Cover Page for Statistical Analysis Plan

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Official title of study:	A Phase 3, Randomised, Open-label, Cross-over Study to Confirm the Clinical Efficacy and Safety of Dasiglucagon Versus Glucagon for the Treatment of Severe Hypoglycaemia in Asian Adults with Type 1 Diabetes (T1D) Including an Investigation of Dasiglucagon in a Japanese Adolescent Cohort
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Statistical Analysis Plan

A Phase 3, Randomised, Open-label, Cross-over Study to Confirm the Clinical Efficacy and Safety of Dasiglucagon Versus Glucagon for the Treatment of Severe Hypoglycaemia in Asian Adults with Type 1 Diabetes (T1D) Including an Investigation of Dasiglucagon in a Japanese Adolescent Cohort

Substance Name: Dasiglucagon

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Version History

This Statistical Analysis Plan (SAP) for study NN9515-7675 is based on the protocol version 2.0 dated 27JUN2024.

SAP Version	Date	Change	Rationale
1.0	20-08-2024	Not Applicable	Original version
2.0	14-10-2025	Appendix 1: Documentation of Pharmacokinetic and Pharmacodynamic profile decisions is added.	To include documentation of decisions regarding pharmacokinetic and pharmacodynamic profiles made before unblinding.

List of abbreviations

CI	confidence interval
DBL	data base lock
ECG	electrocardiogram
i.m.	intramuscular
IMP	investigational medicinal product
PD	pharmacodynamic
PG	plasma glucose
PK	pharmocokinetic
SAP	statistical Analysis Plan
s.c.	subcutaneous
SD	standard deviation
T1D	type 1 diabetes mellitus

1 Introduction

There are no changes to the analyses described in the protocol.

Specifications of tables, figures and listings (TFL) and other specifications not included in this SAP will be described in the mock TFL.

1.1 Objectives, Endpoints, and Estimands

Objectives and Endpoints

Table 1 Objectives and endpoints

Objectives	**Endpoints**		
Primary	**Title**	**Time frame**	**Unit**
To confirm the effect on glycaemic response of a single 0.6 mg s.c. injection of dasiglucagon for the treatment of insulin-induced hypoglycaemia in Asian adults with T1D. This includes comparing the difference in time to plasma glucose (PG) recovery between dasiglucagon s.c. and glucagon i.m. to a non-inferiority margin of 3 minutes.	Primary:		
	Time to PG recovery, where PG recovery is defined as the first increase in PG of ≥20 mg/dL (1.1 mmol/L) from baseline	From 0 to 90 minutes after investigational medicinal product (IMP) injection	min
Secondary	**Title**	**Time frame**	**Unit**
• To investigate the clinical efficacy of dasiglucagon assessed by the glycaemic response of a single 0.6 mg s.c. injection of dasiglucagon for the treatment of insulin-induced hypoglycaemia in Asian participants with T1D	Supportive secondary:		
	[a]Time to PG recovery, where PG recovery is defined as the first increase in PG of ≥20 mg/dL (1.1 mmol/L) from baseline	From 0 to 90 minutes after investigational medicinal product (IMP) injection	min
	PG recovery within 30 minutes after IMP injection (yes/no)	From 0 to 30 minutes after IMP injection	Count of participant
	PG recovery within 20 minutes after IMP injection (yes/no)	From 0 to 20 minutes after IMP injection	Count of participant
	PG recovery within 15 minutes after IMP injection (yes/no)	From 0 to 15 minutes after IMP injection	Count of participant
	PG change from baseline at 15 minutes after IMP injection	From 0 to 15 minutes after IMP injection	mg/dL
	PG change from baseline at 20 minutes after IMP injection	From 0 to 20 minutes after IMP injection	mg/dL
	$AUC_{0\text{-}30min}$: Area under the plasma dasiglucagon concentration time curve from 0 to 30 minutes after IMP injection	From 0 to 30 minutes after IMP injection	h*pmol/L

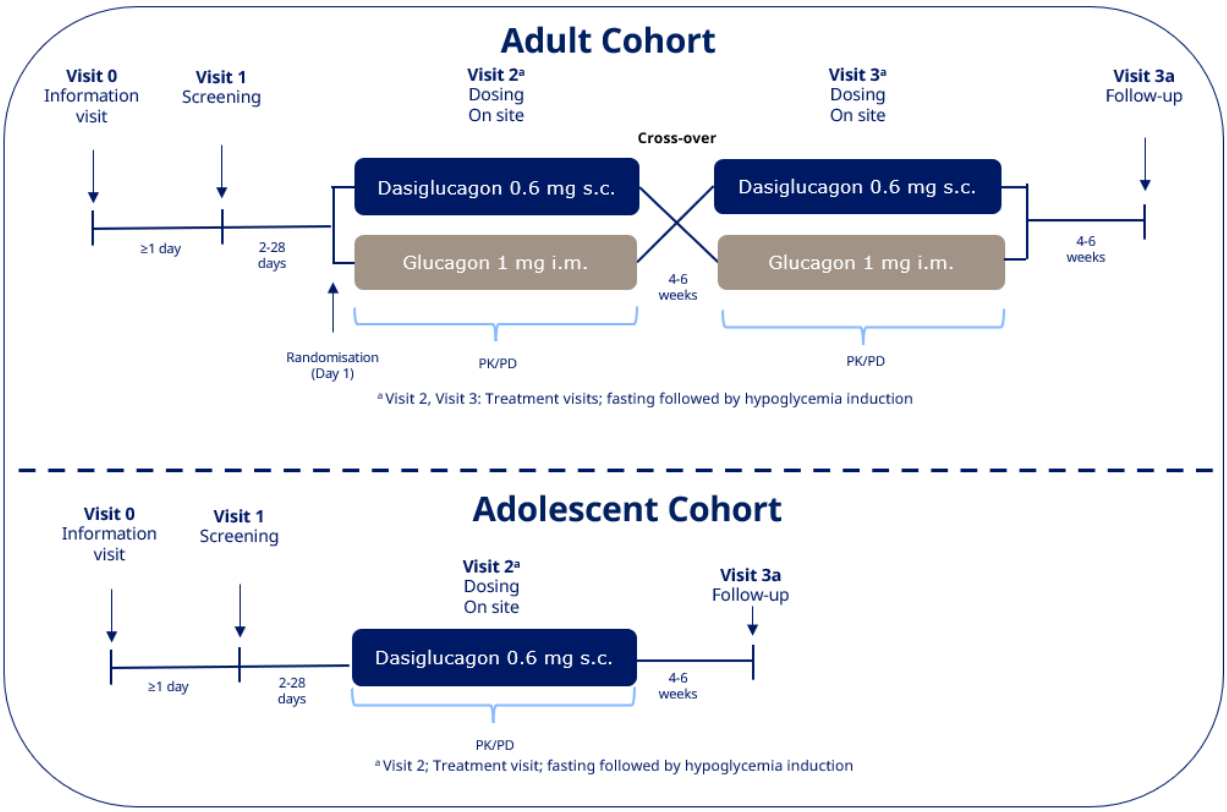
Objectives	Endpoints		
• To investigate the pharmacokinetic properties of a single 0.6 mg s.c. injection of dasiglucagon for the treatment of insulin-induced hypoglycaemia in Asian participants with T1D	C_{max}: Maximum observed plasma dasiglucagon concentration	From 0 to 5 hours after IMP injection	pmol/L
• To investigate the safety of a single 0.6 mg s.c. injection of dasiglucagon for the treatment of insulin-induced hypoglycaemia in Asian participants with T1D	Number of adverse events (AEs)	From IMP injection (visit 2 day 1 and visit 3 day 1) until 28 days after IMP injection.	Number of events
	Number of hypoglycaemic episodes	From IMP injection (visit 2 day 1 and visit 3 day 1) until 12 hours after IMP injection	Number of episodes

[a] applicable to Japanese adolescent cohort only.

1.2 Study Design

This is an interventional, single-centre, randomised, open-label, 2-period crossover study to investigate the PD (efficacy), PK and safety of s.c. injection of dasiglucagon 0.6 mg versus i.m. injection of glucagon 1 mg for the treatment of severe hypoglycaemia in Asian non-Japanese and Japanese adults with T1D including an investigation of dasiglucagon in a Japanese adolescent cohort. The overall study design is shown in Figure 1.

Figure 1 Study design



Thirty-four (34) adult Asian participants (with at least 27 Japanese participants) will be randomised 1:1 to each treatment sequence, receiving a single s.c. injection of 0.6 mg dasiglucagon and a single i.m. injection of 1 mg glucagon in an insulin-induced hypoglycaemic state (using a manual hypoglycaemic clamp procedure). Among them, at least 30 participants (with at least 24 Japanese participants) are planned to complete the dosing visits.

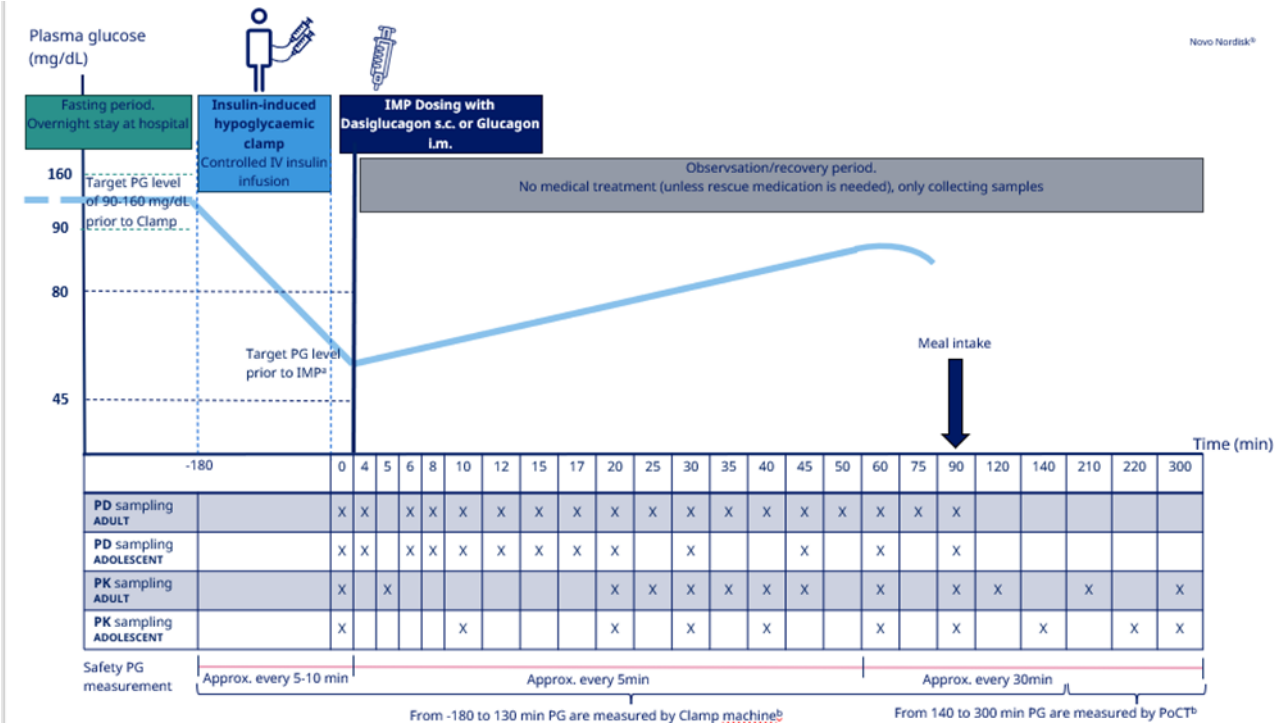
Four (4) adolescent Japanese participants will receive a single s.c. injection of 0.6 mg dasiglucagon in an insulin-induced hypoglycaemic state (using a manual hypoglycaemic clamp procedure).

The study duration for adult participants is approximately 17 weeks and consists of 5 visits: An information visit (visit 0) to obtain the informed consent, a screening visit (visit 1) to determine the eligibility of the participants (2-28 days before visit 2), 2 dosing visits (visits 2 and 3), and a follow-up visit (end of study, visit 3a, 4-6 weeks after dosing) (Figure 1). The randomisation of the participants will take place on the first dosing visit (visit 2 day 1). The dosing visits will be separated by wash-out periods of 4 to 6 weeks, during which the participants will resume their normal insulin treatment. Samples for anti-drug antibodies will be collected prior to the insulin infusion at visit 2, visit 3 and at follow-up visit.

The study duration for adolescent participants is approximately 11 weeks and consists of 4 visits: An information visit (visit 0) to obtain the informed consent, a screening visit (visit 1) to determine the eligibility of the participants (2-28 days before visit 2), 1 dosing visit (visit 2), and a follow-up visit (end of study, visit 3a, 4-6 weeks after dosing). Samples for anti-drug antibodies will be collected prior to the insulin infusion at visit 2 and at follow-up visit (Figure 1).

At each dosing visit, a hypoglycaemic clamp procedure will be performed. The hypoglycaemic state will be induced by controlled IV insulin infusion targeting a PG level of 55 mg/dL (3.1 mmol/L; and range: ≥45 to <60 mg/dL [2.5 to 3.3 mmol/L]) for adults and a PG level of 65 mg/dL (3.6 mmol/L; and range: ≥54 to <80 mg/dL [3.0 to 4.4 mmol/L]) for adolescents prior to investigational medicinal product (IMP) injection. During insulin -induced hypoglycaemia, PG levels will be monitored closely at site for safety reasons. In cases of persistent hypoglycaemia after IMP injection, participants will be treated with rescue glucose infusion. Glucose plasma PD samples and dasiglucagon/glucagon PK samples will be collected prior to and up to 300 minutes after IMP injection (Figure 2). Ninety (90) minutes after the IMP injection, the participants will be allowed to eat a light snack.

Figure 2 Clamp Design



PoCT = Point of care testing

[a] Target PG range of ≥45 to 60 mg/dL (2.5-3.3 mmol/L) for adults and ≥54 to <80 mg/dL (3.0-4.4 mmol/L) for adolescents.

[b] For adolescents, all safety PG measurements (from -180 to 300 min) will be performed by PoCT method.

2 Statistical hypotheses

The primary hypothesis to be tested is that dasiglucagon s.c. is non-inferior to glucagon i.m. in terms of the primary endpoint, time to PG recovery, for adults with T1D.

Formally, let D be the treatment difference 'dasiglucagon s.c.' minus 'glucagon i.m.' of time to PG recovery. The null hypothesis (H_0) will be tested against the alternative hypothesis (H_A) of non-inferiority as given by,

H_0: $D \geq 3$ minutes against H_A: $D < 3$ minutes

Non-inferiority of dasiglucagon will be considered confirmed if the upper boundary of the 2-sided 95% confidence interval for D is below 3 minutes.

The non-inferiority margin of 3 minutes ensures that a large fraction of the Glucagon treatment effect against placebo on time to PG recovery is preserved. In a confirmatory trial in adults with T1D comparing Dasiglucagon to Glucagon s.c. and placebo (ZP4207-16137), the median time to PG recovery was 12 minutes for Glucagon s.c. and 40 minutes for placebo. The effect of glucagon on the blood glucose response has been seen to be similar for s.c. and i.m. administration routes.[26, 27]

No confirmatory testing will be performed for the secondary endpoints nor the adolescent cohort.

2.1 Multiplicity Adjustment

Not applicable for this study.

3 Analysis Sets

Participant Analysis Set	Description
Full analysis set (adults)	All randomised adult participants who received at least one dose of IMP.
Full analysis set (adolescents)	All adolescent participants who received one dose of IMP.
Safety analysis set	All participants who received at least one dose of IMP.

The adult and the adolescent cohort will be evaluated separately. The evaluation of the PD and PK endpoints and parameters will be based on full analysis set. Safety will be evaluated on safety analysis set.

Obviously erroneous data points may be excluded from the analysis or re-analysed. Furthermore, individual PD and PK profiles will be examined prior to data base lock (DBL) to establish whether it is possible to calculate all PD and PK endpoints and parameters. If samples are missing that are important for the derivation of the endpoints and parameters, certain endpoints and/or parameters will not be calculated, and they will be excluded from analysis. If a participant receives rescue treatment (IV glucose), the PD samples collected after administration of rescue treatment will be excluded from analysis.

The participants, derived endpoints/parameters or observations to be excluded, and the reason for their exclusion must be documented before DBL. The participants, derived endpoints/parameters and observations excluded from analysis, and the reason for this, will be described in the CSR.

4 Statistical Analyses

4.1 General Considerations

The PD endpoints and parameters specified for this study will be derived for both treatments separately. The PK endpoints and parameters for glucagon corresponding to the PK endpoints and parameters specified for dasiglucagon in Table 2 and Table 5 will also be derived.

All endpoints and parameters will be summarised descriptively for the adult and the adolescent cohort separately. For the adolescent cohort, all PD and PK endpoints and parameters will be evaluated only descriptively.

If not otherwise specified, a baseline measurement is defined as the last available measurement prior to first IMP administration (visit 2 day 1).

The PG baseline measurement used for derivation of PG endpoints and parameters is defined as the last PG sample taken prior to IMP injection (visit 2 day 1/visit 3 day 1). The baseline adjustment for PD parameters will be performed on the PG-time profile by subtracting the PG baseline value from the PG-time profile.

An AE is considered treatment emergent if it occurs in the period from time of IMP injection (visit 2 day 1 and visit 3 day 1) until 28 days after IMP injection. A hypoglycaemic episode is considered treatment emergent if it occurs in the period from time of IMP injection (visit 2 day 1 and visit 3 day 1) until 12 hours after IMP injection. Please refer to Appendix 6 (Section 10.6) in the protocol for details on definition and recording of hypoglycaemic episodes from initiation of hypoglycaemic clamp procedure until 45 min after IMP administration.

4.2 Primary endpoint analysis

4.2.1 Definition of Endpoint

The primary endpoint that will be used to address the primary objective is time to PG recovery. PG recovery is defined as the first increase in PG of ≥20 mg/dL (1.1 mmol/L) from baseline. Time to PG recovery will be derived using linear interpolation between the last observed time point before recovery and the first observed time point after recovery, based on actual sampling time points with time zero as time of IMP injection.

4.2.1.1 Main analytical approach

The primary objective is to confirm the effect on glycaemic response of a single 0.6 mg s.c. injection of dasiglucagon for the treatment of insulin-induced hypoglycaemia in Asian adults with T1D. This includes comparing the difference in time to PG recovery between dasiglucagon s.c. and glucagon i.m. to a non-inferiority margin of 3 minutes as per Section 2. The primary endpoint analysis will be based on full analysis set (adults) (Section 3).

The primary endpoint will be compared between dasiglucagon and glucagon for the adult cohort using a linear mixed effects model with treatment and period as fixed effects and participant as a random effect. Estimated treatment means and the estimated treatment difference together with the 2-sided 95% CI and corresponding 2-sided p-value will be derived.

4.2.1.2 Sensitivity analysis

Not applicable for this study.

4.2.1.3 Supplementary analysis

Not applicable for this study.

4.2.2 Secondary endpoints analysis

Table 2 Supportive secondary endpoints

Parameter	Parameter title	Time frame	Description
Time to PG recovery (increase ≥20 mg/dL)	[a]Time to plasma glucose (PG) recovery, where PG recovery is defined as the first increase in PG of ≥20 mg/dL (1.1 mmol/L) from baseline	From 0 to 90 minutes after IMP injection	Time to PG recovery will be derived using linear interpolation between the last observed time point before recovery and the first observed time point after recovery, based on actual sampling time points with time zero as time of IMP injection.
PG recovery within 30 min	PG recovery within 30 minutes after IMP injection (yes/no)	From 0 to 30 minutes after IMP injection	Dichotomous outcome variable: 'Yes' if an increase in PG of ≥20 mg/dL (1.1 mmol/L) from baseline is observed within 30 minutes after IMP injection. Otherwise 'No'
PG recovery within 20 min	PG recovery within 20 minutes after IMP injection (yes/no)	From 0 to 20 minutes after IMP injection	Dichotomous outcome variable: 'Yes' if an increase in PG of ≥20 mg/dL (1.1 mmol/L) from baseline is observed within 20 minutes after IMP injection. Otherwise 'No'
PG recovery within 15 min	PG recovery within 15 minutes after IMP injection (yes/no)	From 0 to 15 minutes after IMP injection	Dichotomous outcome variable: 'Yes' if an increase in PG of ≥20 mg/dL (1.1 mmol/L) from baseline is observed within 15 minutes after IMP injection. Otherwise 'No'
PG change from baseline at 15 min	PG change from baseline at 15 minutes after IMP injection	From 0 to 15 minutes after IMP injection	Calculated as the difference of the PG value at 15 minutes after IMP injection and the PG value at baseline.
PG change from baseline at 20 min	PG change from baseline at 20 minutes after IMP injection	From 0 to 20 minutes after IMP injection	Calculated as the difference of the PG value at 20 minutes after IMP injection and the PG value at baseline.

$AUC_{Dasi,0-30min}$	Area under the plasma dasiglucagon concentration time curve from 0 to 30 minutes after IMP injection	From 0 to 30 minutes after IMP injection	Calculated as the area under the plasma dasiglucagon concentration-time curve using the linear trapezoidal technique based on observed values and actual measurement times from 0 to 30 minutes after IMP injection.
$C_{max,Dasi}$	Maximum observed plasma dasiglucagon concentration after IMP injection	From 0 to 5 hours after IMP injection	The maximum of all valid plasma dasiglucagon concentration measurements, for each measurement series after a single dose.

[a] applicable to adolescent cohort only.

The endpoints PG change from baseline at 15 and 20 minutes will be compared between dasiglucagon and glucagon for the adult cohort using a linear mixed effects model with baseline PG as covariate, treatment and period as fixed effects and participant as a random effect. Estimated treatment means and the estimated treatment difference with corresponding 95% CI will be derived.

Adverse events

All AEs will be coded using the latest version of the Medical Dictionary for Regulatory Activities. AEs considered treatment emergent will be summarised by system organ class, preferred term, seriousness, severity, outcome and relation to investigational medicinal product using descriptive statistics.

Hypoglycaemic episodes

All hypoglycaemic episodes will be classified according to Appendix 6 (Section 10.6) in the protocol. Hypoglycaemic episodes considered treatment emergent will be presented descriptively.

4.2.3 Exploratory analysis

Not applicable for this study.

4.2.4 Other safety analyses

The other safety assessments (safety laboratory parameters, vital signs and ECG) will be presented descriptively.

4.2.5 Other analyses

In addition to the primary and supportive secondary endpoints, the following parameters will be derived.

Efficacy (pharmacodynamic) parameters

Table 3 Overview of pharmacodynamic parameters

Parameter	Parameters title	Time frame	Unit	Description

PG recovery within 10 min	Plasma glucose recovery within 10 minutes after IMP injection (yes/no)	From 0 to 10 minutes after IMP injection	Count of participants	Dichotomous outcome variable: 'Yes' if an increase in PG of ≥20 mg/dL (1.1 mmol/L) from baseline is observed within 10 minutes after IMP injection. Otherwise 'No'
PG change from baseline at 10 min	Plasma glucose change from baseline at 10 minutes after IMP injection	From 0 to 10 minutes after IMP injection	mg/dL	Calculated as the difference of the PG value at 10 minutes after IMP injection and the PG value at baseline.
PG change from baseline at 30 min	Plasma glucose change from baseline at 30 minutes after IMP injection	From 0 to 30 minutes after IMP injection	mg/dL	Calculated as the difference of the PG value at 30 minutes after IMP injection and the PG value at baseline.
$AUE_{0\text{-}30min}$	Area under the baseline-adjusted effect curve from 0 to 30 minutes after IMP injection	From 0 to 30 minutes after IMP injection	h*mg/dL	Calculated as the area under the PG-time curve using the linear trapezoidal technique based on baseline adjusted values and actual measurement times from 0 to 30 minutes after IMP injection.
$AUE_{0\text{-}60min}$	Area under the baseline-adjusted effect curve from 0 to 60 minutes after IMP injection	From 0 to 60 minutes after IMP injection	h*mg/dL	Calculated as the area under the PG-time curve using the linear trapezoidal technique based on baseline adjusted values and actual measurement times from 0 to 60 minutes after IMP injection.
$AUE_{0\text{-}90min}$	Area under the baseline-adjusted effect curve from 0 to 90 minutes after IMP injection	From 0 to 90 minutes after IMP injection	h*mg/dL	Calculated as the area under the PG-time curve using the linear trapezoidal technique based on baseline adjusted values and actual measurement times from 0 to 90 minutes after IMP injection.
CE_{max}	Maximum observed baseline adjusted PG level	From 0 to 90 minutes after IMP injection	mg/dL	Maximum observed baseline adjusted PG level.
TE_{max}	Time to maximum observed baseline adjusted PG level	From 0 to 90 minutes after IMP injection	h	Time to maximum observed baseline adjusted PG level (measured as actual time since IMP injection). If not uniquely determined, the lowest value is chosen

The baseline adjusted parameters $AUE_{0\text{-}30min}$, $AUE_{0\text{-}60min}$, $AUE_{0\text{-}90min}$ and CE_{max} will be compared between dasiglucagon and glucagon for the adult cohort. The parameters will be log-transformed and analysed using a linear mixed effects model with log baseline PG as covariate, treatment and period as fixed effects and participant as a random effect. The estimated treatment means and the estimated treatment difference with corresponding 95% CI will be back-transformed to the original scale resulting in estimated geometric means and estimated treatment ratio with corresponding 95% CI.

Pharmacokinetic parameters

Table 4 Overview of pharmacokinetic parameters

Parameter	Parameters title	Time frame	Unit	Description
$AUC_{Dasi,0\text{-}300min}$	Area under the plasma dasiglucagon concentration time curve from 0 to 300 minutes after IMP injection	From 0 to 300 minutes after IMP injection	h*pmol/L	Calculated as the area under the plasma dasiglucagon concentration-time curve using the linear trapezoidal technique based on observed values and actual measurement times from 0 to 300 minutes after IMP injection.
$AUC_{Dasi,0\text{-}inf}$	Area under the plasma dasiglucagon concentration time curve from 0 minutes to infinity after IMP injection	From 0 minutes to infinity after IMP injection	h*pmol/L	Calculated as the sum of two areas; namely $AUC_{Dasi,0\text{-}tz}$, the area under the curve from zero to last quantifiable concentration at time t_z, and $AUC_{Dasi,tz\text{-}inf}$, the area from t_z to infinity. $AUC_{Dasi,0\text{-}tz}$ will be calculated using the linear trapezoidal technique based on observed values and actual measurement times from 0 to t_z. $AUC_{Dasi,tz\text{-}inf}$ will be approximated by the area from t_z to infinity under an exponential curve with terminal rate constant rate $\lambda_{z,Dasi}$ and with a value in t_z equal to $\hat{C}(t_z)$, where $\hat{C}(t_z)$ is the concentration at time t_z predicted from the linear part of the log-transformed curve. This area equals $\hat{C}(t_z)/\lambda_{z,Dasi}$.
$t_{max,Dasi}$	Time to maximum observed plasma dasiglucagon concentration after IMP injection	From 0 to 5 hours after IMP injection	h	Time to the maximum of all valid plasma dasiglucagon concentration measurements after a single dose (measured as actual time since IMP injection). If not uniquely determined, the lowest value is chosen
$t_{1/2,Dasi}$	Terminal half-life for plasma dasiglucagon after IMP injection	Terminal part of the plasma dasiglucagon concentration-time curve where the curve is well approximated by a straight line on logarithmic scale after IMP injection	h	Calculated as $\log(2)/\lambda_{z,Dasi}$ $\lambda_{z,Dasi}$ is the negative of the slope of a linear regression with the logarithm to dasiglucagon concentrations as the response variable and actual measurement times as the explanatory variable (if the slope is negative $\lambda_{z,Dasi}$ = -slope, otherwise $\lambda_{z,Dasi}$ cannot be determined). Valid observations from the terminal part of the curve, where it is well approximated by a straight line on logarithmic scale, will be used for the determination

MRT_{Dasi}	Mean residence time for plasma dasiglucagon after IMP injection	From 0h to infinity after IMP injection	h	Calculated as $AUMC_{Dasi,0\text{-}inf}/AUC_{Dasi,0\text{-}inf.}$ The numerator is the area under the first moment dasiglucagon concentration-time curve which is calculated as: $AUMC_{Dasi,0\text{-}inf}=AUMC_{Dasi,0\text{-}tz}+AUMC_{Dasi,tz\text{-}inf}$, where $AUMC_{Dasi,0\text{-}tz}$ is calculated using the linear trapezoidal technique based on observed values and actual measurement times from 0 to t_z and $AUMC_{Dasi,tz\text{-}inf}$ is calculated as $(\hat{C}(t_z)\cdot t_z/\lambda_{z,Dasi})+\hat{C}(t_z)/\lambda_{z,Dasi}^2$, where $\hat{C}(t_z)$ is the concentration at time t_z predicted from the linear part of the log-transformed curve
Cl/F_{Dasi}	Apparent serum clearance of plasma dasiglucagon after IMP injection	From 0h to infinity after IMP injection	L/h	Calculated as $Dose_{Dasi}/AUC_{Dasi,0\text{-}inf}$
Vz/F_{Dasi}	Apparent volume of distribution of plasma dasiglucagon after IMP injection based on plasma concentration values	From 0h to infinity after IMP injection	L	Calculated as $(CL/F_{Dasi})/\lambda_{z,Dasi}$

Immunogenicity data

Immunogenicity data will be analysed descriptively by treatment and time. No statistical tests are planned. Anti-drug antibody-positive participants will be calculated as a percentage of the total number of participants tested for anti-drug antibody. Titre will be reported as median and interquartile range.

Pharmacokinetic and/or pharmacodynamic modelling

Data from the study may be used for exploratory PK and/or pharmacodynamic analysis as needed.

4.3 Interim analysis

Not applicable for this study.

4.4 Sample size determination

The sample size is based on having sufficient power for confirming non-inferiority of dasiglucagon in terms of time to PG recovery for adults with T1D. In a 2-period crossover trial (ZP4207-17084) in adults with T1D comparing different batches of dasiglucagon, the within-participant standard deviation (SD) in time to PG recovery was 1.6 minutes. In a dose finding study in adults with T1D, the within-participant SD in time to PG recovery when comparing dasiglucagon s.c. (0.6 mg) and glucagon s.c. (1.0 mg) (Group 3 in study ZP4207-15126) was 1.9 minutes. Assuming a within-participant SD between 1.6 and 2.2 minutes, Table 5 shows the number of participants needed to complete both treatment periods in order to have power above 90% for confirming non-inferiority in terms of the primary endpoint for dasiglucagon s.c. as compared to glucagon i.m., assuming a

true treatment difference of 1 minute in favour of glucagon i.m. (a conservative estimate) and using a one-sided test with 2.5% level of significance.

Table 5 Number of adult participants needed to complete both dosing periods

Non-inferiority margin (min)	Within-participant SD for the primary endpoint (min)			
	1.6	**1.8**	**2.0**	**2.2**
3	16	20	24	28

Assuming a within-participant SD for the primary endpoint of 2.0 minutes, a total of 24 adults completing both treatment periods are needed for having power above 90% for confirming non-inferiority for the primary endpoint for dasiglucagon s.c. as compared to glucagon i.m., assuming a true treatment difference of 1 minute in favour of glucagon i.m.

To allow for inclusion of Asian non-Japanese participants, a total of 30 Asian adults with T1D are planned to complete both treatment periods. Of the 30 completers, a minimum of 24 will be Japanese. This gives power of 96% for confirming non-inferiority for the primary endpoint for dasiglucagon s.c. as compared to glucagon i.m. To ensure that at least 30 adults complete both treatment periods, a total of 34 adults will be randomly assigned to a treatment sequence. Of the 34 randomised adults, a minimum of 27 will be Japanese.

To investigate the clinical efficacy, PK properties, and safety of dasiglucagon in Japanese adolescents, 4 Japanese adolescents with T1D in the age 12 to 15 years (both inclusive) will be included as a separate cohort in this study. This is not based on a formal sample size calculation. Considering the feasibility of recruitment, the adolescent cohort will be exposed only to dasiglucagon during insulin induced hypoglycaemia in a single-arm, open-label design.

Approximately a total of 76 participants will be screened to achieve 34 adult participants (Asian non-Japanese and Japanese) randomly assigned to a treatment sequence and 4 Japanese adolescent participants included in this study.

5 Supporting documentation

5.1 Appendix 1: Documentation of Pharmacokinetic and Pharmacodynamic profile decisions

The below PK related decisions are based on a pharmacokinetic profiles review of blinded pharmacokinetic data using nominal time and blinded dummy IDs.

All adult Dasiglucagon PK profiles have been reviewed. At this time point it hasn't been possible for the lab to perform valid analyses of the adolescent Dasiglucagon samples and hence no PK adolescent data has been received or reviewed by the PK review group before DBL.

For glucagon it has been decided to also calculate baseline-adjusted glucagon PK endpoints since the glucagon assay measures both exogenous glucagon and endogenous glucagon. Baseline-adjustment is performed on the glucagon-time profile by subtracting the value of the baseline measurement from the glucagon-time profile. The baseline-measurement is the last measurement before IMP administration.

All glucagon profiles as well as baseline-adjusted glucagon profiles have been reviewed by the PK review group.

The below PD decisions are based on a pharmacodynamic profiles review of blinded pharmacodynamic data using scrambled treatment and actual time and subject id.

5.1.1 PK datapoints, profiles and endpoints excluded from analysis

No PK datapoints, profiles or endpoints are excluded from analysis.

5.1.2 Tail estimation

The general rule for selecting datapoints for tail estimation of Dasiglucagon PK profiles is to include all datapoints with nominal time equal to or greater than 90 minutes. During PK profiles review, tail fit for each profile is evaluated. Adjustments to the datapoint selection for tail estimation can be seen in the table below.

The general rule for selecting datapoints for tail estimation of glucagon PK profiles is to include all datapoints with nominal time equal to or greater than 60 minutes. During PK profiles review, tail fit for each profile is evaluated. Adjustments to the datapoint selection for tail estimation can be seen in the table below.

Datapoints excluded from tail estimation

Profile ID	Nominal time	Decision
Dasiglucagon X1038	300	Record excluded from tail fit estimation
Dasiglucagon X1098	90	Record excluded from tail fit estimation

Profile ID	Nominal time	Decision
Glucagon X1003	300	Record excluded from tail fit estimation
Glucagon X1030	300	Record excluded from tail fit estimation
Glucagon X1031	300	Record excluded from tail fit estimation
Glucagon X1032	300	Record excluded from tail fit estimation
Glucagon X1033	300	Record excluded from tail fit estimation
Glucagon X1036	300	Record excluded from tail fit estimation
Glucagon X1037	300	Record excluded from tail fit estimation
Glucagon X1038	300	Record excluded from tail fit estimation
Glucagon X1062	300	Record excluded from tail fit estimation
Glucagon X1064	300	Record excluded from tail fit estimation
Glucagon X1066	300	Record excluded from tail fit estimation
Glucagon X1067	300	Record excluded from tail fit estimation
Glucagon X1068	300	Record excluded from tail fit estimation
Glucagon X1069	300	Record excluded from tail fit estimation
Glucagon X1080	300	Record excluded from tail fit estimation
Glucagon X1081	300	Record excluded from tail fit estimation
Glucagon X1082	300	Record excluded from tail fit estimation
Glucagon X1083	300	Record excluded from tail fit estimation
Glucagon X1084	300	Record excluded from tail fit estimation
Glucagon X1085	210 and 300	Record excluded from tail fit estimation
Glucagon X1086	300	Record excluded from tail fit estimation
Glucagon X1087	300	Record excluded from tail fit estimation
Glucagon X1088	300	Record excluded from tail fit estimation
Glucagon X1089	300	Record excluded from tail fit estimation

Profile ID	Nominal time	Decision
Glucagon X1090	300	Record excluded from tail fit estimation
Glucagon X1091	300	Record excluded from tail fit estimation
Glucagon X1093	300	Record excluded from tail fit estimation
Glucagon X1094	300	Record excluded from tail fit estimation
Glucagon X1096	300	Record excluded from tail fit estimation
Glucagon X1096	210	Record excluded from tail fit estimation
Glucagon X1097	300	Record excluded from tail fit estimation
Glucagon X1099	300	Record excluded from tail fit estimation
Glucagon (baseline-corrected) X1003	300	Record excluded from tail fit estimation
Glucagon (baseline-corrected) X1030	300	Record excluded from tail fit estimation
Glucagon (baseline-corrected) X1031	300	Record excluded from tail fit estimation
Glucagon (baseline-corrected) X1032	300	Record excluded from tail fit estimation
Glucagon (baseline-corrected) X1033	300	Record excluded from tail fit estimation
Glucagon (baseline-corrected) X1036	300	Record excluded from tail fit estimation
Glucagon (baseline-corrected) X1037	300	Record excluded from tail fit estimation
Glucagon (baseline-corrected) X1038	300	Record excluded from tail fit estimation
Glucagon (baseline-corrected) X1062	300	Record excluded from tail fit estimation
Glucagon (baseline-corrected) X1064	300	Record excluded from tail fit estimation

Profile ID	Nominal time	Decision
Glucagon (baseline-corrected) X1066	300	Record excluded from tail fit estimation
Glucagon (baseline-corrected) X1067	300	Record excluded from tail fit estimation
Glucagon (baseline-corrected) X1068	300	Record excluded from tail fit estimation
Glucagon (baseline-corrected) X1069	300	Record excluded from tail fit estimation
Glucagon (baseline-corrected) X1080	300	Record excluded from tail fit estimation
Glucagon (baseline-corrected) X1081	300	Record excluded from tail fit estimation
Glucagon (baseline-corrected) X1082	300	Record excluded from tail fit estimation
Glucagon (baseline-corrected) X1083	300	Record excluded from tail fit estimation
Glucagon (baseline-corrected) X1084	300	Record excluded from tail fit estimation
Glucagon (baseline-corrected) X1085	210 and 300	Record excluded from tail fit estimation
Glucagon (baseline-corrected) X1086	300	Record excluded from tail fit estimation
Glucagon (baseline-corrected) X1087	300	Record excluded from tail fit estimation
Glucagon (baseline-corrected) X1088	300	Record excluded from tail fit estimation
Glucagon (baseline-corrected) X1089	300	Record excluded from tail fit estimation
Glucagon (baseline-corrected) X1090	300	Record excluded from tail fit estimation

Profile ID	Nominal time	Decision
Glucagon (baseline-corrected) X1091	300	Record excluded from tail fit estimation
Glucagon (baseline-corrected) X1093	300	Record excluded from tail fit estimation
Glucagon (baseline-corrected) X1094	300	Record excluded from tail fit estimation
Glucagon (baseline-corrected) X1096	300	Record excluded from tail fit estimation
Glucagon (baseline-corrected) X1096	210	Record excluded from tail fit estimation
Glucagon (baseline-corrected) X1097	300	Record excluded from tail fit estimation
Glucagon (baseline-corrected) X1099	300	Record excluded from tail fit estimation

Notice that in the above table the profile ID for identification of the individual PK profile includes the blinded dummy IDs eg. X1038. After unblinding the blinded dummy IDs will not appear in ADaM but the real subject ID will. A file linking the blinded dummy ID and the real subject ID becomes available after unblinding.

5.1.3 PD datapoints, profiles and endpoints excluded from analysis

No PD datapoints, profiles or endpoints are excluded from analysis.

6 References

26. Mühlhauser I, Koch J, Berger M. Pharmacokinetics and bioavailability of injected glucagon: differences between intramuscular, subcutaneous, and intravenous administration. Diabetes Care. 1985;8(1):39-42.
27. Zealand Pharma A/S. Protocol (ZP4207-14013). A randomized, double-blinded trial of single ascending doses of ZP4207 administered s.c. or i.m. to healthy volunteers and a single dose of ZP4207 administered s.c. to hypoglycemic Type 1 diabetic subjects to evaluate the safety, tolerability, pharmacokinetics and pharmacodynamics of ZP4207 as compared to an active comparator. version 3.0. 20-02-2015 2015.