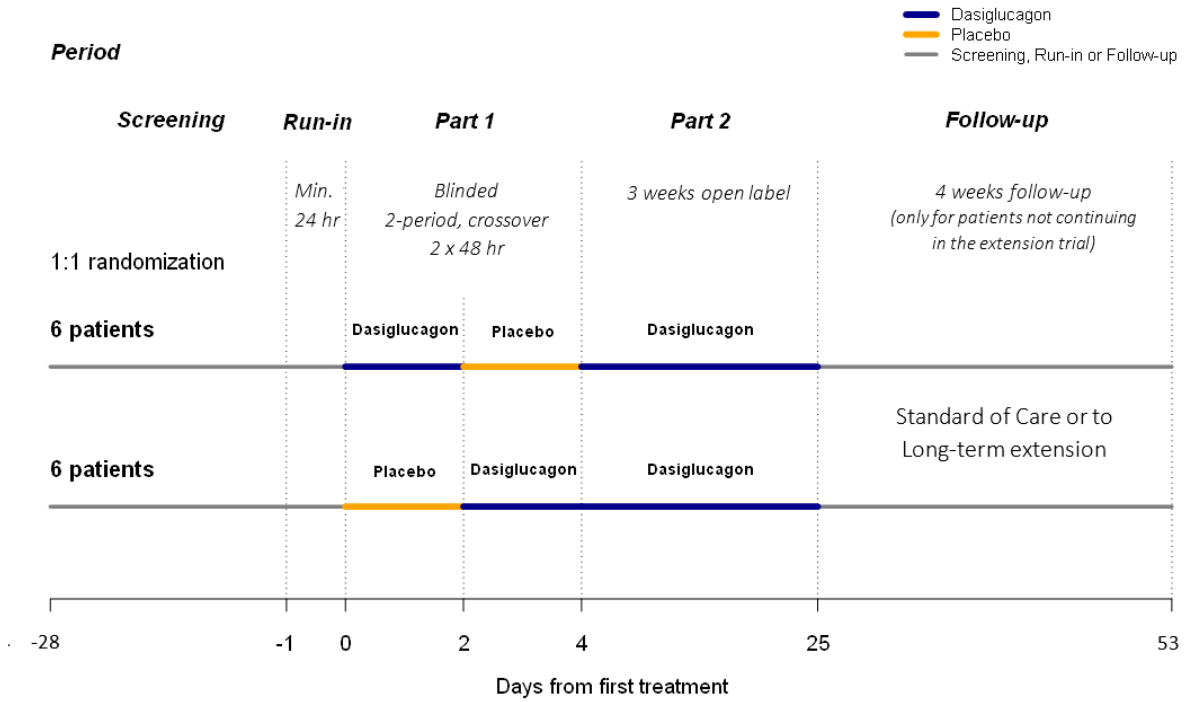
This is a combined phase 2 and 3, randomized, multinational trial to evaluate the efficacy and safety of individually titrated dasiglucagon in children ≥7 days and <1 year of age who have been diagnosed with CHI, comprising 2 parts, a crossover (2 periods, 48 hours each), double-blind, placebo-controlled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

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

and titrated accordingly.
There will be no washout between the 2 periods in Part 1 to limit the length of time in which patients are reliant on IV GIR as their only means of preventing/treating hypoglycemia.
During Part 1, the trial drug and IV GIR should be adjusted according to the protocol-specified algorithm. Non-nutritional carbohydrates and/or carbohydrate fortification of feeds during Part 1 is only allowed when the maximum tolerable volume and concentration of IV glucose for the patient is reached, and should be limited to the minimum needed to ensure the patient's safety. All feedings (administered as parenteral nutrition, by nasogastric [NG] tube, gastrostomy, or normal route), will be recorded during this period.
After Part 1, patients will continue in open-label Part 2 to receive dasiglucagon for 21 days. Additional CHI treatments can be introduced during Part 2 if needed, if up-titration of dasiglucagon is not possible because of undesirable side effects or if the maximum dose level (70 µg/hr) has been reached. Gradual transfer from IV glucose to oral and gastric carbohydrates should be initiated in Part 2, enabling weaning of IV glucose and hospital discharge. Patients will continue to be hospitalized until IV GIR is weaned off; however, as soon as local site criteria for discharge are met, patients can be discharged to continue the treatment period at home. Planned visits can be converted to telephone visits if appropriate, at the investigator's discretion. On Day 25, the End of Treatment Visit will take place, and based on the investigator's confirmation of continued positive benefit-risk balance, patients will be offered the opportunity to participate in the long-term extension trial (Trial ZP4207-17106) to continue dasiglucagon treatment. Patients who do not enter the long-term extension trial will have a Follow-up Visit 28 days after their last dose of trial drug. Figure 1 describes the study design.

No Interim Analysis is planned.

Figure 1



Abbreviations: Min = minimum

3.1. Overall Design

3.2. Sample Size and Power

A total of 12 patients is planned to be randomized and exposed to trial drug in this crossover trial on the basis of the following considerations. The GIR results from 40 infants treated with IV glucagon for 24 hours published in the JIMD Research Report "The Effect of Continuous Intravenous Glucagon on Glucose Requirements in Infants with Congenital Hyperinsulinism" [3](Hawkes et al 2019).

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

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

3.3. Study Population

The study population consists of male and female patients between the ages of 7 days and 12 months with established CHI diagnosis and requiring continuous IV glucose to prevent

hypoglycemia.

3.4. Treatments Administered

During the part 1, cross-over period, patient will receive Dasiglucagon and Placebo. During Part 2, patients will receive only Dasiglucagon.
Part 1 dispensing unit configuration: 3 vials containing dasiglucagon, 4 mg/mL in a 3 mL vial containing 1 mL of drug product or placebo in a 3 mL vial containing 1 mL of drug product. Dasiglucagon and matching placebo will be provided in the form of solution for injection for subcutaneous administration through an infusion pump.
As long as the patient is receiving IV glucose, the PG will be measured and reviewed hourly using a hand-held PG meter. The IV GIR will be titrated to achieve glycemia of at least 70 mg/dL (3.9 mmol/L) (a minimum GIR will be established as the rate up-titrated after the patient drops at least once below 70 mg/dL [3.9 mmol/L]). When the patient is no longer on IV GIR, the PG will be checked according to local practice, but at least 3 times daily. The PG will be measured using the same trial-supplied hand-held PG meter during the entire trial.
Additionally, blinded CGM will be started 24 hours prior to randomization (using the Dexcom G4 system) and continued until the end of Part 2.
Dosing of dasiglucagon will approximate continuous infusion by delivering small doses at frequent intervals via the infusion pump. The adjustment of trial drug dosing is closely linked to the PG level achieved, which in turn will govern the IV GIR. In Part 1 the algorithm in Table 1 should be used.
The pump administers 0.000025 mL/dose ~ 0.1 µg/dose (4 mg/mL formulation):

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

Table 1 Algorithm to maintain plasma glucose

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

Abbreviations: GIR = glucose infusion rate; IV = intravenous

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

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

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

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

The 2-hour dose-adjustment interval will allow drug plasma levels to approach steady-state before the dose is further increased. The cumulative dose will not exceed 1.26 mg over the first 24 hours and 1.68 mg for each of the subsequent 24-hour periods.
At the time of crossover from the first 48-hour period to the second 48-hour period, the trial drug will again be titrated from the starting dose of 10 µg/hr, and IV GIR will be set to the rate obtained at the end of the run-in periodv which corresponds to the last value obtained before randomization and titrated accordingly.

Part 2 (Open label)

After Part 1, patients will continue in open-label Part 2 to receive dasiglucagon for a further 21 days. Since treatment allocation during Part 1 remains blinded, all patients are required to initiate dasiglucagon dosing at 10 µg/hr in Part 2, while IV GIR should be started at the rate obtained at the end of the run-in period which corresponds to the last value obtained before randomization. During Part 2, the dasiglucagon dose, the amount and route of administration of carbohydrates (IV GIR, parenteral nutrition, gastric carbohydrate infusions, and carbohydrates from oral feeds) can be adjusted at the discretion of the investigator. The PG should be monitored at least hourly to adjust the IV GIR as appropriate and to avoid hypoglycemia. Additional concomitant medications, including somatostatin analogs and/or sirolimus/mTOR inhibitors, may be introduced at the investigator's discretion if the maximum dose level of dasiglucagon (70 µg/hr) has been reached or if further up-titration is not possible due to undesirable side effects. Adjustment of total carbohydrate administered (IV glucose, parenteral nutrition, carbohydrate infusions, and carbohydrates from oral feeds) should continue throughout this period according to local practice to maintain PG levels in the range of 70 mg/dL to 120 mg/dL (3.9-6.7 mmol/L). The aim will be to obtain stable glucose levels with minimum rescue/hypoglycemia-preventative carbohydrates administered by invasive routes, and to encourage/optimize oral feeds, and achieve weaning off IV glucose. Regardless of their discharge status, all patients will be offered the opportunity to participate in the long-term safety extension trial (ZP4207-17106), providing the investigator attests to the positive benefit-risk balance of continued dasiglucagon treatment.

3.5. Method of Assigning Subjects to Treatment Groups

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

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

Randomization will be performed using a block randomization scheme stratified by region (US/non-US). The randomization scheme will be generated prior to the initiation of the trial by an independent statistician/programmer who will not be a member of the trial team; investigators will not be aware of the block size of the randomization scheme.
Patients will be randomly assigned to a trial treatment sequence using an IWRS that has been validated for the intended use under the International Society of Pharmaceutical Engineers Good automated manufacturing practice guidelines, 21CFR Part 11 (FDA regulation for Electronic Records and Electronic Signatures), and the ICH Guidance E6 for Industry on Good Clinical Practice.
After completing Part 1, patients will continue in Part 2 to receive active treatment for a maximum of 21 days.

3.6. Blinding and Unblinding

Part 1 of this trial will be blinded. Part 2 will be open-label. During the conduct of the study, patients will have CGM performed but the results will be masked.
Unblinding will be permitted in a medical emergency that requires immediate knowledge of the patient's treatment assignment. Treatment unblinding is discouraged if knowledge of the treatment assignment will not materially change the planned management of a medical emergency.
For emergency unblinding, the investigator will use the measures provided through the IWRS. Unblinding should be discussed in advance with the medical monitor if possible. If the investigator is not able to discuss treatment unblinding in advance, then he or she must notify the medical monitor as soon as possible about the unblinding incident without revealing the patient's treatment assignment. The investigator or designee must record the date and reason for unblinding on the appropriate eCRF page for that patient

3.7. Schedule of Events

A detailed schedule of events for the study is provided in Table 2.

Table 2: Schedule of Events

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

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

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

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

a The Follow-up Visit will only be performed for patients who will not enter the extension trial.
b After screening, eligible patients will complete a minimum 24-hour run-in period to confirm the IV glucose randomization requirement (≥10 mg/kg/min). All non-nutritional carbohydrates and carbohydrate fortification of feeds are prohibited during this 24-hour run-in period.
c Visits 5 (Day 11) and 6 (Day 18) can be converted to phone visits at the investigator's discretion.
d Patients who are discharged from the hospital before Day 25 will be contacted by the investigator by telephone the day after discharge. The investigator will ask the parent(s)/guardian if they have any questions about the trial procedures and if their child has experienced any AEs.
e Body length will be measured at screening and at the End of Treatment visit.
f For CHI diagnosis: Data on biochemical parameters, genotyping results, and information about PET-CT should be captured when available.
g An echocardiogram performed within 1 month of screening can be used.
h Vital signs should be measured at 6 ± 1, 12 ± 2, and 24 ± 4 hours after initiation of the trial drug and every 8 ± 2 hours hereafter.
i Fluid balance assessments are to be performed and documented every 8 hours as long as the patient is receiving IV glucose.

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

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

4. Statistical Analysis and Reporting

4.1. Introduction

Data processing, tabulation of descriptive statistics, calculation of inferential statistics, and graphical representations will be performed primarily using SAS (release 9.4 or higher). If the use of other software is warranted, the final statistical methodology report will detail what software was used for what purposes.

Continuous (quantitative) variable summaries will include the number of subjects (n) with non-missing values, mean, standard deviation (SD), median, minimum, and maximum.

Categorical (qualitative) variable summaries will include the frequency and percentage of subjects who are in the particular category or each possible value. In general, the denominator for the percentage calculation will be based upon the total number of subjects in the study population for the within each treatment group, unless otherwise specified. The denominator for by-visit displays will be the number of patients in the relevant study population with non-missing data at each visit.

The minimum and maximum will be reported with the same degree of precision (ie, the same number of decimal places) as the observed data. Measures of location (mean and median) will be reported to 1 degree of precision more than the observed data and measures of spread (SD) will be reported to 2 degrees of precision more than the observed data.

Percentages will be presented to 1 decimal place, unless otherwise specified.

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

4.2. Interim Analysis and Data Monitoring

No interim analyses are planned.

4.2.1. Data Monitoring Committee

A Data Monitoring Committee (DMC) will be established to conduct reviews of patient safety. It is expected that DMC meetings will occur 3 times a year during the course of the study. The reviews will monitor for issues that may justify modifying, suspending, or terminating the study.

The sponsor may request additional reviews, e.g., should any other findings/issues pertaining to safety or efficacy emerge requiring DMC review. Details of the operation of the DMC will be developed in conjunction with the members of the DMC before the first meeting and will be modified as required.

The DMC analysis will be performed by an independent statistician. The study team (excluding the project statistician and programming team) will see only DMC summary tables aggregated over all patients (i.e., not split by treatment) until the final database lock.

4.2.2. Zealand Pharma 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 serious AEs (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.

The data package will be delivered based on Appendix 6 of the Pharmacovigilance Agreement; the shells for the data package will be provided in a separate document.

5. Analysis Populations

The following analysis populations are planned for this study:

- **Safety Set (SAF):** The Safety Set includes all patients administered any randomized treatment. Patients will be summarized by treatment period according to treatment received.
- **Full Analysis Set (FAS):** The Full Analysis Set includes all patients in the Safety Set who have a valid baseline primary efficacy assessment (i.e. baseline IV GIR assesment). Patients will be analyzed by treatment period according to planned treatment.
- **Per protocol (PP) Set:** The PP Set includes 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.
- **Pharmacokinetic Set (PK):** The PK Set will include all patients in the Safety Set who have at least 1 measurement with quantifiable plasma concentration of dasiglucagon. This population will be used to provide descriptive summaries of PK data. Patients will be summarized according to treatment received.

6. General Issues for Statistical Analysis

6.1. Statistical Definitions and Algorithms

6.1.1. Baseline

6.1.1.1. Efficacy

For the primary endpoint, baseline is defined as IV GIR rate obtained at the end of the run-in period which corresponds to the last value obtained before randomization.

According to statistical analysis described in Section 8, no other baseline definition is needed for efficacy endpoint.

6.1.1.2. Safety

For safety endpoints, baseline is defined as the last observation recorded prior to the first dose (Placebo or Dasiglucagon) on Day 1.

6.1.2. Adjustments for Covariates

No adjustment planned.

6.1.3. Multiple Comparisons

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

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

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

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

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

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

The test hierarchy is:

Part 1

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

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

Inferential analysis will be performed for the secondary endpoint on Weighted Mean GIR during 48-hour period and Mean GIR below 10mg.kg/min in the last 12 hours of each treatment period during Part 1. Results of those analysis are not included in hierarchical testing.

For other secondary endpoints, no inferential statistical analysis is planned, only descriptive.

6.1.4. Handling of Dropouts or Missing Data

6.1.4.1. Premature Withdrawal from Trial Treatment

If the patient is discontinued from trial treatment, he/she (or the parent) should be asked to continue in the trial by following the planned visit schedule and to have trial assessments performed according to the schedule of events. This is especially important during Part 1 of the trial where the primary endpoint is evaluated. As a minimum, the patient (or the parent) will be asked to attend the Follow-up Visit 28 days (± 3 days) after discontinuation of trial treatment.

6.1.4.2. Premature Withdrawal from Study

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

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

6.1.5. Analysis Visit Windows

For all analyses, unscheduled and/or repeated measurements will only be included if a scheduled measurement is not available, and the unscheduled/repeated measurement falls within the analysis visit windows as described in Table 3. Otherwise, visits will be analyzed as scheduled, and unscheduled/repeated measurements falling outside of the visit windows will be excluded from analysis.

Table 3: Analysis Visit Windows

Analysis Visit	Target Day	Lower Limit	Upper Limit
Baseline		-28	-1
Day 1	1	1	1
Day 2	2	2	2
Day 3	3	3	3
Day 4	4	4	4
Day 5	5	5	5
Day 6	6	6	6
Day 11	11	9	13
Day 18	18	16	20
Day 25	25	22	28
Day 53 (FUP)	53	50	56

Abbreviations: FUP = Follow-up

6.1.6. Pooling of Sites

Not applicable; sites are pooled into regions as part of randomization stratification.

6.1.7. Derived Variables

- **Day 1** = First day of Treatment (Placebo or Dasiglucagon) during Part 1. Study day will be calculated with respect to Day 1.

- **Treatment period**

As primary and secondary endpoint assessments are not performed at planned visits but continuously during the study, we have to define treatment periods. Those will then be used to assign a treatment period to an evaluation.

Treatment periods created will be:

- Part 1 – Treatment period 1: First 48-hours treatment period
- Part 1 – Treatment period 2: Second 48-hours treatment period
- Part 1 – Last 12 hours per treatment period
- Part 2

Table 4 describes the derivation for each of those treatment periods.

Table 4: Derivation of Treatment Periods

Part	Time Point	Treatment Period (TP)	Start date	End date
Part 1		TP1	Day 1	Last exposure treatment period 1
		TP2	First exposure treatment period 2	Last exposure treatment period 2
Part 1	Last 12-hour/24-hour per TP	TP1	End date minus 12 hours/24 hours	Last exposure treatment period 1
		TP2	End date minus 12 hours/24 hours	Last exposure treatment period 2
Part 2			Day 5 first exposure	End of study exposure

Abbreviations: TP1 = treatment Part 1, TP2 = treatment Part 2

- **Study Week:** to be defined for Part 2 only

  - A 7-day period derived for displays of events related to CGM and/or SMPG results, gastric carbohydrate administrations, health care outcomes, and other assessments that may occur between clinic visits where a defined study week is needed.

    The study weeks will be derived as follows:

Table 5: Study weeks

Study Week	Start	End
Week 1	Day 5 first exposure	Start + 167 hours 59 minutes
Week 2	End date week 1 + 1 minute	Start + 167 hours 59 minutes
Week 3	End date week 2 + 1 minute	Start + 167 hours 59 minutes

- **Change from baseline** = value at current time point – value at baseline.

- **TEAE** = an AE with an onset at the time of or following the start of treatment with the trial drug (dasiglucagon or placebo) through the Follow-up visit or Early Termination visit, whichever occurs first.

- **Weighted Mean IV GIR:** AUC will be calculated using linear trapezoidal method and divided by the length of the time period in minutes. The weighted mean IV GIR endpoint is expressed as glucose in mg/kg/min.This will be calculated over last 12 hours of each treatment period and over last 48 hours of each treatment period during Part 1.

- **Weighted Mean IV GIR threshold:** This variable will be assigned “Yes” when the Weighted Mean IV GIR calculated over 12 hours period is not missing and < 10mg/kg/min and will be assigned “No” when value calculated will be >= 10mg/kg/min.

- **Z-scores:** For subjects who are less than 2 years of age, WHO growth charts will be used to calculate Z-scores, as described here: 'www.cdc.gov/nccdphp/dnpao/growthcharts/resources/sas-who.htm'. For subjects who are greater than or equal to 2 years of age, Z-scores will be derived based on the SAS program in link 'www.cdc.gov/nccdphp/dnpao/growthcharts/resources/sas.htm' by using SEXN, Age in months ((VSDAT-ADSL.BRTHDT)/30.4375) and corresponding weight/height at baseline measurements.

- **PK Clearance:**

$$CL/f\ (L/h) = R0/Css$$

where R0= infusion rate (pmol/h) and Css = concentration at steady state (pmol/L), weight (1µg/kg/hr = 295.7 nmol/kg/h)

- **Primary endpoint IV GIR:** Values from SDTM.EX where EXTRT="IV GLUCOSE INFUSION", not including the "IV GLUCOSE BOLUS".

- **Total amount of carbohydrates received:**

  Calculated as the sum of carbohydrates received via IV glucose infusion or bolus, as part of total parenteral nutrition, via oral route and via NG tube or gastrostomy. This average daily value will be used in analysis calculated as the sum of carbohydrates received divided by number of days in corresponding period. As IV glucose infusion is recorded in mg/kg/min, for each analysis period, the total amount received will be calculated as:

  IV glucose infusion (mg) = Infusion rate recorded in CRF x WGT x TIME

  with WGT = weight at beginning of the analysis period, TIME = duration of analysis period in minutes

  IV glucose infusion or bolus are reported in SDTM.EX with EXTRT in "IV GLUCOSE INFUSION" or "IV GLUCOSE BOLUS".

  Carbohydrates received via oral route, via NG tube or gastrostomy are reported under Carbohydrates administration eCRF.

  Total parenteral nutrition is reported under Carbohydrates administration eCRF selecting observations specified as part of TPN.

- **Nightly gastric carbohydrates to treat hypoglycemia =** amount of gastric carbohydrates (g) given between midnight and 6 am inclusive to treat hypoglycemia.
- **Gastric carbohydrates received to treat hypoglycemia:** those will be the Carbohydrates received via NG tube or gastrostomy reported under Carbohydrate's administration eCRF and identified as used to treat a hypoglycemic episode in the same eCRF form. Oral route carbohydrates are not included in this endpoint.
- **SMPG-detected Hypoglycemia episodes = PG <70 mg/dL or 3.9 mmol/L**, reported in the eCRF. 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. No programming is needed to detect the events as they are reported in the eCRF.

- **SMPG-detected Clinically significant hypoglycemia episode =** PG <54 mg/dL or 3.0 mmol/L, reported in the eCRF. A single clinically significant hypoglycemia episode is defined as up until 60 minutes from the start of the episode even if normoglycemia (>54

mg/dL) is not reached within this time. A new episode of clinically significant hypoglycemia is to be reported when the next PG value below 54 mg/dL (3.0 mmol/L) is measured. No programming is needed to detect event as they are reported in the eCRF.

- **CGM-detected Hypoglycemic episode** = PG <70 mg/dL (3.9 mmol/L) for 15 minutes or more, as measured from external data. 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. Event will be defined by programming using external CGM data.

- **CGM-detected Clinically significant hypoglycemia episode** = PG <54 mg/dL or 3.0 mmol/L for 15 minutes or more, as measured from external data. A single clinically significant hypoglycemia episode is defined as up until 60 minutes from the start of the episode even if normoglycemia (>54 mg/dL) is not reached within this time. A new episode of clinically significant hypoglycemia is to be reported when the next PG value below 54 mg/dL (3.0 mmol/L) is measured. Event will be defined by programming using external CGM data.

- **Event rate** = average of the weekly number (sum) of events/episodes, based on the number of weeks in the relevant period. Events include hypoglycemia, clinically significant hypoglycemia, gastric carbohydrate administrations, and SMPG readings.

- **Percent time (CGM)** = (number of minutes where PG at a pre-defined level / total number of minutes patient is wearing CGM) * 100%. For this calculation, one assessment of CGM will be considered having a 5-minute duration. The possible levels are:

  - **In range:** PG between 70-180 mg/dL (3.9-10.0 mmol/L), inclusive
  - **Hypoglycemia:** PG <70 mg/dL (3.09 mmol/L)
  - **Clinically significant hypoglycemia:** PG <54 mg/dL (3.0 mmol/L)
  - **Hyperglycemia:** PG >180 mg/dL (10.0 mmol/L)

- **Time to complete weaning off IV GIR**: Time from first exposure during Part 2 to stop of Glucose infusion. Complete weaning off IV GIR is defined as the first point in time when the patient has been off IV GIR for 12 hours. This definition is based on clinical considerations that 12 hours off IV GIR is considered a reasonable indication that patients can manage without IV GIR, even though some of them may need re-initiation of IV glucose at later point. In case of study withdrawal before having the event, patient will be censored at time of study withdrawal.

- **Time to actual hospital discharge:** Time from first exposure during Part 2 to discharge of hospital. In case of study withdrawal before having the event, patient will be censored at time of study withdrawal.

- **Time to pancreatic surgery = T**ime from first exposure during Part 2 to pancreatic surgery. Pancreatic surgery event will be retrieved from Adverse Event or Medical History Form. Pancreatic surgery is defined as sub-total or total pancreatectomy with a cutoff of 95% or more. In case of study withdrawal before having the event, patient will be censored at time of study withdrawal.

- **Extent of hypoglycemia ($AOC_{glucose}$) =**

$$\sum_{k=0}^{X-1} \frac{(UL - PG_k) + (UL - PG_{k+1})}{2} \times (t_{k+1} - t_k)$$

where *UL* is the upper limit for the hypoglycemia definition (70 mg/dL or 54 mg/dL); *k* is the k^{th} time point that glucose is measured using CGM; PG_k is the PG value in mg/dL on the k^{th} time point; and *X* is the end time point of the 3-week treatment period (Weeks 2-4). When $k = 0$, t_k is set to 0 and PG_k is the PG value corresponding to the start of the 3-week treatment period. Only PG values less than UL will be included in the calculation. If PG ≥ UL at the k^{th} time point and PG < UL at the k^{th}+1 time point, then the extent of hypoglycemia for that interval will be determined as follows:

1. Estimate time point on x-axis where PG = UL – 0.01, as if a straight line were drawn between time points by using the following formula:

$$\hat{k} = \frac{(UL - 0.01) - b}{m}$$

Where *b* = y-intercept of line created by the coordinates (k^{th} time point,PG_k) and (k^{th}+1 time point,PG_{k+1}) and *m* = slope of that line.

2. Substituting PG_k with UL and t_k with $t_{\hat{k}}$, the formula for the estimated interval below UL is as follows:

$$\frac{(UL - UL) + (UL - PG_{k+1})}{2} \times (t_{k+1} - t_{\hat{k}})$$

Similar methodology would be utilized if PG < UL at the k^{th} time point and PG ≥ UL at the k^{th}+1 time point, with the estimate of the time point where PG crosses the UL threshhold being estimated for $t_{\hat{k}+1}$.

If the time period between two timepoints is greater than 1 hour then no interpolation will be performed and no area will be calculated over this timeperiod.

If PG results from the k^{th} time point are missing, then *k-1* scores will be used and the weight will be t_{k+1}-t_{k-1}.

The extent of hypoglycemia calculated as above will then be divided by the total duration of CGM assessments calculating as summing all $t_{k+1} - t_k$ duration used in previous formulae. Time points for which PG>UL will be included in the total duration. This variable will then be used the endpoint evaluation.

6.1.8. Data Adjustments/Handling/Conventions

All collected data will be presented in listings. Some data not subject to analysis according to this plan will not appear in any tables or graphs but will be included only in the data listings.

All *P* values will be displayed in four decimals and rounded using standard scientific notation (eg, 0.XXXX). If a *P* value less than 0.0001 occurs it will be shown in tables as <0.0001.

Adverse events (AE) will be coded using the MedDRA version 21.1 thesaurus.

A treatment-related AE is any AE with a relationship to the study drug of possible or probable.

If partial medication/AE dates occur, the convention for replacing missing dates for the purposes of calculating derived variables is as follows:

For partial medication/AE start dates:

- If the year is unknown, then do not impute the date but assign a missing value.
- If the month is unknown, then:
    - If the year matches the year of first dose date and the end date (if present) is after first dose date, or medication is ongoing, then impute as the month and day of the first dose date. If this produces a date after the medication end date, assign 01 January.
    - Otherwise, assign 01 January.
- If the day is unknown, then:
    - If the month and year match the month and year of the first dose date, then impute as the day of the first dose date. If this produces a date after the medication end date, assign 01.
    - Otherwise, assign 01.

For partial medication/AE end dates:

- If the year is unknown, then do not impute the date but assign as missing value.
- If the month is unknown, then assign December.
- If the day is unknown, then assign the last day of the month.

7. Study Patients/Subjects and Demographics

7.1. Disposition of Patients/Subjects and Withdrawals

The numbers of patients enrolled, randomized, completing, and withdrawing from treatment and the trial, along with reasons for withdrawal for treatment and trial, respectively, will be tabulated overall and by treatment sequence (Part 1). The number of patients in each analysis set will be reported, as well as the number of patients at each visit. A CONSORT diagram showing patient disposition will be provided.

All disposition information will be listed.

7.2. Protocol Violations and Deviations

Protocol deviations will be listed.

7.3. Demographics and Other Baseline Characteristics

Demographic and baseline characteristics (including age, sex, race, ethnicity, weight, weight z-scores, length/height, and length/height z-scores) will be summarized at screening using descriptive statistics for patients in FAS by treatment sequence. Z-scores (based on the World Health Organization [WHO] growth charts) will be derived using a patient's age and sex. No formal statistical analyses will be performed.

Focal CHI will be described from the general medical history form.

7.4. Exposure and Compliance

Study drug administration will be summarized separately for total dasiglucagon exposure (µg/kg) and length of exposure (days), respectively for Part 1 and Part 2. Moreover, the duration of drug use (days) will be presented. It will be calculated as length of exposure minus number of days without IMP exposure. Additionally, the infusion rate, defined as the average hourly weight-adjusted infusion rate of study drug over the study period analyzed (i.e., µg/kg/hr and µg/hr), will be summarized for each Part. For Part 1, the two days of duration will be used for calculation and for Part 2, it will be calculated for each week.

7.5. COVID impact assessment

For all subjects under the study during the COVID pandemic, an assessment of visits missed, visits delayed, visits done remotely, efficacy and/or safety assessments missed will be performed. All protocol deviations related to COVID will be listed.

8. Efficacy Analysis

8.1. Primary Efficacy Analysis

The weighted mean IV GIR in the last 12 hours of each treatment period during Part 1 will be analyzed. The hypothesis:

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

The weighted mean IV GIR will be based on the last 12 hours of each treatment period, calculated as a weighted mean across the 12 hours, taking the actual time periods between the measurements into account (corresponding to calculating the area under the curve [AUC] and dividing by the length of the time period, i.e., weighted mean IV GIR). The weighted mean IV GIR endpoint is expressed as glucose in mg/kg/min, i.e., data reported from different concentrations of the glucose used for infusion will be transformed to this standardized unit prior to analysis. When transitioning each patient between the parts (cross-over from Day 2 to Day 3 and from Day 4 to Day 5), it required from sites approximately 15 minutes of IP disconnection. Because the subject would be disconnected from all IP during this approximately 15-minute

period and to prevent any hypoglycemia, study team from site 101 restarted the subjects on their baseline GIR to provide them with dextrose support while the infusion sets were being changed. Because of this return to baseline GIR prior to the start of the next phase/ start of next IP dosing, it appears in database that for some patients, GIR values are back to baseline before the new IP has been set. As those values are artificial and not linked to any effect of IP, they will have to be removed from statistical analysis. Those GIR values are clearly described in a protocol deviation form and will be clearly identified in blinded data review meeting minutes as non analyzable. They will not be included in any statistical analysis based on IV GIR.

The primary analysis is defined by the estimand based on the treatment policy (de-facto) strategy, where the actual GIR measurement reported irrespective of adherence to treatment (also considering data not under treatment) or use of subsequent therapy is used. The difference in weighted mean IV GIR between placebo and dasiglucagon will be estimated.
The weighted mean IV GIR will be analyzed using a mixed-model regression approach using this programming:

```
PROC MIXED;
 CLASS PATIENT PERIOD TRT;
 MODEL OUTCOME= PERIOD TRT / DDFM=KR;
 RANDOM PATIENT;
 LSMEANS TRT / PDIFF CL E;
RUN;
```

with treatment (TRT) and period as fixed effects and patient as a random effect. Denominator degrees of freedom will be calculated according to Kenward Roger method. The 2-sided 95% confidence interval (CI) for the treatment difference will be calculated from the mixed regression model.

Missing data will be imputed as detailed below:

- if the weighted mean IV GIR is missing for the placebo period, it will be imputed using the baseline IV GIR;
- if the weighted mean IV GIR is missing for the dasiglucagon period, it will be imputed using the placebo-weighted mean IV GIR for that patient.

The primary efficacy analysis will be based on the Full Analysis dataset.

8.1.1. Sensitivity and Supportive Analyses for the Primary Endpoint

As a sensitivity analysis, in case of missing data for primary endpoint, it will also be analyzed:

- using only complete cases cases (i.e., weighted mean IV GIR is non-missing for both placebo and dasiglucagon)- an assessment performed not under treatment will not be considered as missing,
- imputing missing weighted mean IV GIR, for both dasiglucagon and placebo values, using the baseline IV GIR,
- impute missing dasiglucagon values during one period with the placebo value for that patient, imputing missing placebo values with the mean of placebo patients for that period.
- Using tipping point analysis: a 2-way tipping point sensitivity analysis will be performed to investigate the sensitivity for the assumptions on missing data in the

primary analysis for the primary endpoint. Missing GIR values in the dasiglucagon period in part 1 will be imputed with the value for the same patient in the placebo period plus a penalty. Missing GIR values in the placebo period will be imputed using baseline for the same patient plus a penalty. The penalty will be varied independently in the two treatment periods including no penalty in the placebo periods together with increasing penalties in the dasiglucagon periods (penalties will be 0.5, 1, 1.5). For each combination the statistical analysis similar to the primary analysis will be carried out. Scenarios for which statistical significance change will be evaluated with respect to plausibility of the penalties.

- Sensitivity analysis using a placebo multiple-imputation method will be performed. As for each period of cross-over we have only baseline and end of period value, non-monotone imputation is not needed. Monotone missing values will be imputed using the imputation model built from the placebo group in corresponding period, i.e., assuming the missing data in the Dasiglucagon group will have a profile that equals the profile of the Placebo group. The missing data imputation will be implemented using PROC MI in SAS 9.4 using a monotone regression model approach for each period independantly. The baseline GIR will be included as explanatory variable and the value at the end of the period will be included as the outcome variable in the model. The seed to be used in the analysis is 12255070, and 100 imputed datasets will be created. Once the completed data sets are formed, the same analysis model as specified for the primary analysis will be applied to each completed set and the inference drawn using Rubin's combination rules (SAS proc MIANALYZE).

As a sensitivity analysis, as some subjects did not have the same GIR infusion rate at start of each cross-over period, the same analysis as primary analysis will be run adding as covariate period specific baseline (BASEP) and Mean baseline for subject over the two periods (MEANBASE):

```
PROC MIXED;
CLASS PATIENT PERIOD TRT;
MODEL OUTCOME= PERIOD TRT BASEP MEANBASE/ DDFM=KR;
RANDOM PATIENT;
LSMEANS TRT / PDIFF CL E;
RUN;
```

As supportive analysis:

- the primary endpoint analysis will be repeated on the PP Set.
- the primary endpoint will be analyzed using a generalized linear model using a log-link and assuming a normal distribution/ With this analysis, exponentiated estimate for treatment effect would give you the mean(GIR) dasi / mean (GIR) placebo/. Following program will be used:

```
PROC GENMOD;
        CLASS PERIOD TRT;
        MODEL OUTCOME= PERIOD TRT / LINK=LOG DIST=NORMAL ;
 REPEATED SUBJECT=PATIENT/ TYPE=CS;
 ESTIMATE "DASIGLUCAGON versus PLACEBO" TRT 1 -1 / EXP;
RUN;
```

These sensitivity and supportive analyses will not be included in the fixed-sequence hierarchical testing strategy.

8.2. Key Secondary Efficacy Analysis

8.2.1. Primary Analyses for the Key Secondary Endpoint

For the primary analysis of the key secondary endpoint, missing dasiglucagon values during one period will be imputed with the placebo value for that patient, missing placebo values will be imputed with the mean of placebo patients for that period.

Total amount of carbohydrates administered per day

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

This hypothesis will be analyzed by using a mixed-model regression approach, with treatment and period as fixed effects and patient as a random effect. The total amount of carbohydrates administered per day is the sum of the last 48 hours of each period in Part 1 divided by number of days followed.

8.2.2. Sensitivity Analyses for the Key Secondary Endpoints

In addition, a sensitivity analysis will be performed for the key secondary endpoint using complete cases.

8.3. Secondary Efficacy Analysis

The secondary efficacy analysis for this study includes the below summaries and inferential analyses. These are continuous endpoints and one binary endpoint and will be summarized using descriptive statistics by study visit (if applicable), treatment group (Part 1) and overall (Part 2) for patients in the FAS. Inference will be performed for Part 1 secondary endpoint as described below. For all the other endpoints no inference will be performed.

Missing data will be imputed for Weighted Mean IV GIR (continuous) as per what is described for primary analysis. The imputed Weighted Mean IV GIR will be used for derivation of Weighted Mean IV GIR below 10 mg/kg/min endpoint. No other imputation for missing data performed and no sensitivity analysis.

8.3.1. Primary Analyses for Secondary Endpoints

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

- Weighted Mean IV GIR for each 48-hour treatment period during Part 1 (dasiglucagon or placebo administration). This endpoint will be analyzed using the same mixed-model regression approach as the one used for primary endpoint and removing IV GIR data that are not valid for site 101. No sensitivity analysis on this endpoint.

- Weighted Mean IV GIR below 10 mg/kg/min in the last 12 hours of each treatment period during Part 1 (yes/no) (dasiglucagon or placebo administration). For this analysis a generalized estimating equation (GEE) method with logit link function will be used using below program and removing IV GIR data that are not valid for site 101.

```
PROC GENMOD;
        CLASS PATIENT PERIOD TRT;
        MODEL OUTCOME= PERIOD TRT / LINK=LOGIT DIST=BINOMIAL ;
    REPEATED SUBJECT=PATIENT/ WITHIN=PERIOD TYPE=CS;
    LSMEANS TRT / ILINK CL EXP DIFF;
    ESTIMATE "DASIGLUCAGON versus PLACEBO" TRT 1 -1 ;
RUN;
```

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

If patient needs a pancreatectomy, this will occur only during the Part 2 of the study. If pancreatectomy was done before randomization, it will not be taken into account. Only patients with pancreatectomy during the study will have censored values.

The evaluation of the Part 2 endpoints will then be impacted by pancreatectomy. To be able to assess only treatment effect as primary analysis the following rules will be applied:

- SMPG-detected episode, Carbohydrates administrated, CGM-detected episode, CGM percent time endpoint and extent of hypoglycemia will be set to missing after pancreatectomy (whatever percentage of pancreatectomy).
- Time to complete weaning off IV GIR and time to actual hospital discharge will be censored at time of pancreatectomy (whatever percentage of pancreatectomy).

Following endpoints will be described by week and over the three weeks period.

- Time to complete weaning off IV GIR. Only patient without complete weaning off IV GIR at start of Part 2 will be included in this analysis. Kaplan-Meier estimates will be given for each of the three weeks treatment period.
- Rate of SMPG-detected hypoglycemia episode, defined as number of hypoglycemic events (PG <70 mg/dL or 3.9 mmol/L).
- Rate SMPG-detected Clinically significant hypoglycemia episode, defined as number of events <54 mg/dL (3.0 mmol/L).
- Time to actual hospital discharge. Kaplan-Meier estimates will be given for each of the three weeks treatment period.
- Time to pancreatic surgery (sub-total or total pancreatectomy, cutoff 95% or more).
- Total amount (g) of carbohydrates administered (regardless of route) per day, together with amounts (g) of carbohydrates administered per day:
    - via IV glucose infusion or bolus (not as part of total parental nutrition),
    - as part of total parenteral nutrition (if applicable),
    - via oral route, and
    - via NG tube or gastrostomy.
- CGM percent time in range 70-180 mg/dL (3.9-10.0 mmol/L).

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

8.3.2. Sensitivity Analyses for the Key Secondary Endpoints

In addition, a sensitivity analysis will be performed for all secondary endpoints related to Part 2 not applying any missing rule when pancreatectomy (whatever percentage of pancreatectomy). is performed. Time to pancreatic surgery will not be included in this sensitivity analysis as not impacted by missing rules.

As paracetamol is a prohibited medication that can impact CGM endpoint evaluation for Dexcom G4 users a sensitivity analysis on CGM percent time in hypoglycemia (< 70mg/dL) will be run excluding following CGM data:

- For each use of paracetamol (from concomitant medication form preferred term =”PARACETAMOL”) for G4 users exclude CGM data if paracetamol start date <= CGM date <= paracetamol end date + 4 hours

8.4. Other Efficacy Analysis

8.4.1. CHI Related Hospitalization

The frequencies and percentage of patients with admissions/emergency department visits for hypoglycemia, hospitalizations due to CHI or CHI-related events, visits to health care providers (family doctor, specialists, etc.) caused by CHI or CHI-related events and need for home visits by paramedics due to hypoglycemia will be summarized by week for Part 2.

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 due to hypoglycemia will be summarized by week for Part 2 and for the patients in FAS.

9. Safety and Tolerability Analysis

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. No formal inferential analyses will be conducted for safety variables, unless otherwise specified.

All safety analyses will be summarized by treatment received within treatment period and part also by active treatment (Dasiglucagon pooling data of part 1 and part 2) and by study visit, if applicable.

9.1. 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. A treatment-emergent AE (TEAE) is defined as an AE with an onset at the time of or following the start of treatment with the trial drug (Dasiglucagon or Placebo) through the Follow-up Visit or Early Termination Visit, whichever occurs first. An AE will be assigned to the treatment period the AE started.

The causal relationship of the AE to the study drug is determined by the investigator as Not Related, Unlikely, Possible, and Probable. These will be mapped to Unrelated (*Not Related* or *Unlikely*) and Related *(Possible* or *Probable*) for summarization purposes. Any missing relationship will be mapped to “Related”.

Adverse event severity grades are reported as mild, moderate, or severe.

Each patient will be counted only once within each summation level system organ class (SOC) and preferred term (PT). The summary will be presented in descending order of frequency of SOC and then PT within SOC based on overall patients.

The incidence of TEAEs will be summarized with frequencies and percentages for patients in Safety Set (including number of TEAEs occurring, as well as rate of events [number of events /

100 years of exposure in each part]), by part and treatment group and also overall for Dasiglucagon group for the following:

- SOC and PT
- SOC, PT, and severity
- SOC, PT, and relationship to study drug
- SAEs by SOC and PT

9.1.1. Adverse Events Leading to discontinuation of study drug

A summary of incidence rates (frequencies and percentages) of TEAEs leading to discontinuation of study drug, by part and treatment group, overall for Dasiglucagon group, SOC, and PT will be prepared for patients in Safety Set. No inferential statistical tests will be performed.

A data listing of TEAEs leading to discontinuation of study drug will also be provided displaying details of the event(s) captured on the CRF.

9.1.2. Deaths and Serious Adverse Events

Any deaths that occur during the study will be listed.

Serious TEAEs and Related Serious TEAEs will be listed and also tabulated by SOC and PT and presented by part and treatment group and overall for Dasiglucagon group.

9.1.3. Other Significant Adverse Events

Four types of AEs of special interest (AESI) are collected in this study. They are:

- Risk of liver injury
- Neurological events,
- Suspicion of necrolytic migratory erythema,
- Post-Dose Hemodynamic Changes

All Treatment Emergent AESI types occurring in at least 1 patient will be presented.

Incidence of Treatment Emergent AESI will be summarized by frequencies and percentages by part, treatment group and overall for Dasiglucagon group for patients in Safety Set by the following:

- AESI type, SOC, PT
- AESI type, SOC, PT, and relationship to the study drug

Additional information describing each AESI type, captured separately from date in the AE CRF, will be listed.

9.2. Clinical Laboratory Evaluations

Samples for hematology and chemistry will be collected at the Screening and Day 6, Day 25 and Follow-up.

Descriptive summaries of observed and changes from baseline values will be presented for clinical laboratory values by study visit and treatment group within part and also overall for the Dasiglucagon group.

Shifts from baseline for clinical laboratory values below, within, or above the normal range will be provided for hematology and chemistry results.

Frequencies of clinically significant abnormal laboratory values for hematology and chemistry results will be presented by study visit and treatment group within part and also overall for the Dasiglucagon group.

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.

Immunogenicity data will be analyzed descriptively from data at baseline, Day 25, and Follow-up. 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. Antidrug antibodies will be listed.

9.3. Vital Signs

Vital signs will be collected at Screening, Days 1, 2, 3, 4, 5, 6, 11, 18, and 25.

Descriptive summaries of observed and change from baseline values will be calculated for systolic blood pressure, diastolic blood pressure, pulse, respiratory rate, and oxygen saturation (SpO_2) by study visit and treatment group within treatment period for patients in Safety Set.

Frequencies and percentages of vital sign interpretation results (normal, abnormal clinically significant, and abnormal not clinically significant) will be summarized by part, treatment group and overall Dasiglucagon group and study visit.

Shifts from baseline for vital sign interpretation results normal, abnormal not clinically significant and abnormal clinically significant will be provided by treatment group within treatment period and study visit.

9.4. Electrocardiograms

An ECG will be performed at Screening and Days 1, 2, 3, 4, 5; 6; 11, 18, and 25. Descriptive summaries of observed and change from baseline values will be presented for continuous ECG measures of heart rate, PQ interval, QRS duration, QT interval, and QTcF interval by study visit and treatment group within part and overall for Dasiglucagon group.

Frequencies and percentage of patients of investigator interpretation of ECG results (normal,

abnormal clinically significant, and abnormal not clinically significant) will be summarized for patients in Safety Set by part, for each treatment group, overall for Dasiglucagon group and by study visit.

Additionally, frequencies and percentage of patients with observed QTcF >450, >480, and >500 msec, as well as change from baseline in QTcF >30, >50, and >60 msec, will be summarized for patients in the Safety Set by part, for each treatment group, overall for Dasiglucagon group and by study visit.

9.5. Echocardiography

An echocardiogram will be performed at Screening and Follow-up.

Frequencies and percentage of echocardiography interpretation results (normal, abnormal clinically significant, and abnormal not clinically significant) will be summarized for patients in Safety Set by study visit.

9.6. Physical Examination

A complete physical examination of body systems 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 at Screening, Days 1, 2, 3, 4, 5, 6, 11, 18, 25 and Follow-up.

Frequencies and percentages of physical examination interpretation results (normal, abnormal clinically significant, and abnormal not clinically significant) will be summarized by part, treatment, overall Dasiglucagon group and by study visit for each body system on patients in the Safety Set.

In addition, physical examination results will be summarized in listing.

9.7. Local Tolerability

Local tolerability data will be collected separately from AEs at Days 1, 2, 3, 4, 5, 6, 11, 18, and 25. Data will be collected on the nature of any reaction (including spontaneous pain, pain on palpitation, itching, redness, edema, induration/infiltration, and other type of reaction; other type of reaction will be coded using MedDRA, same version as for AEs), if reaction at injection site, the severity (i.e., mild, moderate, or severe), and any action taken (i.e., no action, interruption of infusion, and other). The likely cause of the reaction will also be collected (i.e., insertion site, drug, adhesive dressing, or other).

The frequencies and percentages of patients with local tolerability findings (nature of reaction, if reaction at injection site, severity, and action taken), collected separately from AEs, will be summarized by part and study visit. Description will be performed only on the Dasiglucagon group.

9.8. Prior/Concomitant Medication and Procedure

Concomitant medications will be coded using World Health Organization Drug Dictionary Enhanced (WHO-DDE) (Version September 01, 2018 or later) thesaurus.

Medications that started before first dose of dasiglucagon or placebo will be considered prior medications if they were stopped before first dose of dasiglucagon/placebo. Any medications ongoing at start of trial will be considered to be concomitant medication ongoing at start. If a medication starts or changed during the trial it will be considered as medication starting during the trial. Prior medication and procedure will be described by treatment sequence on the FAS.

Concomitant Medication and Procedures will be described by treatment (Part 1) and overall (Part 2) as well as for all Dasiglucagon exposure on the FAS.

Frequencies and percentages will be summarized descriptively by Anatomical Therapeutic Chemical (ATC) classification Level 4 and Preferred Name. The summary will be presented in descending order of frequency of ATC and then Preferred Name within ATC based on overall patients.

10. Changes from Planned Analysis

- To be consistent over all studies, wording of hypoglycemia episodes has been updated as “SMPG-detected hypoglycemia episodes” and “CGM-detected hypoglycemia episodes”.
- For site 101, remove IV GIR values that are assessed in between parts from statistical analysis
- Add tipping point analysis on primary endpoint
- Add sensitivity analysis on primary endpoint due to different GIR values at start of each cross-over for few patients
- Add sensitivity analysis for CGM percent time in hypoglycemia due to use of paracetamol for patients using Dexcom G4.

11. Other Planned Analysis

11.1. Pharmacokinetic Analysis

Plasma concentrations are collected on Day 5 (applicable for patients with a body weight of ≥4kg, sample will only be collected if it does not compromise the total amount of blood drawn), Day 6 and Day 25. Descriptive summaries will be presented for plasma PK concentrations by study visit and infusion rate (in µg/kg/hr). Information pertaining to PK data collection will be listed.

PK analysis will be performed using PK population according to actual treatment received.

12. References

1. US Federal Register. (1998) International Conference on Harmonization; Guidance on Statistical Principles for Clinical Trials. Department of Health and Human Services: Food and Drug Administration [Docket No. 97D-0174]. Federal Register Volume 63, Number 179, pages 49583-49598. September 16, 1998.
2. ASA. (2016) Ethical Guidelines for Statistical Practice. Prepared by the Committee on Professional Ethics, April 2016. http://www.amstat.org/about/ethicalguidelines.cfm
3. Hawkes CP, Lado JJ, Givler S, De Leon DD. The Effect of Continuous Intravenous Glucagon on Glucose Requirements in Infants with Congenital Hyperinsulinism. JIMD Rep. 2019;45:45-50.
4. The Royal Statistical Society: Code of Conduct, 2014.

13. Tables, Listings, and Figures

All listings, tables, and graphs will have a header showing the sponsor company name and protocol and a footer showing the version of SAS, the file name and path, and the source of the data (CRF page or listing number).

The following reporting conventions will be adopted for the presentation of study data. These conventions will enhance the review process and help to standardize the presentation with common notations.

General Reporting Conventions

- All tables and data listings will be developed in landscape orientation.
- Specialized text styles, such as bolding, italics, borders, shading, and superscripted and subscripted text will not be used in tables, figures, and data listings unless they add significant value to the table, figure, or data listing.
- Only standard keyboard characters should be used in tables and data listings. Special characters, such as nonprintable control characters, printer-specific characters, or font specific characters, will not be used on a table, figure, or data listing.
- Adverse events with missing MedDRA coding will have their system organ class and/or preferred term presented as “Not Coded” in the tables. The “Not Coded” frequencies will be sorted to the end of the tables. This will only be applicable for any deliveries sent before database lock (e.g., for dry runs).
- Programming notes may be inserted into the shells, these notes will not appear in the final output.

Population Summary Conventions

- Population sizes may be presented for each classification factor as totals in the column header as (N=xx), where appropriate.
- All population summaries for categorical variables will include all categories that were planned and for which the subjects may have had a response. Percentages corresponding to null categories (cells) will be suppressed; however, counts and percentages of missing values may be needed.
- All population summaries for continuous variables will include n, mean, SD, median, minimum, and maximum. Other summaries (e.g., number missing, geometric mean, median, quartiles, 95% CIs, and coefficient of variation [CV] or % CV) may be used as appropriate. The precision of the maximum and minimum will match the maximum precision in the data. The mean and median will have 1 additional decimal place. The SD will have 2 additional decimal places.
- All percentages are rounded and reported to a single decimal point (xx.x%).

Appendix 1: List of Abbreviations

Abbreviation	Definition
AE	adverse event
AESI	adverse events special interest
ATC	anatomical therapeutic chemical
CGM	continuous glucose monitoring
CHI	congenital hyperinsulinism
CI	confidence intervals
CRF	case report form
CSR	clinical study report
DBL	database lock
DMC	data monitoring committee
ECG	electrocardiogram
eCRF	electronic case report form
EDC	electronic data capture
EMA	European medicines agency
eTMF	electronic trial master file
EU	European Union
FAS	full analysis set
FDA	food and drug administration
GCP	good clinical practice
HR	heart rate

Abbreviation	Definition
IC or ICF	informed consent or informed consent form
ICH	international council for harmonization
ID	Identification
IDM	independent drug monitoring
IWRS	interactive web response system
MedDRA	medical dictionary for regulatory activities
N	Number
NA	not applicable
NCS	non-clinically significant
PD	protocol deviation
PE	physical examination
PG	plasma glucose
PK	pharmacokinetic
PP	per-protocol
QA	quality assurance
SAE	serious adverse event
SAP	statistical analysis plan
SAS®	a software system used for data analysis
SD	standard deviation
SDTM	study data tabulation model
SF	screen failure

Abbreviation	Definition
SMPG	self-monitored plasma glucose
SOC	system organ class
SOP	standard operating procedure
TEAE	treatment-emergent adverse event
USA	United States of America
WHO	world health organization
WHO-DD	world health organization drug dictionary