Cover Page for Protocol

Sponsor name:	Novo Nordisk A/S
NCT number	NCT06588504
Sponsor trial ID:	NN9515-7675
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
Document date:	14 November 2024

Protocol

Protocol Title:
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

Short Title:
Research study in Japan to compare dasiglucagon with glucagon in treating very low levels of blood sugar in Asian adults with type 1 diabetes and testing of dasiglucagon for the same condition in Japanese adolescents

Substance Name: Dasiglucagon

Protocol Number: NN9515-7675
Protocol Version Number: Version 4.0
Protocol Version Applicability: Global

Universal Trial Number: U1111-1300-1855

NCT Number: NCT06588504

jRCT number: jRCT2071240050

Study Phase: 3

Development Stage: Pivotal stage

Sponsor Name: Novo Nordisk A/S
Sponsor Legal Address: Novo Allé, 2880 Bagsvaerd, Denmark

In the following, Novo Nordisk A/S and its affiliates will be stated as "Novo Nordisk".

This ~~confidential~~ document is the property of Novo Nordisk. ~~No unpublished information contained herein may be disclosed without prior written approval from Novo Nordisk. Access to this document must be restricted to relevant parties.~~

Protocol amendment summary of changes table

Document version	Date
Protocol version 4.0	14 November 2024
Protocol version 3.0	24 September 2024
Protocol version 2.0	27 June 2024
Original protocol version 1.0	05 June 2024

Protocol version 4.0 (14 November 2024)

This amendment is considered to be substantial based on the criteria set forth in Article 2(13) of Regulation (EU) No 536/2014 of the European Parliament and of the Council of 16 April 2014;[1] because it might substantially impacts the safety or rights of the participants or the reliability or robustness of the data generated in the study.

Overall rationale for preparing protocol, version 4.0:

Section # and name	Description of change	Brief rationale
Section 1.1 Synopsis	Under key inclusion criteria, a new inclusion criterion with body weight requirement has been added for adolescent participants.	To enrol broader adolescent participants reflecting actual population with T1DM in Japan.
Section 1.2, Schedule of activities tables	For adults table: The footnote 'j' - BMI is calculated at screening for adults only - specified in bracket For adolescents table: The footnote 'i' - BMI is calculated at screening for adults only - specified in bracket	To emphasize that the BMI calculation is only for adults.
Section 4.1 Overall design	Figure 4-2 has been updated with 'From -180 to 130 min PG are measured by Clamp machine, PoCT method or another appropriate method'.	To allow flexible selection of the most appropriate method for confirmation of PG concentration.
Section 5.1 Inclusion criteria	The term 'local laboratory' has been revised as 'subcontracted laboratory by the site' in inclusion criteria #5.	For clarity
	A new inclusion criterion with body weight requirement has been added for adolescent participants.	To enrol broader adolescent participants reflecting actual population with T1DM in Japan.
Section 5.2 Exclusion criteria	Exception has been added to exclusion criterion #32 in alignment with dosing day exclusion criteria #2.	Exception has been added as per the allowed concomitant medication and to be in alignment with dosing day exclusion criterion #2.

Section 5.5.1 Dosing day exclusion criteria	A new dosing day exclusion criterion (#17) is included for the use of oral antidiabetic medications time period before initiation of the Clamp procedure.	To clarify the handling of OADs
Section 7.2.1 Replacement of participants	The term 'at least' has been deleted.	For correctness
Section 8 Study assessments and procedures	The maximum amount of blood volume collected during the study is increased from 345 mL to 462 mL for adults. The maximum amount of blood volume collected on any visit is increased from 165 mL to 225 mL for adults. Table 8-1 is also updated accordingly.	As there is a change in method for confirmatory and safety PG measurement, the blood volume has been revised.
Section 8.2.1 Hypoglycaemic clamp procedure	The method to confirm PG concentration has been changed to 'PoCT method or another appropriate method'.	To allow flexible selection of the most appropriate method for confirmation of PG concentration.
Section 8.3.3 Plasma glucose measurements	The text has been updated with PoCT method to confirm the safety PG measurements.	To clarify that PoCT method can also be used at any time as necessary during hypoglycaemic clamp procedure.
Section 8.4.2 Body measurements	The BMI bullet has been added with 'only for adults'	To emphasize that the BMI calculation is only for adults.

Table of contents

Page

Protocol amendment summary of changes table **3**

Table of contents **5**

1 Protocol summary **9**
1.1 Synopsis 9
1.2 Schedule of activities 12

2 Introduction **28**
2.1 Study rationale 28
2.2 Background 29
2.3 Benefit-risk assessment 30
2.3.1 Risk assessment 30
2.3.2 Benefit assessment 35
2.3.3 Overall benefit-risk conclusion 35

3 Objectives and endpoints **37**

4 Study design **39**
4.1 Overall design 39
4.2 Scientific rationale for study design 41
4.2.1 Patient input into design 41
4.3 Justification for dose 41
4.4 End of study definition 42

5 Study population **43**
5.1 Inclusion criteria 43
5.2 Exclusion criteria 43
5.3 Lifestyle considerations 45
5.3.1 Meals and dietary restrictions 45
5.3.2 Caffeine, alcohol and tobacco 45
5.3.3 Activity 46
5.3.4 Other restrictions 46
5.4 Screen failures 46
5.5 Dosing day exclusion criteria 47
5.5.1 Dosing day exclusion criteria 47
5.6 Stand-by participants 48

6 Study intervention(s) and concomitant therapy **49**
6.1 Study intervention(s) administered 49
6.2 Preparation, handling, storage and accountability 51
6.3 Measures to minimise bias: Randomisation and blinding 51
6.4 Study intervention compliance 52
6.5 Dose modification 52
6.6 Continued access to study intervention after end of study 52
6.7 Treatment of overdose 52
6.8 Concomitant therapy 53
6.8.1 Rescue medicine 53

7 Discontinuation of study intervention and participant discontinuation/withdrawal **55**
7.1 Discontinuation of study intervention 55
7.1.1 Temporary discontinuation of study intervention 55
7.1.2 Rescue criteria 55
7.2 Participant discontinuation/withdrawal from the study 55
7.2.1 Replacement of participants 56

7.3 Lost to follow-up 57

8 Study assessments and procedures 58
8.1 Screening/baseline assessments and procedures 59
8.2 Dose adjustment guidelines 60
8.2.1 Hypoglycaemic clamp procedure 60
8.3 Efficacy assessments 63
8.3.1 Efficacy-related assessments 63
8.3.2 Clinical efficacy or Pharmacodynamic laboratory assessments 63
8.3.3 Plasma glucose measurements 63
8.4 Safety assessments 64
8.4.1 Physical examinations 64
8.4.2 Body measurements 64
8.4.3 Vital signs 65
8.4.4 Eye examination 65
8.4.5 Electrocardiograms 65
8.4.6 Clinical safety laboratory assessments 66
8.4.7 Pregnancy testing 66
8.5 Adverse events and other safety reporting 66
8.5.1 Time period and frequency for collecting AE information 67
8.5.2 Method of detecting AEs 67
8.5.3 Follow-up of AEs 67
8.5.4 Regulatory reporting requirements for SAEs 68
8.5.5 Pregnancy 68
8.5.6 Cardiovascular and death events 68
8.5.7 Disease-related events and/or disease-related outcomes not qualifying as AEs or SAEs 68
8.5.8 Adverse event of special interest 69
8.5.9 Technical complaints 69
8.6 Pharmacokinetics 69
8.7 Pharmacodynamics 70
8.8 Genetics 70
8.9 Biomarkers 70
8.10 Immunogenicity assessments 70

9 Statistical considerations 72
9.1 Statistical hypotheses 72
9.1.1 Multiplicity adjustment 72
9.2 Analysis sets 72
9.3 Statistical analyses 73
9.3.1 General considerations 73
9.3.2 Primary endpoint analysis 73
9.3.2.1 Definition of endpoint 73
9.3.2.2 Main analytical approach 73
9.3.2.3 Sensitivity analysis 74
9.3.2.4 Supplementary analysis 74
9.3.3 Secondary endpoints analysis 74
9.3.4 Exploratory analysis 75
9.3.5 Other safety analyses 75
9.3.6 Other analyses 76
9.4 Interim analysis 78
9.5 Sample size determination 79

10 Supporting documentation and operational considerations 80
10.1 Appendix 1: Regulatory, ethical and study oversight considerations 80
10.1.1 Regulatory and ethical considerations 80

10.1.2 Financial disclosure 80
10.1.3 Informed consent process 81
10.1.4 Recruitment and information to participants during the study 81
10.1.5 Data protection 82
10.1.6 Committees structure 83
10.1.6.1 Novo Nordisk safety committee 83
10.1.6.2 Study safety group 83
10.1.6.3 Data monitoring committee 83
10.1.6.4 Event adjudication committee 83
10.1.7 Dissemination of clinical study data 83
10.1.8 Data quality assurance 83
10.1.8.1 Case report forms 83
10.1.8.2 Monitoring 84
10.1.8.3 Protocol compliance 85
10.1.9 Source documents and source data 85
10.1.10 Retention of clinical study documentation 85
10.1.11 Study and site start and closure 86
10.1.12 Responsibilities 86
10.1.13 Indemnity statement 87
10.1.14 Publication policy 87
10.1.14.1 Communication of results 88
10.1.14.2 Authorship 88
10.1.14.3 Site-specific publication(s) by investigator(s) 89
10.1.14.4 Investigator access to data and review of results 89
10.2 Appendix 2: Clinical laboratory tests 90
10.3 Appendix 3: Adverse Events and Serious Adverse Events: Definitions and procedures for recording, evaluating, follow-up and reporting 93
10.3.1 Definition of AE 93
10.3.2 Definition of an SAE 93
10.3.3 Description of AEs requiring additional data collection and other events requiring collection of additional information 94
10.3.4 Recording and follow-up of AEs and/or SAEs 95
10.3.4.1 AE and SAE recording 95
10.3.4.2 Assessment of severity 96
10.3.4.3 Assessment of causality 96
10.3.4.4 Outcome of AEs 97
10.3.4.5 Follow-up of AE and SAE 97
10.3.5 Reporting of SAEs 98
10.4 Appendix 4: Contraceptive guidance and collection of pregnancy information 100
10.4.1 Definitions 100
10.4.2 Contraceptive guidance 100
10.4.3 Collection of pregnancy information 102
10.5 Appendix 5: Technical complaints: Definition and procedures for recording, evaluation, follow-up and reporting 103
10.5.1 Definition of technical complaint 103
10.5.2 Recording and follow-up of technical complaints 103
10.5.3 Reporting of technical complaints for products not included in the technical complaint form 104
10.6 Appendix 6: Hypoglycaemic episodes 105
10.7 Appendix 7: Hyperglycaemic episodes 108
10.8 Appendix 8: Mitigations to ensure participant safety and data integrity during a crisis 110
10.8.1 Definition and scope of appendix 110
10.8.2 Visits 110
10.9 Appendix 9: Abbreviations 111
10.10 Appendix 10: Protocol amendment history 114

11 References 116

Protocol attachment I list of key staff and relevant departments and key suppliers of clinical relevance

1 Protocol summary

1.1 Synopsis

This is an interventional, single-centre, open-label, randomised, 2-period cross-over study designed 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 (brand name: Glucagon G Novo; hereafter referred to as glucagon) for the treatment of insulin-induced hypoglycaemia in Asian non-Japanese and Japanese adults with T1D including an investigation of dasiglucagon in a Japanese adolescent cohort with T1D.

Rationale:

The main purpose of this study is to confirm the effect on glycaemic response of a single 0.6 mg s.c. injection of dasiglucagon for treatment of insulin-induced hypoglycaemia in adult Asian non-Japanese and Japanese participants with T1D including an investigation of dasiglucagon in a Japanese adolescent cohort with T1D. The primary hypothesis to be tested in terms of the primary endpoint, time to plasma glucose (PG) recovery, for adults with T1D is that dasiglucagon s.c. is non-inferior to glucagon i.m. Data from this study will support the regulatory applications of dasiglucagon for the treatment of severe hypoglycaemia in Japan and other Asian countries.

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 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

Objectives	Endpoints		
	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
• 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.	$AUC_{0\text{-}30min}$: 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
	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.

Overall design:

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,
- two dosing visits (visits 2 and 3); randomisation on the first dosing visit (visit 2 day 1), and
- a follow-up visit (end of study, visit 3a).

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,
- a dosing visit (visit 2), and
- a follow-up visit (end of study, visit 3a).

Study intervention groups and duration:

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. Furthermore, 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 planned total study duration (including screening) for the individual adult and adolescent participant is approximately 17 and 11 weeks, respectively.

Number of participants:

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

Participant characteristics:

Key inclusion criteria:

- **For Adults:** Asian male or female; **For Adolescents:** Japanese male or female.
- Age at the time of signing the informed consent:
  For Adults: Age 18-75 years (both inclusive):
  For Adolescents: Age 12-15 years (both inclusive).
- Diagnosed with T1D >1 year before screening.
- HbA1c <10.0% (86 mmol/mol) as assessed by local laboratory on the day of screening.
- **For adults:** BMI between 18.5 and 29.9 kg/m^2 (both inclusive).
  For adolescents: Body weight ≥ 33.4 kg
- Treated with stable insulin treatment (based on the investigator's discretion preferably no more than a 10-unit daily variation in total daily insulin dose) 30 days prior to screening.
- **For Japanese participants:** Japanese passport or equivalent
  For non-Japanese participants: Asian (non-Japanese passport or equivalent).

Key exclusion criteria:

- Known or suspected hypersensitivity to study intervention(s) or related products (glucagon or its derivatives).
- Exposure to an investigational medicinal product (IMP) within 30 days or 5 times the half-life of the IMP (if known), whichever is longest before screening.
- Severe hypoglycaemia in the last month prior to screening.
- Hospitalisation for diabetic ketoacidosis (DKA) in the last month prior to screening.
- Presence or history of any clinically relevant respiratory, metabolic, renal, hepatic, gastrointestinal, endocrinological conditions (except conditions associated with diabetes mellitus).
- History[a] of epilepsy or seizure disorder.
- Known presence or history[a] of pheochromocytoma (i.e., adrenal gland tumour) or insulinoma (i.e., insulin-secreting pancreas tumour).
- Clinically significant abnormal ECG at screening as evaluated by investigator.
- Any disorder, unwillingness or inability which in the investigator's opinion, might jeopardise the participant's safety or compliance with the protocol.

  [a] As declared by the participant or in the medical records.

Efficacy and safety data will be collected at regular intervals throughout the study.

Data monitoring committee:

No

1.2 Schedule of activities

For Adults:

Procedure	Protocol section	Information visit	Screening	In-house dosing visits				End of study
Visit short name		V0	V1	V2		V3		V3a[e]
Timing of Visit (Day) within each dosing period				-1	1[d]	-1	1[d]	
Visit window (days)		≥ 1 day before V1	2-28 days before V2 Day 1			28-42 days after V2 Day 1		28-42 days after V3 Day 1
PARTICIPANT RELATED INFORMATION AND ASSESSMENTS								
Informed Consent[a]	10.1.3	X						
Demography[b]	8		X					
Eligibility Criteria	5.1, 5.2		X	X[c]				
Medical History/ Concomitant Illness[f]	8.1		X	X	X			
Concomitant Medication	6.8		X	X	X	X	X	X
Tobacco, E-cigarettes, and Nicotine[g]	5.3.2		X	X	X	X	X	
Randomisation	5.5, 6.3				X			
Dosing Day Exclusion Criteria	5.5				X		X	

Procedure	Protocol section	Information visit	Screening	In-house dosing visits				End of study
Visit short name		V0	V1	V2		V3		V3a[e]
Timing of Visit (Day) within each dosing period				-1	1[d]	-1	1[d]	
Visit window (days)		≥ 1 day before V1	2-28 days before V2 Day 1			28-42 days after V2 Day 1		28-42 days after V3 Day 1
Discontinuation Criteria	7			X	X	X	X	
Hypoglycaemia Unawareness[h]	5.2		X					
Childbearing Potential	8.4.7, 10.4.1		X					
Pregnancy Test[g,i]	8.4.7		X	X		X		X
Screen for alcohol[g]	5.3.2		X		X		X	
Screen for drug[g]	5.3.2		X		X		X	
Body Measurements[j]	8.4.2		X		X		X	X
REMINDERS								
Attend Visit Fasting	5.3.1, 5.5				X		X	
Hand out and instruct in BG-meter	8.3.3			X				

Procedure	Protocol section	Information visit	Screening	In-house dosing visits				End of study
Visit short name		V0	V1	V2		V3		V3a[e]
Timing of Visit (Day) within each dosing period				-1	1[d]	-1	1[d]	
Visit window (days)		≥ 1 day before V1	2-28 days before V2 Day 1			28-42 days after V2 Day 1		28-42 days after V3 Day 1
Hand out and instruct in Diary	8.3.3			X		X		
Collect, review, and transcribe Diary[k]	8.3.3				X	X	X	X
SAFETY								
Vital Signs[l]	8.4.3		X		X		X	X
ECG[l]	8.4.5		X		X		X	X
Physical Examination	8.4.1		X	X		X		X
Hypoglycaemic episode[m]	10.6		X	X	X	X	X	X
Adverse Events	8.5, 10.3				X	X	X	X
EFFICACY								
Insulin-induced hypoglycaemia	8.2.1				X		X	

Procedure	Protocol section	Information visit	Screening	In-house dosing visits				End of study
Visit short name		V0	V1	V2		V3		V3a[e]
Timing of Visit (Day) within each dosing period				-1	1[d]	-1	1[d]	
Visit window (days)		≥ 1 day before V1	2-28 days before V2 Day 1			28-42 days after V2 Day 1		28-42 days after V3 Day 1
PD sampling (plasma glucose)[n]	8.3				X		X	
PK sampling[o]	8.6				X		X	
TRIAL MATERIAL								
Administration of IMP (dasiglucagon/glucagon)	6.1				X		X	
Drug dispensing	6.1				X		X	
Drug accountability	6.2				X		X	
LABORATORY ASSESSMENTS								
Haematology	8.4.6, 10.2		X					X
Coagulation parameters	8.4.6, 10.2		X					
Biochemistry	8.4.6, 10.2		X					X

Procedure	Protocol section	Information visit	Screening	In-house dosing visits				End of study
Visit short name		V0	V1	V2		V3		V3a[e]
Timing of Visit (Day) within each dosing period				-1	1[d]	-1	1[d]	
Visit window (days)		≥ 1 day before V1	2-28 days before V2 Day 1			28-42 days after V2 Day 1		28-42 days after V3 Day 1
Lipids	8.4.6, 10.2		X					X
Serology[g]	8.4.6, 10.2		X					
Urinalysis	8.4.6, 10.2		X					X
Glucose metabolism[p]	8.4.6, 10.2		X					
Antibodies[q]	8.10, 10.2				X		X	X

[a] Information visit can be performed at the same day as screening visit (visit 1). Informed consent must be obtained before any study-related activities. The informed consent will be checked for signature and signature date and will be recorded in eCRF at the screening visit (visit 1).

[b] Demography (consisting of year of birth, sex [at birth], race, and ethnicity according to local regulation) is collected at the screening visit (visit 1). Ethnicity and race must be self-reported by the participant.

[c] Confirmation that the participant is eligible for continuation in the study.

[d] Procedures at dosing days must be performed prior to dosing unless otherwise noted.

[e] For a withdrawn/discontinued participant after dosing, the investigator must aim to complete procedures for the end of study visit (visit 3a) approximately 28-42 days after last product administration (Section 7.2).

[f] Report conditions identified before first administration of investigational intervention as medical history/concomitant illness in accordance with Section 8.1. Report conditions identified after first administration of investigational intervention as AEs in accordance with Section 8.5.

[g] Data will not be collected in the electronic case report form (eCRF), but only in the source data.

[h] Information on hypoglycaemia unawareness will be recorded in the eCRF according to Clarke's questionnaire, question 8.[2] The investigator must ask the participant in the following way: "To what extent can you tell by your symptoms that your blood glucose is low" Participants answering 'never, rarely or sometimes' are considered to have impaired awareness of hypoglycaemia, whereas those answering 'often or always' are not.

[i] Only woman of childbearing potential by serum or urine stick pregnancy test as described in Section 10.2.

[j] At screening: body weight, height and BMI (only for adults). At other visits only body weight will be recorded.

[k] At day 1, (visits 2 and 3) diaries will be reviewed and information from site visit transcribed to eCRF. Diaries will be collected and reviewed at visit 3 day -1 for information since visit 2 day 1 and will be transcribed to eCRF. Finally, at visit 3a diaries will be collected and information since from previous visit period (visit 3 day 1) will be transcribed to eCRF.

[l] During in-house dosing visits (visit 2 and visit 3): Assessment of vital signs and ECG will be performed at pre-clamp, during clamp and end of clamp according to Table 1-1.

[m] Definitions (including definition of hypoglycaemic episodes from initiation of hypoglycaemic clamp procedure until 45 min after IMP administration), classification and reporting requirements for hypoglycaemic episodes are described in Appendix 6 (Section 10.6). For screening visit (visit 1), refer to Section 5 for eligibility criteria for hypoglycaemic episodes.

[n] Plasma glucose for safety will be measured by study staff during the in-house visits (visit 2 + visit 3). Refer to Table 1-1.

[o] Blood pharmacokinetic (PK) sampling for measurement of dasiglucagon and glucagon. Refer to Table 1-1.

[p] Glycated Haemoglobin (HbA1c) is assessed at screening (visit 1).

[q] Blood sampling for measurement of anti-dasiglucagon antibodies will be performed pre-dose at either dosing visits (visit 2 or visit 3) and at the end-of-study visit (visit 3a). Refer to Table 1-1.

For Adolescents:

Procedure	Protocol section	Information visit	Screening	In-house dosing visits		End of study
Visit short name		V0	V1	V2		V3a
Timing of Visit (Day) within each dosing period				-1	1[d]	
Visit window (days)		≥ 1 day before V1	2-28 days before V2 Day 1			28-42 days after V2 Day 1
PARTICIPANT RELATED INFORMATION AND ASSESSMENTS						
Informed Consent[a]	10.1.3	X				
Demography[b]	8		X			
Eligibility Criteria	5.1, 5.2		X	X[c]		
Medical History/ Concomitant Illness[e]	8.1		X	X	X	
Concomitant Medication	6.8		X	X	X	X
Tobacco, E-cigarettes and Nicotine[f]	5.3.2		X	X	X	
Dosing Day Exclusion Criteria	5.5				X	
Discontinuation Criteria	7			X	X	
Hypoglycaemia Unawareness[g]	5.2		X			

Procedure	Protocol section	Information visit	Screening	In-house dosing visits		End of study
Visit short name		V0	V1	V2		V3a
Timing of Visit (Day) within each dosing period				-1	1[d]	
Visit window (days)		≥ 1 day before V1	2-28 days before V2 Day 1			28-42 days after V2 Day 1
Childbearing Potential	8.4.7, 10.4.1		X			
Pregnancy Test[f,h]	8.4.7		X	X		X
Screen for alcohol[f]	5.3.2		X		X	
Screen for drug[f]	5.3.2		X		X	
Body Measurements[i]	8.4.2		X		X	X
REMINDERS						
Attend Visit Fasting	5.3.1, 5.5				X	
Hand out and instruct in BG-meter	8.3.3			X		
Hand out and instruct in Diary	8.3.3			X		
Collect, review, and transcribe Diary[j]	8.3.3				X	X

Procedure	Protocol section	Information visit	Screening	In-house dosing visits		End of study
Visit short name		V0	V1	V2		V3a
Timing of Visit (Day) within each dosing period				-1	1[d]	
Visit window (days)		≥ 1 day before V1	2-28 days before V2 Day 1			28-42 days after V2 Day 1
SAFETY						
Vital Signs[k]	8.4.3		X		X	X
ECG[k]	8.4.5		X		X	X
Physical Examination	8.4.1		X	X		X
Hypoglycaemic episode[l]	10.6		X	X	X	X
Adverse Events	8.5, 10.3				X	X
EFFICACY						
Insulin-induced hypoglycaemia	8.2.1				X	
PD sampling (plasma glucose)[m]	8.3				X	
PK sampling[n]	8.6				X	

Procedure	Protocol section	Information visit	Screening	In-house dosing visits		End of study
Visit short name		V0	V1	V2		V3a
Timing of Visit (Day) within each dosing period				-1	1[d]	
Visit window (days)		≥ 1 day before V1	2-28 days before V2 Day 1			28-42 days after V2 Day 1
TRIAL MATERIAL						
Administration of IMP (dasiglucagon/glucagon)	6.1				X	
Drug dispensing	6.1				X	
Drug accountability	6.2				X	
LABORATORY ASSESSMENTS						
Haematology	8.4.6, 10.2		X			X
Coagulation parameters	8.4.6, 10.2		X			
Biochemistry	8.4.6, 10.2		X			X
Lipids	8.4.6, 10.2		X			X
Serology[f]	8.4.6, 10.2		X			

Procedure	Protocol section	Information visit	Screening	In-house dosing visits		End of study
Visit short name		V0	V1	V2		V3a
Timing of Visit (Day) within each dosing period				-1	1[d]	
Visit window (days)		≥ 1 day before V1	2-28 days before V2 Day 1			28-42 days after V2 Day 1
Urinalysis	8.4.6, 10.2		X			X
Glucose metabolism[o]	8.4.6, 10.2		X			
Antibodies[p]	8.10, 10.2				X	X

[a] Information visit can be performed at the same day as screening visit (visit 1). Informed consent must be obtained before any study-related activities. The informed consent will be checked for signature and signature date and will be recorded in eCRF at the screening visit (visit 1).

[b] Demography (consisting of year of birth, sex [at birth], race, and ethnicity according to local regulation) is collected at the screening visit (visit 1). Ethnicity and race must be self-reported by the participant.

[c] Confirmation that the participant is eligible for continuation in the study.

[d] Procedures at dosing days must be performed prior to dosing unless otherwise noted.

[e] Report conditions identified before first administration of investigational intervention as medical history/concomitant illness in accordance with Section 8.1. Report conditions identified after first administration of investigational intervention as AEs in accordance with Section 8.5.

[f] Data will not be collected in the electronic case report form (eCRF), but only in the source data.

[g] Information on hypoglycaemia unawareness will be recorded in the eCRF according to Clarke's questionnaire, question 8.[2] The investigator must ask the participant in the following way: "To what extent can you tell by your symptoms that your blood glucose is low" Participants answering 'never, rarely or sometimes' are considered to have impaired awareness of hypoglycaemia, whereas those answering 'often or always' are not.

[h] Only woman of childbearing potential by serum or urine stick pregnancy test as described in Section 10.2.

[i] At screening: body weight, height and BMI (only for adults). At other visits only body weight will be recorded.

[j] At visit 2 day 1, diaries will be reviewed and information from site visit transcribed to eCRF. At visit 3a, diaries will be collected and information since from previous visit period (visit 2 day 1) will be transcribed to eCRF.

[k] During in-house dosing visits (visit 2): Assessment of vital signs and ECG will be performed at pre-clamp, during clamp and end of clamp according to Table 1-2.

[l] Definitions (including definition of hypoglycaemic episodes from initiation of hypoglycaemic clamp procedure until 45 min after IMP administration), classification and reporting requirements for hypoglycaemic episodes are described in Appendix 6 (Section 10.6). For screening visit (visit 1), refer to Section 5 for eligibility criteria for hypoglycaemic episodes.

[m] Plasma glucose for safety will be measured by study staff during the in-house visit (visit 2). Refer to Table 1-2.

[n] Blood pharmacokinetic (PK) sampling for measurement of dasiglucagon. Refer to Table 1-2.

[o] Glycated Haemoglobin (HbA1c) is assessed at screening (visit 1).

[p] Blood sampling for measurement of anti-dasiglucagon antibodies will be performed pre-dose at dosing visit (visit 2) and at the end-of-study visit (visit 3a). Refer to Table 1-2.

Table 1-1 Visits 2 and 3- PK/PD Sampling Timepoints-for Adults

Intervention[a]	Nominal Time (min)[b]	Pharmacokinetic samples (PK)[c]	Pharmacodynamic samples (PD)[c]	Plasma Glucose for safety[d]	Insulin infusion for clamp procedure[e]	Urine drug test, alcohol screen and Antibodies sampling	12-lead safety ECG[f]	Vital signs[g]
Assessments prior to Clamp[h]	-210 min					X	X	X
Clamp starts[i]	-180 min			Approx. every 5-10 min	X			
Clamp stops[j]	0 min							
IMP administration[k]	0 min	X (pre-dose)	X (pre-dose)	Approx. every 5 min				
Collecting sampling	4 min		X					
	5 min	X						
	6 min		X					
	8 min		X					
	10 min		X					
	12 min		X					
	15 min	X	X					
	17 min		X					
	20 min	X	X				X	
	25 min	X	X					
	30 min	X	X					X
	35 min	X	X				X	
	40 min	X	X					
	45 min	X	X				X	
	50 min		X					

Intervention[a]	Nominal Time (min)[b]	Pharmacokinetic samples (PK)[c]	Pharmacodynamic samples (PD)[c]	Plasma Glucose for safety[d]	Insulin infusion for clamp procedure[e]	Urine drug test, alcohol screen and Antibodies sampling	12-lead safety ECG[f]	Vital signs[g]
	60 min	X	X	Approx. every 30 min			X	
	75 min		X					
	90 min	X	X					X
	120 min	X						
	210 min	X						
	300 min	X					X	X

[a] The STG-55 may be started at the investigator's discretion at visit 2 day-1 and visit 3 day -1 for safety monitoring of PG levels prior to the initiation of hypoglycaemic procedures.

[b] Nominal timing should be followed except for pre-clamp and run-in period (during clamp) for which nominal time will be variable, depending on the time for stabilization of PG.

[c] Allowed sampling window: ±1 min.

[d] For PG above 110 mg/dL (6.1 mmol/L): Safety measurement every 10 min.
For PG at or below 110 mg/dL (6.1 mmol/L): Safety measurement every 5 min.
For PG from 60 min time point: Safety measurement should be checked every 30 min.

[e] For further details on Clamp procedure, please refer to Section 8.2.1.

[f] Allowed sampling window: ±5 min at timepoints outlined after IMP administration.

[g] Allowed sampling window: ±10 min at timepoints outlined after IMP administration.

[h] Assessments prior to the Clamp (IV insulin infusion) should be completed 30 min before the start of Clamp (IV insulin infusion). Dosing day exclusion criteria and randomization should be performed before Clamp procedure starts (IV insulin infusion).

[i] Clamp starts refers to IV insulin infusion starts.

[j] Clamp ends refers to when IV insulin infusion stops.

[k] Baseline (pre-dose) PD/PK sample must be taken 5 min after clamp (IV insulin infusion) stops given PG-target is confirmed (see Section 8.2.1). Allowed administrations window for IMP is within 2 min after baseline (pre-dose) PD/PK sample. The IMP could be administered as soon as PG level is ≥45 to <60 mg/dL (2.5-3.3 mmol/L).

Table 1-2 Visit 2 - PK/PD Sampling Timepoints-for Adolescents

Intervention	Nominal Time (min)[a]	Pharmacokinetic samples (PK)[b]	Pharmacodynamic samples (PD)[b]	Plasma Glucose for safety[c]	Insulin infusion for clamp procedure[d]	Urine drug test, alcohol screen and Antibodies sampling	12-lead safety ECG[e]	Vital signs[f]
Assessments prior to Clamp[g]	-210 min					X	X	X
Clamp starts[h]	-180 min			Approx. every 5-10 min	X			
Clamp stops[i]	0 min							
IMP administration[j]	0 min	X (pre-dose)	X (pre-dose)	Approx. every 5 min				
Collecting sampling	4 min		X					
	6 min		X					
	8 min		X					
	10 min	X	X					
	12 min		X					
	15 min		X					
	17 min		X					
	20 min	X	X				X	
	30 min	X	X					X
	40 min	X						

Intervention	Nominal Time (min)[a]	Pharmacokinetic samples (PK)[b]	Pharmacodynamic samples (PD)[b]	Plasma Glucose for safety[c]	Insulin infusion for clamp procedure[d]	Urine drug test, alcohol screen and Antibodies sampling	12-lead safety ECG[e]	Vital signs[f]
	45 min		X				X	
	60 min	X	X	Approx. every 30 min			X	
	90 min	X	X					X
	140 min	X						
	220 min	X						
	300 min	X					X	X

[a] Nominal timing should be followed except for pre-clamp and run-in period (during clamp) for which nominal time will be variable, depending on the time for stabilization of PG.

[b] Allowed sampling window: ±1 min.

[c] For PG above 110 mg/dL (6.1 mmol/L): Safety measurement every 10 min.

For PG at or below 110 mg/dL (6.1 mmol/L): Safety measurement every 5 min.

For PG from 60 min time point: Safety measurement should be checked every 30 min.

[d] For further details on Clamp procedure, please refer to Section 8.2.

[e] Allowed sampling window: ±5 min at timepoints outlined after IMP administration.

[f] Allowed sampling window: ±10 min at timepoints outlined after IMP administration.

[g] Assessments should be completed 30 min before the start of Clamp (IV insulin infusion). Dosing day exclusion criteria and randomization should be performed before Clamp procedure starts (IV insulin infusion).

[h] Clamp starts refers to IV insulin infusion starts.

[i] Clamp ends refers to when IV insulin infusion stops.

[j] Baseline (pre-dose) PD/PK sample must be taken 5 min after clamp (IV insulin infusion) stops given PG-target is confirmed (see Section 8.2.1). Allowed administrations window for IMP is within 2 min after baseline (pre-dose) PD/PK sample. The IMP could be administered as soon as PG level is ≥54 to <80 mg/dL (3.0-4.4 mmol/L).

2 Introduction

Hypoglycaemia in patients with diabetes is defined as episodes of an abnormally low PG concentration.[3] This is a common, unpredictable, and potentially dangerous side effect of treatment of diabetes mellitus, especially with insulin or sulfonylureas.[4] Symptoms and signs of hypoglycaemia are not specific. Patients undergoing a hypoglycaemic episode may experience unpleasant symptoms such as anxiety, sweating, hunger, tremors, palpitations, paraesthesia, nausea and pallor. Depending on its severity, the hypoglycaemia may lead to mild confusion, behavioural changes, loss of consciousness, seizures, coma, and, if untreated, potentially death.[5] The incidence of hypoglycaemic events or even the fear of hypoglycaemia influences patients' adherence to prescribed treatment regimens.[6]

Despite advances in diabetes treatment, severe hypoglycaemia remains a clinical problem in patients with T1D or T2D with an overall rate of self-reported severe hypoglycaemia worldwide of 1.3-4.9 episodes/patient year.[7-10] Recurrent hypoglycaemia and fear of hypoglycaemia remain some of the greatest barriers for achieving optimal glycaemic control in diabetes patients, especially patients treated with insulin.[11, 12] The ADA Standard of Care in Diabetes guidelines recommend prescribing glucagon to individuals at increased risk of level 2 or 3 hypoglycaemia for emergency treatment.[5] Any individual, including caregivers, school personnel, or family members providing support to these patients, can safely administer glucagon and should know where the glucagon is and when and how to administer it.[13] Japan Diabetes Society and the Ministry of Health, Labour and Welfare have indicated emergency actions for hypoglycaemia outside medical institutions.[14]

Dasiglucagon, a second-generation, ready-to-use glucagon product, has been developed to provide a convenient and effective treatment option for hypoglycaemia without the need for reconstitution that ensures fast and effective recovery from hypoglycaemia. Dasiglucagon offers a ready-to-use alternative to traditional glucagon injection powder for severe hypoglycaemia treatment, making it easier for caregivers of people with diabetes mellitus.

2.1 Study rationale

The main purpose of this study is to confirm the effect on glycaemic response of a single 0.6 mg s.c. injection of dasiglucagon for treatment of insulin-induced hypoglycaemia in adult Asian non-Japanese and Japanese participants with T1D including an investigation of dasiglucagon in a Japanese adolescent cohort with T1D. For adult participants, an i.m. injection of 1 mg glucagon (brand name: Glucagon G Novo); hereafter referred to as glucagon, will be included as active comparator. Data from this study will support the regulatory applications of dasiglucagon for the treatment of severe hypoglycaemia in Japan and other Asian countries.

As a pharmaceutical company, Novo Nordisk has a legitimate interest in conducting research to learn how the medicines and devices we develop work and learn more about how to prevent and/or treat serious chronic diseases and related conditions. As part of conducting research, Novo Nordisk also has a legitimate interest to communicate the results of our research, and interact with competent authorities.

Novo Nordisk may also share data with external researchers who are not part of this study for research purposes, to find out more about dasiglucagon, severe hypoglycaemia, T1D or related conditions and to help improve medical care and scientific understanding.

2.2 Background

Hypoglycaemia

In T1D, hypoglycaemia is experienced throughout the course of established disease.[4] The incidence of hypoglycaemic events or even the fear of hypoglycaemia influences patients' adherence to prescribed treatment regimens for diabetes mellitus.[6] This leads to inadequate glycaemic control, which in turn may lead to an increased risk of diabetic complications.[15] Serious clinically significant hypoglycaemia is defined as PG <54 mg/dL (3.0 mmol/L), while the PG alert value is defined as <70 mg/dL (3.9 mmol/L).[16] Treatment is needed when PG falls below these values.

Glucagon

Glucagon is a naturally occurring hormone, secreted from the alpha cells of the pancreatic islets. Glucagon plays a central role in the regulation of glucose homeostasis and is the counterpart of insulin for controlling blood glucose levels (i.e. it acts in opposition to insulin in terms of effects on blood glucose levels).[17, 18] Insulin decreases blood glucose levels and cases of hypoglycaemia can be reversed by glucagon. Therefore, glucagon is indicated for the treatment of severe hypoglycaemia.

Besides IV glucose administration, an injectable form of glucagon is given as first aid in cases of severe hypoglycaemia, when the patient is unconscious or for other reasons cannot take glucose orally.

In Japan, Glucagon G Novo is marketed and approved for the use for emergency treatment for hypoglycaemia. The approved glucagon dose for emergency treatment of hypoglycaemia in Japan is 1 mg/mL administered intramuscularly or intravenously.

Dasiglucagon

Dasiglucagon is a stable peptide analogue (a peptide of 29 amino acids, with 7 amino acid substitutions compared to native glucagon) of human glucagon, available in a ready-to-use liquid formulation. Dasiglucagon is a specific and full glucagon receptor agonist designed to mimic the effects of glucagon. The main purpose of the substitutions is to increase the physical and chemical stability of the glucagon analogue compared to marketed glucagon products. Dasiglucagon is marketed in the US under the brand name ZEGALOGUE®. Zealand Pharma has applied for marketing authorisation of dasiglucagon in the European Union (EU) in June 2023 with an expected approval in Q3 2024.

Dasiglucagon has been developed for both refrigerated storage and storage at room temperature for prolonged periods. This provides patients and caregivers with a portable and convenient treatment option, allowing flexibility in dasiglucagon storage while ensuring immediate access to an effective, ready-to-use emergency treatment of hypoglycaemia. For further information, please refer to the current dasiglucagon investigator's brochure (IB).

Study population

The study population will consist of adult Asian non-Japanese and Japanese participants and Japanese adolescents with T1D. The study population is further described in Section 5.

2.3 Benefit-risk assessment

The main benefits and risks related to participation in the study are described in the below sections. More detailed information about the known and expected benefits and risks and reasonably expected adverse events of dasiglucagon may be found in the dasiglucagon IB. Please refer to the local approved label in Japan for information on benefits and risks related to glucagon.

2.3.1 Risk assessment

Table 2-1 Risk assessment

Potential risk of clinical significance	Summary of data/rationale for risk	Mitigation and monitoring strategy
Study intervention (dasiglucagon)		
Inappropriate use of the administration device leading to loss of drug benefit'(RMP)	If an involved staff fail to administer the medication correctly to the participant due to lack of compliance with the instructions for use. A failure to administer the medication correctly may result in a failure to deliver the full dose of dasiglucagon required to restore normal blood glucose levels and thus not recover as rapidly from the severe hypoglycaemia episode. Failure to dose or administration of an inadequate dose during a severe hypoglycaemia episode may lead to worsening of the severe hypoglycaemia episode and clinical sequelae such as seizure or coma.	Experienced and trained staff are to perform the injection of IMP and additionally education that includes training for the proper use of the dasiglucagon devices (considered standard of care) will provide knowledge to reduce the risk of drug administration error that leads to loss of benefit

Potential risk of clinical significance	Summary of data/rationale for risk	Mitigation and monitoring strategy
Development of anti-drug antibodies	In the development program supporting the indication of treatment of severe hypoglycaemia in diabetes mellitus, in the studies completed in healthy participants or patients with T1D, a total of 498 participants have been exposed to dasiglucagon. A total of 4 anti-drug antibody positive participants were identified and none of these participants showed an observable clinical consequence from the anti-drug antibodies. Furthermore, there was no effect of anti-drug antibodies on PK, PD and safety in the few participants who had an anti-dasiglucagon antibody response. The anti-drug antibody incidence is <1% and the potential of dasiglucagon to induce anti-drug antibodies at clinically relevant doses in relation to rescue treatment of severe hypoglycaemia is considered to be low. No clinical consequences of anti-drug antibody formation have yet been identified in 28-day non-clinical studies or in short duration clinical studies, which indicate that the clinical immunogenicity risk is low. In the postmarketing setting, patients may use dasiglucagon more than once per year. The long-term immunogenicity risk of repeated dosing over years is currently unknown.	Monitoring of adverse events related to immunogenicity, including hypersensitivity reactions and potential lack of efficacy, will be covered by routine pharmacovigilance activities (Section 8.10).
Allergic reaction including Acute generalized hypersensitivity reactions	Allergic reactions have been reported with glucagon products. Mild or moderate allergic reactions may include symptoms of rash, fever, flu-like symptoms, nausea, headache and myalgia. Acute generalized hypersensitivity reactions are usually very rare but may include symptoms of flushing, sweating, dizziness, changes in blood pressure and difficulties in breathing.	Participants with known or suspected allergies to dasiglucagon, glucagon or related products will be excluded, and appropriate measures will be taken at the investigation site in case of any hypersensitivity reaction.
Acute hypertensive reaction	Glucagon products stimulates the release of catecholamines. In the presence of pheochromocytoma, glucagon products can cause the tumour to release large amounts of catecholamines, which will cause an acute hypertensive reaction. Therefore, dasiglucagon is contraindicated in patients with phaeochromocytoma.	Participant with known presence or history (exclusion criterion number 24; Section 5.2)of pheochromocytoma (i.e., adrenal gland tumour) will be excluded.

Potential risk of clinical significance	Summary of data/rationale for risk	Mitigation and monitoring strategy
Hypoglycaemia	In participants with insulinoma, administration of glucagon products may produce an initial increase in blood glucose. However, dasiglucagon administration may directly or indirectly (through an initial rise in blood glucose) stimulate exaggerated insulin release from an insulinoma and cause hypoglycaemia. A participant developing symptoms of hypoglycaemia after a dose of dasiglucagon should be given glucose intravenously. Dasiglucagon is effective in treating hypoglycaemia only if sufficient liver glycogen is present. To prevent relapse of the hypoglycaemia, oral carbohydrates should be given to restore liver glycogen, when the participant has responded to treatment. Participants in states of starvation, with adrenal insufficiency, chronic alcohol abuse or chronic hypoglycaemia may not have adequate levels of hepatic glycogen for dasiglucagon administration to be effective. Participants with these conditions should be treated with glucose.	To prevent relapse of the hypoglycaemia, oral carbohydrates should be given to restore liver glycogen, when the participant has responded to treatment. Participants with known presence or history of adrenal insufficiency, chronic hypoglycaemia and chronic alcohol abuse will be excluded.

Potential risk of clinical significance	Summary of data/rationale for risk	Mitigation and monitoring strategy
Hemodynamic events (hypo-hypertension and tachy-bradycardia)	Nonclinical evidence suggests that glucagon exerts positive inotropic and chronotropic effects on the heart, resulting in tachycardia and increased blood pressure. Across the clinical studies with dasiglucagon conducted in the severe hypoglycaemia program, hemodynamic events occurred at a low frequency in the dasiglucagon and glucagon groups in adults. Haemodynamic events reported for dasiglucagon included hypo- and hypertension, presyncope, bradycardia, palpitations and orthostatic intolerance. No events in the active treatment groups were reported in the paediatric study (ZP4207-17086). Most events in adults had an onset 1.5−3 hours after administration of active treatment, and most events were related to a decrease in blood pressure. In healthy participants, all haemodynamic events occurred in subjects who had received dasiglucagon or glucagon doses of 1 mg or above. In line with data in the literature for glucagon, the hemodynamic effects of dasiglucagon appear to be transient; in most cases, vital sign values normalized within 2 hours. None of the events was serious or severe. In all treatment groups (dasiglucagon, GlucaGen and placebo), small or no increases in pulse were observed. but this pattern was not consistently observed across the trials. At follow-up, the mean vital sign values had returned to the pre-dose or screening level.	The participants will be examined by experienced staff and their vital signs, such as blood pressure and heart rate, will be monitored continuously throughout the whole procedure to ensure their safety and well-being. After receiving the IMP, the participants will be observed for 300 minutes to see how they react to the IMP and to detect any adverse effects or complications.
Study intervention (glucagon)		
Please refer to the local approved label in Japan for information on benefits and risks related to glucagon.		
Study procedures		
Discomfort related to blood sampling procedures	Discomfort may occur around the site of blood sampling.	Proper techniques will be used by the experienced and trained site personnel to ensure the minimization of discomfort from the study procedures.
Discomfort in relation to i.m. or s.c. administration of drug	Discomfort may occur around the site of administration of drug.	Experienced and trained site personnel will ensure minimization of discomfort caused by study procedures.

Potential risk of clinical significance	Summary of data/rationale for risk	Mitigation and monitoring strategy
COVID-19 infection in relation to participation in study	Participants may be exposed to the risk of COVID-19 transmission and infection in relation to site visits if an outbreak is ongoing in the given country.	The study procedures will be performed by experienced and trained site personnel. The risk of COVID-19 transmission during site visits is low, but it may vary depending on the local outbreak situation. To reduce the risk further, the following measures have been taken: The site visits will be well-prepared. The physical contact between participants and site staff will be limited, and local infection prevention guidelines (e.g., masks, sanitizers, no aerosol-generating procedures, etc.) will be followed. The investigator may perform COVID-19 testing at any time during the study as required by local requirements or deemed necessary by the investigator or by sponsor. If a participant tests positive, they will be excluded from the study and referred to appropriate care.
Other		
Pregnancy and lactation	Dasiglucagon has not been tested in pregnant or breastfeeding women. There is no information on the presence of dasiglucagon in either human or animal milk, or the effects of the drug on the breastfed infant or milk production. However, dasiglucagon is a peptide and would be expected to be broken down to its constituent amino acids in the infant's digestive tract and is therefore unlikely to cause harm to an exposed infant. The hyperglycaemic effect of dasiglucagon was associated with embryo-foetal defects following repeated dosing in rabbits. Hyperglycaemia is a known teratogen in diabetic pregnancies. However, for the use as a rescue treatment in association with hypoglycaemia, the risk is considered minimal because of the short duration of the glucose increase.	Females who are pregnant, breast-feeding or intend to become pregnant or who are of childbearing potential and not using adequate contraceptive methods, will be excluded. During the study, participants will be discontinued in case of pregnancy or intention to become pregnant. Contraceptive guidance and collection of pregnancy information are described in Appendix 4 Section 10.4.

Potential risk of clinical significance	Summary of data/rationale for risk	Mitigation and monitoring strategy
	The risk of embryo-foetal defects as seen in rabbits dosed with dasiglucagon may be potentially relevant in relation to long-term use in participants. However, the findings in animals are considered a result of hyperglycaemia caused by relatively high dasiglucagon exposure levels, whereas the therapeutic aim of using dasiglucagon in a chronic treatment setting is to normalize blood glucose by counteracting insulin-induced hypoglycaemia.	

Abbreviations: IMP = investigational medicinal product

2.3.2 Benefit assessment

Dasiglucagon 0.6 mg is effective in increasing blood glucose levels in a clinically meaningful timeframe without treatment failures. In the postmarket setting, the clinical effect is expected to result in benefits including recovery from neuroglycopenia and prevention of complications of neurologic sequelae or death. The selected dose is appropriate for all indicated patients (age 6 and above) including those with high body mass index.[19] The formulation offers a potentially easier way to administer rescue product for emergency use and may reduce the likelihood of certain medication errors related to the reconstitution step, although this benefit was not directly assessed in the clinical development program. Dasiglucagon can be stored at room temperature up to 25°C for a single period of up to 12 months providing convenience to patients.[19] For specific storage conditions for dasiglucagon for this clinical study, please refer to the current version of the dasiglucagon IB. Benefits are expected to apply to people with T2D as well. The investigators will follow the current guidelines and best practices for T1D management in treating the participants.[19]

Investigators will ensure that participants are treated according to recommended T1D management.

Safety and efficacy will be monitored frequently according to SoA (Section 1.2), and acceptable glycaemic control will be reinforced at all times during the study.

All participants in this study will receive trial product and auxiliary supplies free of charge.

2.3.3 Overall benefit-risk conclusion

Overall, a highly favourable benefit-risk profile has previously been demonstrated for dasiglucagon for the proposed indication and under the labelled conditions of use.[19]

Necessary precautions have been implemented in the design and planned conduct of the study in order to minimise the risks and inconveniences of participation in the study. The safety profile for dasiglucagon generated from the clinical and non-clinical development programme has not revealed any safety issues that would prohibit administration of s.c. dasiglucagon single dose of 0.6 mg (refer to dasiglucagon Investigator's brochure for more information).

Please refer to the local approved label in Japan for information on benefits and risks related to glucagon.

Taking into account the measures taken to minimise risk and burden to participants in this study, the potential risks identified in association with dasiglucagon are justified by the anticipated benefits that may be offered to participants with T1D.

Likewise, the study design elements are not found to negatively impact the participants' rights (hereunder data privacy) and well-being.

3 Objectives and endpoints

Table 3-1 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
• 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	$AUC_{0\text{-}30min}$: 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
	C_{max}: Maximum observed plasma dasiglucagon concentration	From 0 to 5 hours after IMP injection	pmol/L
	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

Objectives	Endpoints		
• 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 hypoglycaemic episodes	From IMP injection (visit 2 day 1 and visit 3 day 1) until 12 hours after IMP injection	Number of episodes

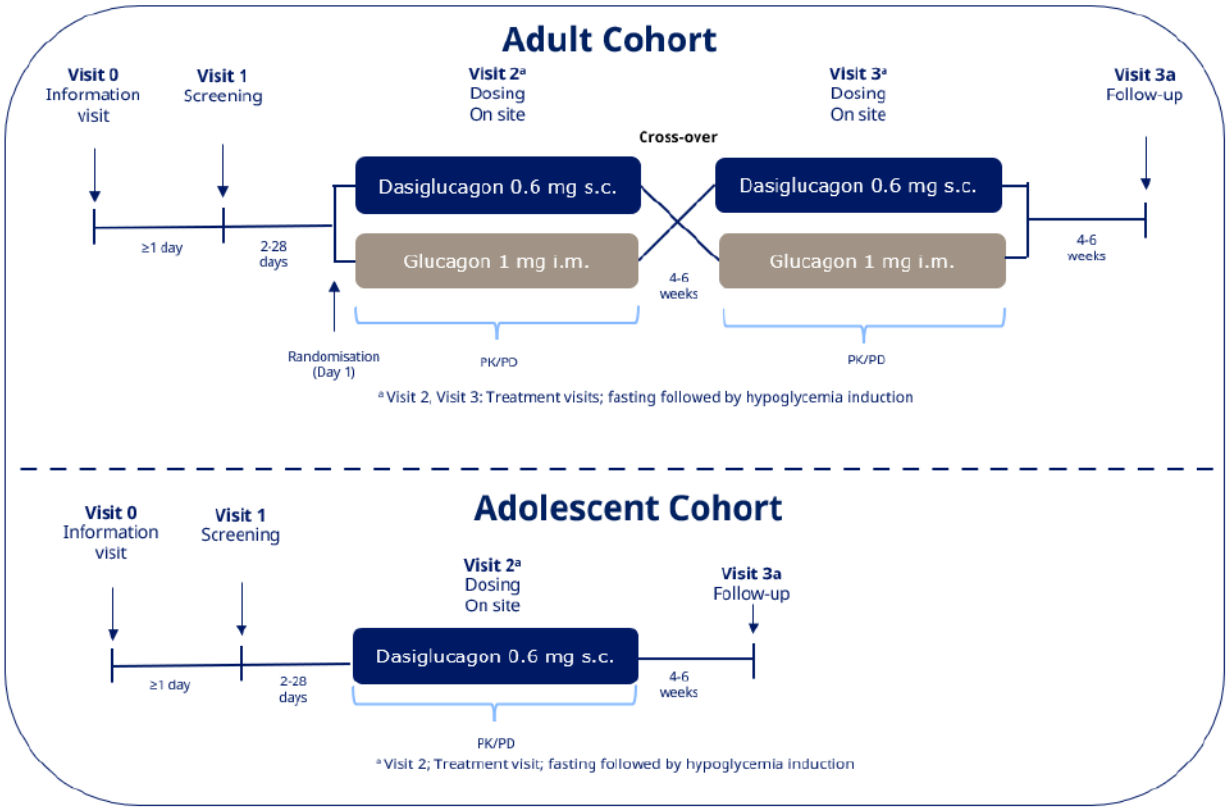
[a] applicable to Japanese adolescent cohort only.

4 Study design

4.1 Overall 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 4-1.

Figure 4-1 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).

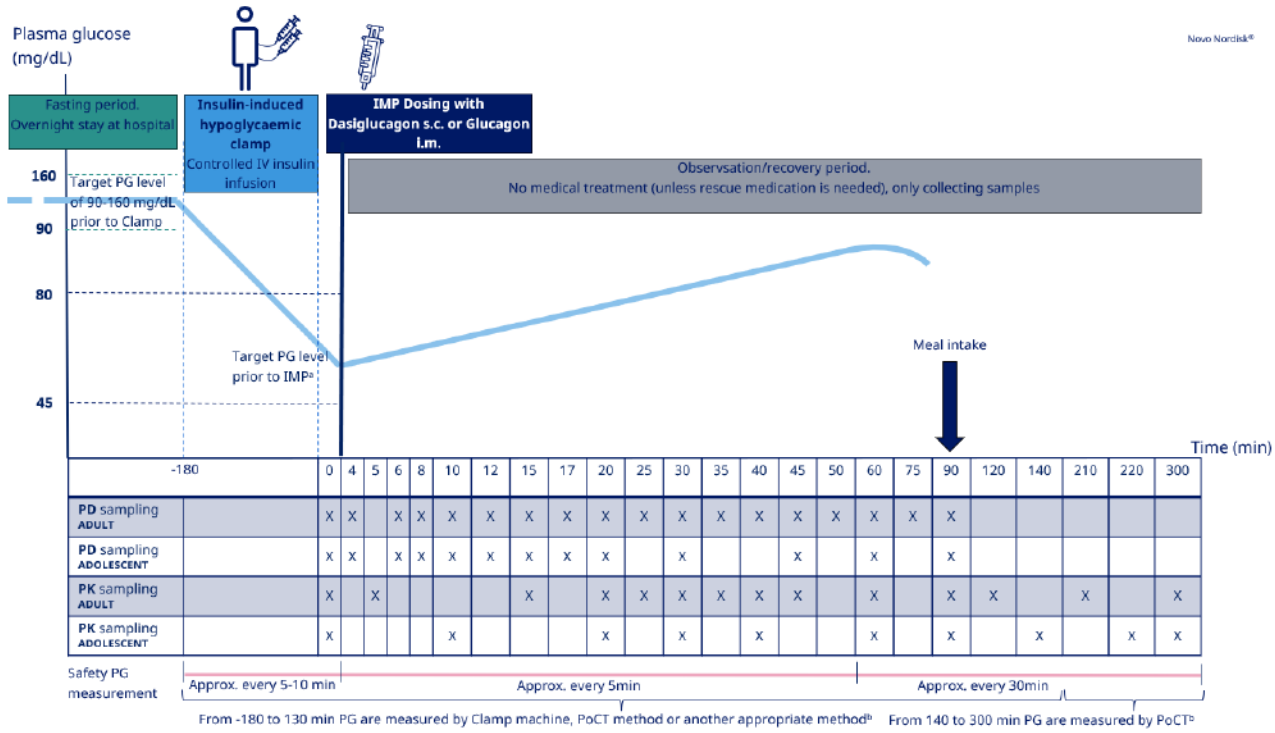
The study duration for adult participants is approximately 17 weeks and consists of 5 visits: An information visit (visit 0) to obtain the informed consent, a screening visit (visit 1) to determine the eligibility of the participants (2-28 days before visit 2), 2 dosing visits (visits 2 and 3), and a follow-up visit (end of study, visit 3a, 4-6 weeks after dosing) (Figure 4-1). 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 4-1).

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 4-2). Ninety (90) minutes after the IMP injection, the participants will be allowed to eat a light snack.

Figure 4-2 Clamp Design



PoCT = Point of care testing; PK = pharmacokinetic; PD = pharmacodynamic; IMP = investigational medicinal product; PG = plasma glucose; IV = intravenous; i.m. = intramuscular; i.v. = intravenous

[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.

4.2 Scientific rationale for study design

This is an open-label study. Dasiglucagon and glucagon are administered through different routes, s.c. and i.m., respectively, which makes it difficult to blind the study staff and participants. Open label study design was chosen as the most appropriate choice for the study given the circumstances (including limitations with blinded-packed products). Randomisation is used in order to avoid bias introduced through an association between treatment sequence allocation and participant characteristics. The cross-over design is chosen to enable the participants to act as their own control, thereby reducing the number of participants needed for a given power. All adult participants will receive both treatments. Novo Nordisk staff involved in the evaluation of PK, PD, and immunogenicity data will be blinded to treatment allocation until after DBL of this data.

A clamp setting is chosen in order to allow evaluation of the PK and PD response of the IMPs under well-defined conditions, thereby minimising the safety risk and allowing repeated blood sampling. After administration of the IMP dose, the participants will be monitored for 300 minutes thereby covering the anticipated total PD effect of dasiglucagon and glucagon.

A time period of 4 to 6 weeks between the in-house dosing visits is introduced to ensure wash-out from the previous treatment period and thereby to avoid any carry-over effect. The length of the wash-out period is considered sufficient based on the expected short half-life of dasiglucagon (28 minutes) and glucagon (45 minutes). Furthermore, the length has been chosen to perform an adequate safety evaluation.

To assess the clinical efficacy of s.c. dasiglucagon and i.m. glucagon, the primary endpoint and supportive secondary efficacy endpoints involve repeated measurements of PG concentration at several time points.

The Japanese adolescent cohort has been included for regulatory purpose to ensure that an investigation of the PD (efficacy), PK and safety after a single s.c. dose administration of 0.6 mg dasiglucagon during insulin-induced hypoglycaemia is performed in a small cohort of Japanese adolescents with T1D.

Both adult and adolescent participants will be followed for 4 to 6 weeks after last dosing in order to perform an adequate safety evaluation.

4.2.1 Patient input into design

Not applicable for this study.

4.3 Justification for dose

Dasiglucagon and glucagon will be administered as fixed doses independent of body weight because this is the intended therapeutic dosing regimen in the emergency treatment of hypoglycaemia. The selected dose of 1 mg/mL of glucagon is the approved dose for emergency treatment for hypoglycaemia in Japan when administered intramuscularly or intravenously. Based on clinical studies in adult participants, it has been demonstrated that 0.6 mg of dasiglucagon s.c.

results in an initial PD response (i.e., acute glucose mobilisation) comparable to 1 mg glucagon s.c. (Section 2.2).

Glucagon will be administrated i.m. to align with the approved route of administration of glucagon for emergency treatment of hypoglycaemia in Japan.

Dasiglucagon dose of 0.6 mg s.c. has been approved by FDA for the treatment of severe hypoglycaemia in paediatric and adult patients with diabetes aged 6 years and above.[20] Based on population PK model predictions and Pop PK/PD simulations (Study 20-046), the 0.6 mg dose is expected to be an appropriate dose to the Japanese adolescent cohort included in this study.[21]

4.4 End of study definition

The end of the study is defined as the date of the last visit of the last participant in the study.

A participant is considered to have completed the study if he/she has completed all visits of the study including the last scheduled procedure shown in the SoA (Section 1.2).

The primary endpoint is evaluated at visits 2 and 3 from 0 to 90 minutes after IMP injection. The primary completion date (PCD) is defined as the date of visit 3 on which the last participant in the clinical study has an assessment for the primary endpoint. If the last participant is withdrawn early, the PCD is considered the date when the last participant would have completed visit 3.

5 Study population

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

Pre-screening is defined as review of the participant's medical records, including handing out participant information, as well as database review. Any pre-screening activities must be documented on site by the investigator.

The inclusion and exclusion eligibility criteria as described below will be assessed at screening (visit 1).

5.1 Inclusion criteria

Participants are eligible to be included in the study only if all the following criteria apply:

1. Informed consent obtained before any study-related activities. Study-related activities are any procedures that are carried out as part of the study, including activities to determine suitability for the study.
2. **For adult participants:** Asian male or female.
   For adolescent participants: Japanese male or female
3. **For adult participants:** Age 18-75 years (both inclusive) at the time of signing the informed consent.
   For adolescent participants: Age 12-15 years (both inclusive) at the time of signing the informed consent.
4. Diagnosed with T1D >1 year before screening.
5. HbA_{1c} <10.0% (86 mmol/mol) as assessed by subcontracted laboratory by the site on the day of screening.
6. Treated with stable insulin treatment (based on the investigator's discretion preferably no more than a 10-unit daily variation in total daily insulin dose) 30 days prior to screening.
7. **For adult participants:** Current total daily insulin treatment between 0.2 and 1.2 (I)U/kg/day (both inclusive)
   For adolescent participants: Current total daily insulin treatment below 2.0 (I)U/kg/day
8. **For adult participants**: Body mass index (BMI) between 18.5 and 29.9 kg/m^2 (both inclusive).
   For adolescent participants: Body weight ≥ 33.4 kg
9. **For Japanese participants:** Japanese passport or equivalent
   For non-Japanese participants: Asian (non-Japanese) passport or equivalent.

5.2 Exclusion criteria

Participants are excluded from the study if any of the following criteria apply:

1. Known or suspected hypersensitivity to study intervention(s) or related products (glucagon or its derivatives).
2. Previous randomisation in this study.
3. Previous rescreening for this study.
4. Female who is pregnant, breast-feeding or intends to become pregnant or is of childbearing potential and not using adequate contraceptive method, as defined in Appendix 4 (Section 10.4).

5. Male of reproductive age who, or whose female partner(s), is not using an adequate contraceptive method, as defined in Appendix 4 (Section 10.4).
6. Current participation (i.e., received study intervention) in any interventional clinical study.
7. Exposure to an IMP within 30 days or 5 times the half-life of the IMP (if known), whichever is longer before screening.
8. Any condition, except for conditions associated with T1D, which in the investigator's opinion might jeopardise participant's safety or compliance with the protocol.
9. Clinically significant illness within 4 weeks before screening, as judged by the investigator.
10. Recurrent severe hypoglycaemia (more than 1 severe hypoglycaemic event within the last year prior to screening)[a] or hypoglycaemic unawareness as judged by the investigator.
11. Severe hypoglycaemia in the last month prior to screening.
12. Hospitalisation for diabetic ketoacidosis in the last month prior to screening.
13. Increased risk of thrombosis, e.g. personal or first-degree relative(s) history of deep vein thrombosis[a].
14. Supine blood pressure at screening outside the range of 90-139 mmHg for systolic or 50-89 mmHg for diastolic.
15. Participant is the investigator or other site staff or relative thereof directly involved in the conduct of the study.
16. Vulnerable participant (e.g. person kept in detention) who may have an increased likelihood of being wronged or of incurring additional harm.
17. Diagnostic test results positive for HIV-1 or HIV-2 infection.
18. Diagnostic test result positive of hepatitis B or hepatitis C infection.
19. Presence or history[a] of any clinically relevant respiratory, metabolic, renal, hepatic, gastrointestinal, endocrinological conditions (except conditions associated with diabetes mellitus).
20. Presence or history[a] of malignant neoplasms or in situ carcinomas (other than basal or squamous cell skin cancer, low-risk prostate cancer, or in-situ carcinomas of the cervix or carcinoma in situ/high grade prostatic intraepithelial neoplasia [PIN]) within 5 years before screening.
21. Presence or history[a] of cardiovascular disease including stable and unstable angina pectoris, myocardial infarction, transient ischaemic attack, stroke, cardiac decompensation, clinically significant arrhythmias or clinically significant conduction disorders.
22. History[a] of epilepsy or seizure disorder.
23. Current bleeding disorder, including use of anticoagulant treatment.
24. Known presence or history[a] of pheochromocytoma/paraganglioma (i.e., adrenal gland tumour/neuroendocrine neoplasm) or insulinoma (i.e., insulin-secreting pancreas tumour).
25. Clinically significant abnormal ECG at screening as evaluated by investigator.
26. The use of prescription or non-prescription medications known to cause QT prolongation.
27. Renal impairment with estimated Glomerular Filtration Rate (eGFR) < 60 mL/min/1.73 m^2 at screening.
28. Impaired liver function defined as ALT ≥ 2.5 times or Bilirubin > 1.5 times upper limit of normal at screening.
29. Mental incapacity, language barriers or unwillingness to comply with the requirements of the protocol, which may preclude adequate understanding or co-operation during the study as judged by the investigator.
30. Use of tobacco and nicotine products, defined as any of the below:
    - Smoking more than 5 cigarettes or the equivalent per day.

- Not able or willing to refrain from smoking and use of nicotine substitute products during the in-house period(s)

31. Abuse or intake of alcohol, defined as any of the below:
    - Known or suspected alcohol abuse within 1 year before screening
    - Positive alcohol test at screening
32. Abuse or intake of drugs, defined as any of the below:
    - Known or suspected drug or chemical substance abuse within 1 year before screening
    - Positive drug of abuse test at screening, except if caused by the use of allowed concomitant medication (see Section 6.8), as judged by the investigator.
33. Current treatment with systemically effective corticosteroids, monoamine oxidase (MAO) inhibitors, systemic beta-blockers, indomethacin, growth hormone, non-routine vitamins or herbal products. Occasional use of paracetamol, loxoprofen, and ibuprofen to treat acute pain is permitted until 24 hours prior to insulin infusion during dosing visits.
34. Surgery or trauma with significant blood loss within the last 2 months before screening.
35. Blood donation, plasma donation or blood draw, defined as any of the below:
    - In excess of 400 mL within 90 days before screening
    - In excess of 50 mL within 30 days before screening
36. Any disorder, unwillingness or inability which in the investigator's opinion, might jeopardise the participant's safety or compliance with the protocol.

[a] As declared by the participant or in the medical records.

5.3 Lifestyle considerations

Eligible participants must be asked to adhere to the below restrictions. The investigator will instruct the participants to follow the study procedures and requirements throughout the study. If any non-compliance is found, the investigator will remind the participants of the importance of following the instructions given.

5.3.1 Meals and dietary restrictions

Participants should:

- refrain from intake of poppy seeds 48 hours prior to initiation of the hypoglycaemic clamp procedure at visits 2 and 3.
- comply with fasting requirements according to the SoA (refer to Section 1.2). Fasting is defined as at least 8 hours overnight without food or liquids, except for water.
- receive meals and snacks at standard times as provided by the site and refrain from intake of other foods during the in-house visit (visits 2 and 3).
- not change lifestyle (such as eating or sleeping pattern) from screening until completion of the end of study visit.

5.3.2 Caffeine, alcohol and tobacco

Tobacco use is defined as smoking at least one cigarette or equivalent daily.

Participants should:

- Refrain from consumption of alcohol or smoking (including use of nicotine substitutes) 24 hours prior to and during the planned in-house visits (refer to Section 1.2)
- Not increase the alcohol consumption or the daily number of cigarettes smoked during the study

- Refrain from excessive consumption of methylxanthine-containing beverages (> 5 cups/day of coffee, tea, soda, mate, guarana, or chocolate) during dosing and 48 hours prior to:
  - initiation of the hypoglycaemic clamp procedure, and
  - end of study visit (visit 3a)

5.3.3 Activity

Participants should:

- Refrain from strenuous exercise 72 hours prior to and during the dosing and end of study visits
- Not change physical exercise pattern from screening until completion of the end of study visit, as judged by the investigator to interfere with study results

5.3.4 Other restrictions

Participants should:

- Adhere to the requirements regarding medication not permitted from screening to the end of study visit (Section 6.8)
- Refrain from donating blood or plasma from screening to the end of study visit
- Refrain from simultaneous participation in another clinical study from screening to the end of study visit
- Not take any illegal drugs or substance of abuse from screening to the end of study visit
- Follow guidelines on contraception from screening to the end of study visit (Appendix 4 [Section 10.4])

5.4 Screen failures

A screen failure occurs when a participant who consents to participate in the clinical study is not subsequently eligible for participation according to the inclusion/exclusion criteria. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants to meet requirements from regulatory authorities. Minimal information includes informed consent date, demography, screen failure details (end of study form), eligibility criteria, results of laboratory safety samples (if collected and analysed) and any serious adverse event (SAE).

Screening and rescreening activities must be stopped if it is confirmed that a participant cannot participate in the study for any reason, to avoid unnecessary procedures being performed.

Individuals who do not meet the criteria for participation in this study may have the failed eligibility criteria/parameter(s) repeated once (repeat procedure) or have all scheduled screening assessments repeated once (rescreening).

One repeat procedure or one rescreening is allowed in the following cases:

- if the individual's condition has changed (e.g., after recovering from an intercurrent illness including recent DKA or severe hypoglycaemia).
- if individual failed one or more eligibility criteria due to practical circumstances (e.g., time since last blood draw or time since participation in a previous study).

If the individual has failed one of the inclusion criteria or fulfilled one of the exclusion criteria related to laboratory parameters, re-sampling is not allowed. However, in case of technical issues (e.g., haemolysed or lost sample), re-sampling is allowed for the affected parameter(s).

Individuals who are rescreened are required to sign a new informed consent form and will be provided a new subject ID.

Date of first assessment of screening visit must be recorded in the electronic case report form (eCRF).

For the first screening, the end of study form must be completed, and the casebook must be signed off. Data from both screening visits will be transferred to the study database or eCRF, as applicable. The first screening participant ID will be noted in the eCRF at the rescreening along with new participant ID. An individual failing rescreening is considered a screen failure.

5.5 Dosing day exclusion criteria

If any of the dosing day exclusion criteria related to laboratory parameters were met, re-sampling is not allowed. However, in case of technical issues (e.g., haemolysed or lost sample), re-sampling is allowed for the affected parameter(s).

5.5.1 Dosing day exclusion criteria

Participants who meet one or more of the following dosing day exclusion criteria on a dosing visit (visits 2 and 3) will be excluded from the dosing visit or withdrawn from the study as specified below:

1. Presence of any medical condition that may confound the results of the study or pose an unacceptable risk to the participant by administering the IMP, as judged by the investigator.
2. Positive drug test, except if caused by the use of allowed concomitant medication (see Section 6.8), as judged by the investigator.
3. Smoking (including use of nicotine substitute products) within 48 h prior to initiation of the hypoglycaemic clamp procedure.
4. Consumption of alcohol within 48 h prior to initiation of the hypoglycaemic clamp procedure or positive alcohol breath test.
5. Consumption of coffee, tea, chocolate or beverages such as cola and energy drinks containing methylxanthine (caffeine, theophylline or theobromine) within 48 h prior to initiation of the hypoglycaemic clamp procedure.
6. Strenuous physical exercise (e.g. weightlifting, football, cycling, swimming or running) within 72 h prior to initiation of the hypoglycaemic clamp procedure, if judged by the investigator to interfere with study results.
7. Use of insulin degludec or insulin glargine U300 within 48 hours prior to dosing; or use of other long-acting insulins (e.g., insulin glargine U100 or insulin detemir) within 24 hours prior to dosing; or use of insulin Neutral Protamine Hagedorn (NPH) within 16 hours prior to dosing.
8. Use of any short acting (bolus) insulin within 6 hours prior to dosing, except NovoRapid.
9. Use of routine vitamins within 48 hours prior to dosing.
10. Use of non-prescription drugs, including herbal products and non-routine vitamins, within 14 days prior to initiation of the hypoglycaemic clamp procedure. Occasional use of paracetamol, loxoprofen, or ibuprofen to treat acute pain is permitted until 24 hours prior to initiation of the hypoglycaemic clamp procedure.
11. Use of systemically effective corticosteroids, MAO inhibitors, systemic beta-blockers, indomethacin, or growth hormone since screening.

12. Use of statins unless the use of these has been stable during the past 3 months prior to the day of screening as judged by the investigator.
13. Approved vaccinations less than 7 days prior to initiation of the hypoglycaemic clamp procedure.
14. Plasma glucose value <50 mg/dL (2.8 mmol/L) within the last 24 hours or PG value <60 mg/dL (3.3 mmol/L) within the last 5 hours before initiation of the hypoglycaemic clamp procedure.
15. Not fasting from 22:00 the evening before dosing, apart from water. Small amounts of carbohydrates (up to 20 g) to prevent hypoglycaemia will be allowed.
16. Body weight change >5% on visit 3 compared with visit 2.
    Dosing day exclusion criterion 16 is only relevant at visit 3 (only applicable for adults).
17. Use of oral antidiabetic medications within 5 times T-half prior to the initiation of the Clamp procedure

Participants who meet one or more of the dosing day exclusion criteria will be excluded from the dosing visit. Participants may be re-scheduled for dosing 1 to 9 days later and within the allowed time window. Each of the dosing visits (for adult participants: visit 2 and visit 3; for adolescents: visit 2) can be rescheduled once. Meeting any of the dosing day exclusion criteria on the rescheduled visit will lead to exclusion from the study. If the participant attends a new dosing visit, data from the first dosing visit should not be transferred to the study database but be kept at site as source data.

The first dosing visit (visit 2 day 1) may be rescheduled once within the allowed time window after screening (Section 1.2). The entire visit should be rescheduled including e.g. visit 2 day -1. All scheduled assessments on the original visit will be performed on the rescheduled visit (visit 2 day -1 and day 1) according to the SoA (Section 1.2). If not possible to reschedule within the allowed time window the participant may be rescreened once according to Section 5.4.

In case the participant does not attend a rescheduled first dosing visit (visit 2) within the allowed 28-days screening window, the participant must be invited to a new screening visit (see Section 5.4). Only if the participant is eligible at rescreening should the participant be invited to a new visit 2. If the participant is not eligible at rescreening, the participant is considered as a screen failure (see Section 5.4).

In case the participant does not attend the rescheduled visit 3 within the allowed 28 to 42 days visit window after visit 2 day 1, the participant will be withdrawn from the study.

Participants who meet dosing day exclusion criterion 2, 11, 12 (visits 2 and 3) or 16 (visit 3) will not be rescheduled but will instead be withdrawn from the study.

5.6 Stand-by participants

Not applicable for this study.

6 Study intervention(s) and concomitant therapy

Study intervention is defined as all pre-specified investigational and auxiliary medicinal products, medical devices, IVDs and other intervention(s) (e.g., surgical and behavioural), intended to be administered to the study participants or otherwise used in relation to treatment or study assessments during the study conduct according to the study protocol.

Investigational interventions are a subset of study interventions that are being tested or used as a control (e.g., placebo or active control).

The term 'trial product' is used in the protocol when referring to specific actions to be taken (e.g., actions related to shipping and storage), that only apply for these products. In this study, 'trial products' comprise IMPs, including active control. The head of the study site or the trial product storage manager assigned by the head of the study site (a pharmacist in principle) is responsible for control and accountability of the trial products.

6.1 Study intervention(s) administered

Table 6-1 provides an overview of the study interventions.

Table 6-1 Study interventions

Intervention name	Dasiglucagon	Glucagon[a]
Intervention type	IMP, test product	IMP, reference therapy
Investigational or non-investigational	Investigational intervention	Investigational intervention
Pharmaceutical form	Solution for injection	Powder for solution for injection. Reconstituted with SWFI[b]
Route of administration	Subcutaneous	Intramuscular
Trial product strength	1 mg/mL (0.6 mg/0.6 mL)	1 mg (reconstituted to 1 mg/mL at administration)
Dose and dose frequency	Administer a single fixed subcutaneous 0.6 mg dose of dasiglucagon.	Administer a single fixed intramuscular 1 mg dose of Glucagon.
Dosing instructions and administration	Administrated by a single use pre-filled syringe	Administrated by a syringe
Sourcing	Manufactured by Zealand Pharma A/S and supplied by Novo Nordisk A/S	Manufactured and supplied by Novo Nordisk A/S
Packaging and labelling	Labelled and packaged by Novo Nordisk A/S Labelled in accordance with EU CTR Annex VI,[22] local regulations and study requirements Dasiglucagon (0.6 mg) is provided in a single use 1.0 mL pre-filled syringe with 0.6 mL	Labelled and packaged by Novo Nordisk A/S Labelled in accordance with EU CTR Annex VI,[22] local regulations and study requirements Glucagon is provided in a vial as a powder for solution for injection.

Authorisation status in Japan	Dasiglucagon 0.6 mg (subcutaneous injection) is not approved in Japan.	Glucagon 1 mg (intramuscular injection) is approved in Japan for emergency treatment of hypoglycaemia.
[a] Commercial name for glucagon in Japan is Glucagon G Novo. The trial product will only be labelled as glucagon. [b] SWFI = Sterile Water for Injection, as per local pharmacopeia specification. SWFI is provided locally by the study site in glass or plastic containers for single use as per site standards for reconstitution of products.		

An appropriately trained and delegated site staff authorized to prepare the dose and administer the treatment in accordance with the randomisation will prepare the treatment required for each participant on each dosing day. The dose will be administered by a trained and delegated site staff. The content of the syringe must be checked for clarity and absence of bubbles.

Investigational medicinal products (IMP)

At dosing visit, the investigator or trained and delegated site staff will administer dasiglucagon via s.c. injection in the thigh and glucagon will be administered via i.m. injection in the thigh. After dose administration, syringes will be discarded.

Auxiliary medicinal products (AxMP)

AxMP(s) that will not be supplied by Novo Nordisk (including any rescue medications considered as concomitant and/or auxiliary medicinal products) will be reimbursed if required according to local regulations.

AxMP(s) used in the clinical study except for IMP(s) that will be provided by the study site include the following:

- Insulin analogue (insulin aspart "NovoRapid®", Novo Nordisk) 100 U/mL; drug to be used in the clinical study except for IMPs and to be used for IV infusion to reduce the participant's blood glucose (BG) during the clamp procedure. Please refer to NovoRapid® Injection 100U/mL local package insert for the latest information.
- Glucose solution (10%); to be used for IV infusion as rescue medication during clamp procedure.
- Sodium chloride isotonic (0.9%); to be used for IV injection/infusion during the clamp procedure.
- Heparin; to be used for the clamp device's PG measurement.
- All other equipment needed for running the glucose clamp procedure.

Other study supplies including non-investigational medical device(s)

Only needles and syringes approved by Novo Nordisk must be used for administration of trial product. Other study supplies comprise supplies other than trial products (including BG meter [ACCU-CHEK®Guide/Roche or FreeStyle Precision Neo/Abbott or equivalent]) will be provided by Novo Nordisk. All other equipment's needed for running the clamp procedure (including syringes and needles) will be provided by the study site.

6.2 Preparation, handling, storage and accountability

Only participants enrolled in the study may use study intervention and only delegated site staff may administer study intervention.
The investigator or delegated site staff will in this section be equal to the head of the study site or the trial product storage manager assigned by the head of the study site (a pharmacist in principle), who is responsible for control and accountability of the trial products. Instructions for use for the dasiglucagon pre-filled syringe and instructions for reconstitution of glucagon are provided in the Trial Materials Manual (TMM).

A sufficient amount of trial product (dasiglucagon and glucagon) will be supplied to the site for the planned number of participants. See TMM for further details on supplies of trial product to site.

The investigator or designee must confirm that appropriate temperature conditions have been maintained during transit for all trial products received and that any discrepancies are reported and resolved before use of the trial products.

All trial products must be stored in a secure, controlled and monitored (manual or automated) area in accordance with the labelled storage conditions with access limited to the investigator and authorised site staff.

The investigator must inform Novo Nordisk immediately if any trial product has been stored outside specified conditions. The trial product must not be dispensed to any participant before it has been evaluated and approved for further use by Novo Nordisk. Additional details regarding handling of temperature deviations can be found in the TMM.

The investigator or designee is responsible for trial product accountability and record maintenance (i.e., receipt, accountability and final disposition records). The dasiglucagon pre-filled syringe and used glucagon vials may be discarded after dose administration according to the local practice. Trial product accountability will be performed on the same day of dosing and empty trial product packaging will be used for the trial product accountability. Drug accountability must be performed at Kit ID/DUN level.

Destruction of trial products can be performed on an ongoing basis and will be done according to local procedures after accountability is finalised by the site and reconciled by the monitor.

All used (not discarded after dose administration), un-used, expired or damaged trial products (for technical complaint samples, see Appendix 5 (Section 10.5)) must be stored separately from non-allocated trial products. No temperature monitoring is required.

Non-allocated trial products, including expired or damaged products, must be accounted by the site and reconciled by the monitor, at the latest at closure of the site.

6.3 Measures to minimise bias: Randomisation and blinding

At screening, each participant will be assigned a unique 6-digit participant ID which will remain the same throughout the study. The site will be assigned a 3-digit number and all Participant ID's will start with the site number. All adult participants will be assigned a unique number (randomisation number) in ascending numerical order at the site on visit 2 day 1. The randomisation number

encodes the participant's assignment to one of the study arms/sequences of the study, according to the randomisation schedule generated before the study. Novo Nordisk will provide blinded and unblinded randomisation lists and the corresponding replacement lists before the study start. Randomisation will be handled by qualified blinded study site staff, using the blinded randomisation list.

This is an open-label study. However, Novo Nordisk staff involved in the evaluation of PK and Immunogenicity data will be blinded to treatment allocation until after DBL of the data. Furthermore, the specific trial product for a participant will be assigned using a randomisation list, to ensure that randomisation is done in a blinded fashion. For further instruction, see the TMM. Trial product will be packed open-labelled and will not be visually identical.

6.4 Study intervention compliance

Drug treatment compliance

Throughout the study, the investigator will remind the participants to follow the study procedures and requirements to encourage participant compliance.

When participants are dosed at the site, they will receive trial product directly from the investigator or designee, under medical supervision. The date and time of each dose administered at the site will be recorded in the source documents.

Site monitoring

The sponsor will monitor the study sites to ensure compliance with the protocol, GCP guidelines, and applicable regulations.

The sponsor will evaluate the adequacy of the site facilities, equipment, personnel, and procedures before the start of the study.

The sponsor will communicate with the site staff regularly and provide feedback and support as needed.

6.5 Dose modification

Not applicable for this study.

6.6 Continued access to study intervention after end of study

Dasiglucagon or glucagon will not be available to participants after end of the study.

6.7 Treatment of overdose

Any dose of dasiglucagon or glucagon greater than the planned dose will be considered an overdose. Novo Nordisk does not recommend specific treatment for an overdose.

Accidental overdose must be reported as a medication error. Intentional overdose must be reported as misuse and abuse, please refer to Section 8.5 and Appendix 3 (Section 10.3) for further details.

In the event of an overdose, the investigator should closely monitor the participant for overdose-related AEs/SAEs for at least 300 minutes, or as specified by Novo Nordisk for each participant, or longer if the investigator deems it clinically necessary.

Decisions regarding dose interruptions or modifications will be made by the investigator based on the clinical evaluation of the participant.

For more information on overdose, also consult the current version of the dasiglucagon IB and the local approved label of glucagon in Japan.

6.8 Concomitant therapy

Any medication or vaccine (including over-the-counter or prescription medicines, recreational drugs, vitamins and/or herbal supplements) that the participant is receiving at the time of the screening visit (visit 1) or receives until end of study (visit 3a) must be recorded as concomitant medication along with:

- Trade name or generic name
- Primary indication
- Dates of administration including start and stop dates
- Dose (unit) and frequency
- Route of administration

Changes in concomitant medication must be recorded at each visit. If a change is due to an AE, then this must be reported according to Section 8.5.

For prohibited medications and restricted use of medications, see the exclusion criteria (Section 5.2) and dosing day exclusion criteria (Section 5.5.1).

Concomitant diabetes medications include anti-diabetic medications taken by the participant during the study and from termination of the clamp and until discharge from site on the dosing visits (visit 2 and visit 3) and does not include NovoRapid and glucose solution used for IV infusion during the clamp procedure. Total daily dose and time of diabetes treatment administrations along with details mentioned earlier to be recorded for concomitant medications above must be recorded in the eCRF from:

- up to 48 hours prior to dosing, and
- from termination of the clamp until the participants are discharged from site on visit 2 and visit 3.

Sponsor should be contacted if there are any questions regarding concomitant or prior medication.

6.8.1 Rescue medicine

Based on the investigator's discretion, glucose solution (10%) may be used for IV infusion as rescue medication during the clamp procedure.

During insulin-induced hypoglycaemia, PG levels will be monitored closely at the site for safety reasons. Participants should receive posttreatment rescue glucose infusion to ameliorate persistent hypoglycaemia as follows, where t is time (in minutes) after IMP administration:

1. Glucose infusion should be initiated if a participant experiences severe alarming escalation of symptoms of hypoglycaemia (e.g., symptoms suggesting a change in consciousness) at any time during the study; glucose infusion should be initiated targeting a PG level >70 mg/dL (3.9 mmol/L).
2. **For Adults:** If PG is <45 mg/dL (2.5 mmol/L) between t=8 and t=44 minutes (both inclusive), rescue glucose infusion (approximately 1-2 mg/kg administered IV over about 5 seconds and glucose solution administration may be repeated) should be initiated to maintain PG between 45 mg/dL and 55 mg/dL (2.5-3.1 mmol/L). Pause glucose infusion if PG is >55 mg/dL (3.1 mmol/L).
   For Adolescents: If PG is <54 mg/dL (3.0 mmol/L) between t=8 and t=44 minutes (both inclusive), rescue glucose infusion (approximately 1-2 mg/kg administered IV over about 5 seconds and glucose solution administration may be repeated) should be initiated to maintain PG between 55 mg/dL and 70 mg/dL (3.1-3.9 mmol/L). Pause glucose infusion if PG is >70 mg/dL (3.9 mmol/L).
3. **For Adults:** If PG is <70 mg/dL (3.9 mmol/L) at t ≥45 minutes, rescue glucose infusion (approximately 2-3 mg/kg administered IV over about 5 seconds and glucose solution administration may be repeated) should be initiated to maintain PG between 70 mg/dL and 80 mg/dL (3.9-4.4 mmol/L). Pause glucose infusion if PG is >75 mg/dL (4.2 mmol/L).
   For Adolescents: If PG is <70 mg/dL (3.9 mmol/L) at t ≥45 minutes, rescue glucose infusion (approximately 2-3 mg/kg administered IV over about 5 seconds and glucose solution administration may be repeated) should be initiated to maintain PG between 70 mg/dL and 90 mg/dL (3.9-5.0 mmol/L). Pause glucose infusion if PG is >80 mg/dL (4.4 mmol/L).

The date and time of rescue medication administration as well as the name and dosage regimen of the rescue medication must be recorded in the eCRF.

7 Discontinuation of study intervention and participant discontinuation/withdrawal

Discontinuation of specific sites or of the study as a whole is detailed in Appendix 1 (Section 10.1.11).

7.1 Discontinuation of study intervention

Not applicable for this study.

7.1.1 Temporary discontinuation of study intervention

Not applicable for this study.

7.1.2 Rescue criteria

Participants with persistent and unacceptable hypoglycaemia should be offered rescue medication at the discretion of the investigator, and in accordance with the guidelines outlined in Section 6.8.1. Refer to Section 6.8.1 for description of rescue medication.

7.2 Participant discontinuation/withdrawal from the study

A participant may be withdrawn from the study at any time at the discretion of the investigator for safety reasons.

Efforts must be made to have participants attend and complete all scheduled visit procedures for end of study visit (visit 3a).

A participant must be discontinued from further study participation by the investigator if the following applies:

- If a protocol deviation or concurrent illness occurs, which, in the clinical judgement of the investigator, may invalidate the study by interfering pharmacokinetically or pharmacodynamically with the IMPs, the participant will be withdrawn by the investigator.
- AEs, which are considered unacceptable by the participant or the investigator.
- Pregnancy or intention of becoming pregnant during the study.
- The participant donates any blood during the study.
- Simultaneous use of an approved or non-approved IMP in another clinical study.

A participant who does not fulfil the eligibility (inclusion/exclusion) criteria must not be enrolled or randomised. Enrolment or randomisation in violation of any of the eligibility criteria is GCP non-compliance and must be reported to the sponsor without delay. This will be handled as an important protocol deviation, and the IRB/IEC and regulatory authorities must be notified according to local requirements. If there are no safety concerns, the study may be continued at the discretion of the investigator after agreement with the sponsor.

A participant may withdraw consent at any time at his/her own request or at the request of the participant's parent(s)/participant's LAR (applicable for adolescents).

If a participant wants to withdraw consent or the investigator wants to withdraw the participant prior to receipt of study intervention, he/she will not be asked to have any follow-up assessments

performed. All data should be kept at the site as source documentation, and all available data including laboratory results must be collected and entered into the eCRF and/or transferred to the study database. The end of study form must be completed.

If a participant wants to withdraw consent or the investigator wants to withdraw the participant after receipt of study intervention, the investigator must ask the participant if he/she is willing, as soon as possible, to have assessments performed according to end of study visit (visit 3a). See the SoA (Section 1.2) for data to be collected. The primary reason for discontinuation of study participation must be specified in the eCRF. The end of study form must be completed.

The withdrawal date is considered to be the participant's last visit, assessment or contact in the study, whichever is the latest.

Final trial product accountability must be performed even if the participant is not able to come to the site.

If the participant withdraws consent, Novo Nordisk may retain and continue to use any data collected up until the withdrawal date for the purpose of the study and scientific research. This will also apply in any country(ies) relying on explicit informed consent as legal basis for processing personal data. If a participant wants to exercise any of his/her rights, he/she must follow the description in the 'Agreement to take part' form (See Section 10.1.3).

If a participant withdraws from the study, he/she may request destruction of any samples taken and not tested; the investigator must document this in the medical record and notify the sponsor as soon as possible.

Although a participant is not obliged to give his/her reason(s) for withdrawing, the investigator must make a reasonable effort to ascertain the reason(s), while fully respecting the participant's rights. Where the reasons are obtained, the primary reason for withdrawal must be specified in the eCRF.

7.2.1 Replacement of participants

In case of discontinuation or withdrawal of randomised/enrolled participants, these may be replaced to ensure enough participants complete the study. Novo Nordisk will decide whether a participant should be replaced or not based on the number of remaining participants at the time of discontinuation or withdrawal. If discontinuation or withdrawal is caused by one or more unexpected AEs possibly or probably related to trial product, the participant will not be replaced.

Participants may be replaced as needed in order to have at least 30 completers in the adult cohort with a minimum of 24 Japanese participants and 4 participants in the adolescent cohort.

The replacement participant will be allocated to the same treatment sequence as the participant that he/she replaces, see the TMM for more information on treatment allocation.

Participant replacement must not exceed a maximum of 8 randomised participants for adult cohort and a maximum of 2 enrolled participants for the Japanese adolescent cohort. A randomisation number can be replaced twice.

7.3 Lost to follow-up

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

The following actions must be taken if a participant fails to return to the site for a required visit:

- The site must attempt to contact the participant or the participant's parent(s)/participant's LAR (applicable for adolescents) and reschedule the missed visit as soon as possible and counsel the participant or the participant's parent(s)/participant's LAR (applicable for adolescents) on the importance of maintaining the assigned visit schedule and ascertain whether the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee must make every effort to regain contact with the participant or the participant's parent(s)/participant's LAR (applicable for adolescents) (where possible, at least three telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts must be documented in the participant's source documents.
- Should the participant or the participant's parent(s)/participant's LAR (applicable for adolescents) continue to be unreachable, the participant will be considered to have withdrawn from the study with a primary reason of 'lost to follow-up' recorded in the end of study form.
- Site personnel, or an independent third party, will attempt to collect the vital status of the participant within legal and ethical boundaries for all participants enrolled or randomised, including those who did not get study intervention. Public sources may be searched for vital status information. If vital status is determined as deceased, this will be documented, and the participant will not be considered lost to follow-up. Sponsor personnel will not be involved in any attempts to collect vital status information.

The participant will be considered 'lost to follow-up' where the site is unable to contact the participant or the participant's parent(s)/participant's LAR (applicable for adolescents) until the last day of the visit window of the End of Study visit 3a.

8 Study assessments and procedures

The following sections describe the assessments and procedures, while their timing is summarised in the SoA (Section 1.2).

Informed consent must be obtained before any study-related activity, see Appendix 1 (Section 10.1.3).

Adherence to the study design requirements, including those specified in the SoA (Section 1.2), is essential and required for study conduct.

Review of diaries, ECG, laboratory reports, etc., must be documented in the source documents or the participant's medical record. If clarification of entries or discrepancies is needed in the diary, the participant must be questioned and a conclusion made in the participant's source documents. Care must be taken not to bias the participant response. In case of detection of an AE, this should be reported if applicable according to the protocol.

The maximum amount of blood collected from each participant over the duration of the study will not exceed 462 mL for adults and 56 mL for adolescents, inclusive of the blood volume required for technical disposal.

The maximum volume of blood collected on any visit is 225 mL for adults and 26.7 mL for adolescents. These values are within the recommended scope provided by the EU.[23] The blood volume will be taken per Table 8-1 and Table 8-2 for each sample at each visit.

Table 8-1 Estimated blood volume for each visit in adults

Sample type	Visit 1 (mL)	Visit 2 (mL)	Visit 3 (mL)	Visit 3a (mL)
Biochemistry/ Lipids including eGFR	3	-	-	3
Haematology	2	-	-	2
Coagulation	1.8	-	-	-
HbA1c	2	-	-	-
Pregnancy[a]	3	-	-	-
Serology	6	-	-	-
Anti-drug antibody	-	4	4	4
PK	-	56 (or 70)	70 (or 56)	-
PD	-	36	36	-
Onsite-PG	-	60	60	-
Clamp procedure	-	32	32	-
Insulin adjustment	-	2	2	-
Number of samplings	1	23	23	1
Sum for each visit	17.8	190	204	9
Total	420.8			

Abbreviations: eGFR; estimated Glomerular Filtration Rate, HbA1c= Haemoglobin A1c; Onsite-PG=Onsite monitoring of plasma glucose; PD=Pharmacodynamics; PK=Pharmacokinetics.

[a] Only in women of childbearing potential (WOCBP), a highly sensitive serum pregnancy test should be performed.

Table 8-2 Estimated blood volume for each visit in adolescents

Sample type	Visit 1 (mL)	Visit 2 (mL)	Visit 3a (mL)
Biochemistry/ Lipids including eGFR	3	-	3
Haematology	2	-	2
Coagulation	1.8	-	-
HbA1c	2	-	-
Pregnancy[a]	3	-	-
Serology	6	-	-
Anti-drug antibody	-	4	4
PK	-	5	-
PD	-	13	-
Onsite-PG	-	2.8	-
Insulin adjustment		1	
Number of samplings	1	18	1
Sum for each visit	17.8	25.8	9
Total	52.6		

Abbreviations: eGFR; estimated Glomerular Filtration Rate, HbA1c=Haemoglobin A1c; Onsite-PG=Onsite monitoring of plasma glucose; PD=Pharmacodynamics; PK=Pharmacokinetics

[a] Only in women of childbearing potential (WOCBP), a highly sensitive serum pregnancy test should be performed.

Repeat samples may be taken for technical issues and unscheduled samples or assessments may be taken for safety reasons. Please refer to Appendix 2 (Section 10.2) for further details on laboratory samples.

8.1 Screening/baseline assessments and procedures

All screening evaluations must be completed and reviewed to confirm that potential participants meet all inclusion criteria and none of the exclusion criteria.

The investigator will maintain a screening log to record details of all participants screened and rescreened and to confirm eligibility or record reason for screen failure, as applicable.

At screening, participants will be provided with a card stating that they are participating in a study and giving contact details of relevant site staff that can be contacted in case of emergency.

The term **medical history** covers medical events that the participant prior to the time point from which AEs are collected.

A **concomitant illness** is any illness that is already present at the time point from which AEs are collected or found as a result of a screening procedure or other study procedures performed before exposure to investigational intervention.

In case of an abnormal and clinically significant finding fulfilling the definition of medical history or concomitant illness, the investigator must record the finding on the medical history/concomitant illness form in the eCRF.

8.2 Dose adjustment guidelines

Forty-eight hours before dosing

The participant's current insulin therapy will be washed out up to visit 2 (for all participants) and visit 3 (adults only): 48 hours prior to dosing and during the dosing visit, treatment with insulin degludec and insulin glargine U300 will not be allowed; 24 hours prior to dosing and during the dosing visit, treatment with other long-acting insulins (e.g., insulin glargine U100 or insulin detemir) is not allowed; 16 hours prior to dosing and during the dosing visit, treatment with insulin NPH is not allowed; 6 hours prior to dosing and during the dosing visit, treatment with any short-acting (bolus) insulin is not allowed. The basal rate of insulin pumps (continuous s.c. insulin infusion) will be discontinued on the morning of the dosing day (if using NovoRapid) OR at least 6 hours prior to dosing (if using other insulins than NovoRapid). Refer to Section 5.5 for dosing day exclusion criteria.

Twenty-four hours before dosing

On the day prior to dosing (visit 2 day -1 and visit 3 day -1), the participants will need to attend the trial site and will be required to stay on site overnight. To target a glucose level of approximately 90 to 160 mg/dL (5.0-8.9 mmol/L) the following morning, participants may be administered NovoRapid and/or glucose solution (10%) at the investigator's discretion by IV infusion. On the morning of the dosing day (visits 2 and 3 day 1), participants will be required to be in a fasting condition, defined as having consumed only water since 22:00 the night before. Participants will be allowed to consume up to 20 g of carbohydrates to prevent hypoglycaemia, targeting a starting PG approximately 90 to 160 mg/dL (5.0-8.9 mmol/L) with minor deviations accepted based on the investigator's discretion (refer to Section 5.5 for dosing day exclusion criteria).

For adults, the STG-55 may be started at the investigator's discretion at visit 2 day-1 and visit 3 day -1 for safety monitoring of PG levels prior to the initiation of hypoglycaemic procedures.

8.2.1 Hypoglycaemic clamp procedure

The hypoglycaemic glucose clamp will be performed by means of a STG-55 (glucose-controlled insulin infusion system: Artificial Endocrine Pancreas, Nikkiso Co., Ltd., Japan) for adults and a basal syringe pump for adolescents. The STG-55 performs continuous PG measurements during the entire procedure.

The participant will remain fasting and will stay in a supine or semi-supine position during the entire glucose clamp. The participant will be allowed to drink water ad libitum during the glucose clamp experiment but will otherwise have no oral intake (Section 8.2).

The following materials will be provided by the study site to be used during the clamp procedure (refer to Section 6.1):

- Insulin analogue (NovoRapid®) 100 Unit/mL to be used for IV infusion to stabilise the participant's PG level in the start of the hypoglycaemic glucose clamp procedure.

- Syringes and needles
- Glucose solution (10%) for IV infusion during the clamp
- Sodium chloride isotonic (0.9%) for IV injection/infusion
- Heparin to be used for the glucose clamp device's PG measurement
- All other equipment needed for running the glucose clamp procedure

Invasive procedures (for Adults)

For sampling of arterialised venous blood for the STG-55's PG concentration measurements, a hand or forearm vein of one arm will be cannulated. This hand will remain under a heating pad throughout the clamp procedure. The heating of the hand results in an arterialisation of the venous blood due to a reflective opening of arterio-venous shunts.[24, 25] A low dose heparin solution (100 U heparin/mL [10,000 U heparin/100 mL saline]) will be used to prevent blood clotting in the STG-55's double lumen catheter. The heparin solution will be taken up together with blood used for the STG-55's PG measurement in the other lumen of the catheter and is aimed to prevent blood clotting in the system.

A catheter will be inserted into the same arm for obtaining blood samples. This catheter will be kept open by use of a 0.9% saline.

For controlled infusion by the STG-55's, a forearm vein of the contralateral arm will be cannulated with a 20-gauge polyetrafluoroethylene catheter.

Dosing day – hypoglycaemic clamp procedure

The STG-55 is used for the clamp procedure including the PG measurements for safety for adults. A basal syringe pump is used for the clamp procedure for adolescents.

An infusion catheter will be inserted into each arm for the hypoglycaemic clamp procedure, with the glucose infusion in one arm and the insulin infusion in the opposite arm preferably, but the same infusion catheter can also be used. If IV NovoRapid has been administered during the night, the same infusion catheter can be used. Another IV catheter, separated from infusion catheter(s), for blood sampling will be placed before trial product administration. The hand of this arm will be warmed (55ºC-65ºC) to arterialize venous blood. If there are issues with blood sampling from, e.g. the metacarpal vein for the purpose of glucose measurements, the investigator may use a new and more proximal IV access.

In the morning (at about 08:00) on the dosing day, IV insulin infusion for the hypoglycaemic induction should be started. The start time and end time of IV insulin infusion will be recorded in eCRF.

Hypoglycaemia will be gradually induced by an IV infusion of fast-acting NovoRapid (for adults: 40 U [100 U/mL] in 98 mL saline and 2 mL of participant's blood or plasma; for adolescents: 15 U [100 U/mL] in 49 mL saline and 1 mL of participant's blood or plasma), initially at 150% of the participant's usual basal rate and can be increased or decreased over a range of 0% to 200% or more as judged necessary by the investigator, targeting a PG level of 55 mg/dL (3.1 mmol/L). The basal rate may be calculated based on the basal infusion rate with insulin pump or an average basal daily dose in multiple daily injection (MDI). If total daily dose of usual basal insulin is 24 (U), the usual

basal rate is 1.0 (U/hour) and the initial recommended rate of insulin for hypoglycaemic clamp procedure is 1.5 (U/hour) (150% of usual basal rate).

After the start of the IV insulin infusion, PG should be checked approximately every 10 minutes while PG is above 110 mg/dL (6.1 mmol/L) and approximately every 5 minutes once PG is at or below 110 mg/dL (6.1 mmol/L).

Once the glucose concentration declines to <60 mg/dL (3.3 mmol/L) for adults or <80 mg/dL (4.4 mmol/L) for adolescents, the insulin infusion will be stopped. Five minutes later, the PG concentration will be confirmed using the PoCT method or another appropriate method and blood samples for baseline assessment of PG (for the PD measurements) and dasiglucagon/glucagon PK will be collected. The samples are the baseline samples and should be collected within 2 minutes before trial product administration.

- **For Adults:** If PG is ≥45 mg/dL and <60 mg/dL (2.5-3.3 mmol/L), trial product will be administered, defining time, t=0. The trial product will be administered in the thigh.
  For Adolescents: If PG is ≥54 mg/dL and <80 mg/dL (3.0-4.4 mmol/L), trial product will be administered, defining time, t=0. The trial product will be administered in the thigh.
- If PG is <45 mg/dL (2.5 mmol/L) for adults or <54 mg/dL (3.0 mmol/L) for adolescents, IV glucose solution will be administered to raise PG to within the target range of 45 to 60 mg/dL (2.5-3.3 mmol/L) for adults or 54 to 80 mg/dL (3.0-4.4 mmol/L) for adolescents. The run-in period will be adequately extended (at least 30 minutes) until the above target is achieved and new baseline samples for PG will be collected. Glucose should not be infused within 10 minutes before trial product administration. If PG is not within target range after the second attempt, the participant should be rescheduled for a new dosing visit, 1 to 9 days later and within the allowed time window.

The trial product could be administered as soon as PG is ≥45 mg/dL and <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. Administration of trial product should not occur later than 12:00 (noon).

During insulin-induced hypoglycaemia, plasma samples will be drawn, and PG levels will be monitored closely at site. For participant safety, PG will continue to be checked every 5 minutes after dosing until the 60-minute time point. Hereafter, PG should be checked approximately every 30 minutes until 300 minutes (5 hours) after dosing.

Drinking of water is allowed ad libitum during the entire procedure. Participants should remain in bed or sit quietly in a chair until completion of the test procedure 300 minutes after dosing (bathroom visits will be allowed). The time of trial product administration will be recorded. At the time when the insulin infusion is stopped, the total insulin dose which was required to induce hypoglycaemia will be recorded. When the 90-minute blood sampling for PG has been collected, the participants will be allowed to eat a light snack and resume their usual diabetes treatment or NovoRapid. The investigator must provide information to the participants on how to resume their usual diabetes treatment.

The participant may be released from the clinical site if the investigator does not have any safety concerns based on the last safety PG value at 300-minute blood sampling timepoint and the general

condition of the participant. However, at the discretion of the investigator or on request of the participant, the participant may stay at the clinical site for a longer period.

8.3 Efficacy assessments

Planned time points for all efficacy assessments are provided in the SoA (Section 1.2).

8.3.1 Efficacy-related assessments

Plasma glucose is the parameter that will be measured to assess all efficacy endpoints. To this end, participants will be brought into a hypoglycaemic state by applying a standard hypoglycaemic clamp procedure, which requires 48 hours' preparation before the actual clamp is started. The participant's current insulin therapy will be washed out as described below and in Section 5.5. For participants using multiple daily injections, the date, time, and the dose of the last basal insulin and the last short-acting insulin administration prior to dosing will be captured. For participants using an insulin pump, the time of discontinuation of the basal rate will be captured.

For the efficacy assessments, samples for PG will be collected as described in Section 1.2, Table 1-1, and Table 1-2.

8.3.2 Clinical efficacy or Pharmacodynamic laboratory assessments

All protocol-required laboratory assessments, as defined in Appendix 2 (Section 10.2), must be conducted in accordance with the SoA (Section 1.2) and the laboratory manual.

8.3.3 Plasma glucose measurements

When using BG meters, the measurement is performed with capillary blood calibrated to plasma equivalent glucose values, i.e., the measurement is performed on blood while the value is reported as plasma; therefore 'PG' or 'self-measured plasma glucose' (SMPG) are the terms to use as descriptor for the value.

The BG meters use test strips calibrated to plasma values. Therefore, all measurements performed with capillary blood are automatically calibrated to plasma equivalent glucose values, which will be shown on the display.

The BG meter provided by Novo Nordisk should be used for the measurements required in the protocol.

For Adults: For safety PG measurements during the clamp procedure, the STG-55 will be used as the primary method. PoCT (Antsense Duo, Horiba Ltd., Kyoto, Japan) will be used for safety PG measurements and will also be used during the clamp procedure, as required, to confirm PG concentration (Table 1-1) and will be documented in the source data.

For Adolescents: For safety PG measurements, PoCT (Antsense Duo, Horiba Ltd., Kyoto, Japan) will be used (Table 1-2) and will be documented in the source data. If use of PoCT is not feasible, BG meter will be used for safety PG measurements based on the investigator's discretion.

Participants should be instructed how to use the BG meter at visit 2 day -1 and should be instructed how to record the results of the SMPG values in the diaries and will be repeated at visit 3 day -1

(only for adult participants). The record of each SMPG value should include date, time and value. All data from the diary collected in between the visits including episodes of hypoglycaemia and hyperglycaemia must be reviewed on visit 2 day 1, visit 3 day -1, visit 3 day 1, and visit 3a and transcribed into the eCRF during or following the contact. If obtained via phone, and a discrepancy is later detected, the values in the eCRF must be corrected.

Occasional review by the investigator of the BG meter values stored in the memory of the BG meter and correct reporting of these in the diary is advised in order to ensure adequacy of the data reported in the study database.

8.4 Safety assessments

Planned time points for all safety assessments are provided in the SoA (Section 1.2), Table 1-1 and Table 1-2.

8.4.1 Physical examinations

A physical examination will include assessments of the cardiovascular, respiratory, gastrointestinal, neurological system and skin, including:

- General appearance
- Head, ears, eyes, nose, throat and neck
- Cardiovascular system
- Abdomen
- Skin
- Respiratory system
- Gastrointestinal including mouth
- Central and peripheral nervous system
- Musculoskeletal system
- Thyroid gland
- Lymph node palpation (on the neck, armpits and groin)

In the eCRF, only data on whether the physical examination was performed will be collected (Yes/No). A physical examination with an abnormal and clinically significant finding must be recorded as an AE, see Section 8.5 and Appendix 3 (Section 10.3).

Investigators should pay special attention to clinical signs related to previous serious illnesses.

8.4.2 Body measurements

Body measurements (e.g., height and weight) will also be measured and recorded as specified in the SoA (Section 1.2) and include:

- Body weight must be measured during the following conditions: on an empty bladder, without shoes, and only wearing light clothing. Body weight should be measured on a digital scale and recorded in kilograms (one decimal) using the same calibrated scale (when possible) throughout the study.
- Height should be measured standing without shoes (in meters with two decimals). Height is only needed at screening.

- BMI (only for adults) will be calculated from screening data as body weight (in kg with one decimal) divided by height (in meters with two decimals) squared. BMI should be recorded in kilograms per meter (kg/m^2) using one decimal and it will be kept in the participant's source documents and will be recorded in the eCRF.

8.4.3 Vital signs

Axillary temperature (Celsius), pulse rate, respiratory rate, as well as systolic and diastolic blood pressure will be assessed.

Vital sign assessment will be preceded by at least 5 minutes of rest for the participant in a quiet setting without distractions (e.g., no use of television, cell phones).

Blood pressure and pulse rate measurements will be assessed in supine position with a completely automated device. Manual techniques must be used only if an automated device is not available.

For both adult and adolescent participants, blood pressure and pulse rate are collected according to SoA (Section 1.2). For adolescent participants, age-appropriate equipment will be used.

Blood pressure will consist of 3 systolic and diastolic blood pressure measurements with intervals of at least 1-2 minutes. An additional fourth blood pressure measurement must be performed if the first two readings on systolic or diastolic blood pressure differ by >10 mmHg. No more than four measurements will be performed. Systolic blood pressure is to be recorded as the mean of the last 2 systolic blood pressure readings, and diastolic blood pressure as the mean of the last 2 diastolic blood pressure readings. Only the last 2 systolic and last 2 diastolic blood pressure readings must be recorded on the eCRF. The mean value of the blood pressure must be used to assess eligibility at screening.

Pulse rate will be measured in connection to the blood pressure measurements. Record the pulse rate for the last 2 measurements including the mean in the eCRF.

Any clinically significant findings must be reported as an AE.

8.4.4 Eye examination

Not applicable for this study.

8.4.5 Electrocardiograms

For both adult and adolescent participants, 12-lead ECG will be obtained as outlined in the SoA (Section 1.2), Table 1-1, and Table 1-2 using an ECG machine that automatically calculates the heart rate and measures PR, QRS, and QT_c intervals. For adolescent participants, age-appropriate equipment will be used.

Following ECG parameters will be collected:

- Time of evaluation
- Heart rate (beats/minute)
- PR interval (msec)
- QRS duration (msec)

- QTcF interval (msec)
- Overall interpretation of ECG

The investigator or designee will perform an overall evaluation of the 12-lead ECG for safety purposes and the recording will be reported as either "normal" or "abnormal". If abnormal, a comment must be given together with an assessment of clinical significance (Yes/No). The original 12-lead ECG recording must be signed and dated by the investigator or designee and be placed in the participant's source documentation. Clinically significant findings at screening (visit 1) and until dosing on visit 2 day 1 should be recorded as concomitant illness. Any clinically significant finding after dosing must be recorded as an AE (Section 8.5).

8.4.6 Clinical safety laboratory assessments

All protocol-required laboratory assessments, as defined in Appendix 2 (Section 10.2), must be conducted in accordance with the SoA (Section 1.2) and the laboratory manual. All safety assessments will be recorded on eCRF.

8.4.7 Pregnancy testing

At screening, information on childbearing potential is to be collected in the eCRF. Women of childbearing potential (WOCBP) will only be included after a negative, highly sensitive serum pregnancy test (refer to Appendix 2 (Section 10.2)).

Pregnancy testing (urine hCG pregnancy test) will be performed according to the SoA (Section 1.2) and whenever a menstruation is missed or when pregnancy is otherwise suspected.

For participants in the adolescent cohort: Pregnancy testing should be performed as soon as possible after a first menstrual period regardless of affirmation on sexual activity. Follow local requirements (if applicable) regarding the rights/safety of the paediatric participant with respect to revealing a positive pregnancy test to parents(s)/LAR.

8.5 Adverse events and other safety reporting

All AEs, irrespective of seriousness and /or severity, should be reported.

The investigator is responsible for detecting, documenting, recording and following up on events that meet the definition of an AE or SAE.

The definitions of AEs and SAEs can be found in Appendix 3 (Section 10.3), along with a description of AEs requiring additional data collection.

Some AEs require additional data collection on a specific event form. The relevant event(s) are listed below in Table 8-3, together with other events requiring collection of additional information.

Table 8-3 AEs requiring additional data collection and other events requiring collection of additional information

Event type	AE requiring additional data collection	Other events requiring collection of additional information
Medication error	X	

Event type	AE requiring additional data collection	Other events requiring collection of additional information
Misuse and abuse	X	
Hypoglycaemic episodes		X
Hyperglycaemic episodes		X

Definitions and reporting timelines for the events mentioned in the above table can be found in Appendix 3 (Section 10.3) and Appendix 6 (Section 10.6) for hypoglycaemic episodes and Appendix 7 (Section 10.7) for hyperglycaemic episodes. For more information on Hypoglycaemic episodes and Hyperglycaemic episodes see Section 8.5.7.

8.5.1 Time period and frequency for collecting AE information

All AEs and SAEs must be collected from the first administration of investigational intervention and until the end of study visit in accordance with the SoA (Section 1.2) or whenever, within the above time period, the site becomes aware of an AE or SAE.

Conditions present prior to the timepoint from which AEs are collected and anticipated day-to-day fluctuations of these conditions, including those conditions identified during screening or identified during other study-related procedures performed before exposure to investigational intervention, will be recorded as medical history/concomitant illness.

AE and SAE reporting timelines can be found in Appendix 3 (Section 10.3). All SAEs must be recorded and reported to Novo Nordisk without undue delay but not later than within 24 hours of obtaining knowledge of the events. Similarly, the investigator must submit any updated data on SAEs to Novo Nordisk without undue delay, but not later than within 24 hours of obtaining knowledge of the information.

Investigators are not obligated to actively seek for AEs or SAEs in former study participants. However, if the investigator learns of any SAE with a suspected causal relationship to the IMP or to study participation, occurring after a participant has discontinued/completed the study, the investigator must notify Novo Nordisk without undue delay.

8.5.2 Method of detecting AEs

The method of recording, evaluating and assessing causality of AEs and SAEs and the procedures for completing and transmitting reports on SAEs are provided in Appendix 3 (Section 10.3).

Care should be taken not to introduce bias when detecting AEs and/or SAEs. Open-ended and non-leading verbal questioning of the participant is the preferred method to inquire about events.

8.5.3 Follow-up of AEs

After the initial AE report, the investigator is required to proactively follow each participant at subsequent visits/contacts. All SAEs will be followed until the outcome of the event is final or until the participant is lost to follow-up as described in Section 7.3. Further information on follow-up and outcome of AEs is given in Appendix 3 (Section 10.3).

8.5.4 Regulatory reporting requirements for SAEs

Prompt notification by the investigator to Novo Nordisk or designee of an SAE is essential so that legal obligations and ethical responsibilities towards the safety of participants and the safety of an investigational intervention are met.

Novo Nordisk has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a study intervention under clinical investigation. Novo Nordisk will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority, IRB/IEC and investigators. This also includes suspected unexpected serious adverse reactions (SUSARs).

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

8.5.5 Pregnancy

Details of pregnancies, occurring between first exposure to IMP and until the end of study visit (visit 3a) in female participants. For details regarding collection and reporting of pregnancy information, please refer to Appendix 4 (Section 10.4).

8.5.6 Cardiovascular and death events

Not applicable for this study.

8.5.7 Disease-related events and/or disease-related outcomes not qualifying as AEs or SAEs

The following disease-related events (DREs) are common in participants with T1D and can be serious/life threatening:

- Hypoglycaemic episodes
- Hyperglycaemic episodes

Hypoglycaemia

Because hypoglycaemic episodes are typically associated with the disease under study, non-serious hypoglycaemic episodes will not be reported according to the standard process for reporting of AEs, even though the episodes meet the definition of an AE. All hypoglycaemic episodes must be recorded on a hypoglycaemic episode form. If the hypoglycaemic episode fulfils the criteria for an SAE, then in addition to the hypoglycaemic episode form, an AE form and a safety information form must be filled in. One AE form and safety information form can cover several hypoglycaemic episode forms, if the participant has not recovered between the episodes, see Appendix 3 (Section 10.3.3).

Definitions (including definition of hypoglycaemic episodes from initiation of hypoglycaemic clamp procedure until 45 min after IMP administration), classification and reporting requirements for hypoglycaemic episodes are described in Appendix 6 (Section 10.6).

Hyperglycaemia

Because hyperglycaemic episodes are typically associated with the disease under study, non-serious hyperglycaemic episodes will not be reported according to the standard process for reporting of AEs, even though the episodes meet the definition of an AE.

Definitions, classification and reporting requirements for hyperglycaemic episodes are described in Appendix 7 (Section 10.7).

Non-serious hyperglycaemia must be reported on a hyperglycaemic episode eCRF form.

If the hyperglycaemic episode fulfils the criteria for an SAE, then in addition to the above, an AE form and a safety information form must also be filled in.

8.5.8 Adverse event of special interest

Not applicable for this study.

8.5.9 Technical complaints

Technical complaints will be collected for all products listed on the technical complaint form on a continuous basis.

Instructions for reporting technical complaints can be found in Appendix 5 (Section 10.5).

In order for Novo Nordisk to perform a complete investigation of reported SAEs, Novo Nordisk might ask the investigator to complete a technical complaint form.

8.6 Pharmacokinetics

Samples will be used to evaluate the PK of dasiglucagon and glucagon.

PK bioanalysis of analyte will be performed at a special laboratory designated by Novo Nordisk. Analyte will be assayed in plasma by a validated assay. The bioanalytical method will be described in a bioanalytical report.

Samples will be collected pre-dose, and at pre-specified time points after dosing (see Table 1-1 and Table 1-2). The nominal time (within the allowed time window) as specified in Table 1-1 for adults and Table 1-2 for adolescents should be followed and the actual time of blood sampling for evaluation of PK sample must be recorded in the eCRF.

Genetic analyses will not be performed on these plasma blood samples. Participant confidentiality will be maintained.

The residual PK samples will be destroyed no later than final CSR.

Approval by clinical pharmacology specialist in Novo Nordisk must be obtained before samples can be destroyed. A destruction certificate must be sent to Novo Nordisk if samples are stored at a special laboratory.

8.7 Pharmacodynamics

The PG profile for evaluation of the primary and secondary clinical efficacy endpoints will be assessed based on plasma concentration data from samples collected at the dosing visits (for adult participants: visit 2 and visit 3, for adolescent participants: visit 2). The samples will be sent to the clinical laboratory and analysed using a sensitive and validated assay for glucose measurements.

Samples will be collected pre-dose, and at pre-specified time points after dosing (see Table 1-1 and Table 1-2). The nominal time (within the allowed time window) as specified in Table 1-1 for adults and Table 1-2 for adolescents should be followed and the actual time of blood sampling for evaluation of PG sample must be recorded in the eCRF.

8.8 Genetics

Not applicable for this study.

8.9 Biomarkers

Not applicable for this study.

8.10 Immunogenicity assessments

Anti-drug antibody serum samples will be collected. All samples must be drawn prior to trial product administration if trial product administration is planned on the sampling day. Analysis for anti-drug antibodies will be done using a validated anti-drug binding antibody assay in a tiered manner with screening, confirmation, titre and cross reactivity to glucagon. In vitro neutralising antibody analysis towards drug/endogenous counterpart will be performed on all confirmed antibody positive samples.

Assessment of antibodies against dasiglucagon in serum will be performed by a special laboratory appointed by Novo Nordisk.

Assessment of in vitro neutralising antibodies against dasiglucagon/glucagon will be performed by a special laboratory appointed by Novo Nordisk.

Sampling, handling, storage, labelling, and shipments of the blood samples will be described in detail in a laboratory manual.

Anti-drug antibody results for the study will be finalised after last participant last visit (LPLV). Hence, the investigator will not be able to review the results of antibody measurements in relation to AEs during the study.

Results from immunogenicity assessments might be available only after DBL, and in this case results may be reported separately from the CSR.

Anti-drug antibody samples may be retained for further characterisation of immune responses towards the trial product, if required by health authorities or for safety reasons. These samples may also be used for assay maintenance or further development of anti-drug antibody assays, or for exploratory investigation of antibodies.

The antibody samples will be stored at a special laboratory designated by Novo Nordisk after end of study until marketing authorisation approval or until the research project terminates, but no longer than 15 years from end of study after which they will be destroyed. The samples might be transferred to other countries, if not prohibited by local regulations. Only relevant Novo Nordisk staff, consultants, auditors, research organisations or laboratories working for or collaborating with Novo Nordisk as well as storage facility employees will be able to access the stored samples and associated data.

In case of a systemic hypersensitivity reaction, the additional blood samples taken in relation to the reaction may be retained to follow-up on the hypersensitivity reaction. If deemed relevant by Novo Nordisk, relevant exploratory tests (including photographs) may be performed, e.g., histamine release (basophil activation). If measured, such data will be reported in a separate report.

9 Statistical considerations

The SAP will be finalised prior to first participant first visit and it will include a more technical and detailed description of the statistical analyses described in this section. This section is a summary of the planned statistical analyses of the most important endpoints including primary and supportive secondary endpoints.

9.1 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.

9.1.1 Multiplicity adjustment

Not applicable for this study.

9.2 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.

9.3 Statistical analyses

9.3.1 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 9-1 and Table 9-4 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) for details on definition and recording of hypoglycaemic episodes from initiation of hypoglycaemic clamp procedure until 45 min after IMP administration.

9.3.2 Primary endpoint analysis

9.3.2.1 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.

9.3.2.2 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 9.1. The primary endpoint analysis will be based on full analysis set (adults) (Section 9.2).

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.

9.3.2.3 Sensitivity analysis

Not applicable for this study.

9.3.2.4 Supplementary analysis

Not applicable for this study.

9.3.3 Secondary endpoints analysis

Table 9-1 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.
$AUC_{Dasi,0\text{-}30min}$	Area under the plasma dasiglucagon concentration time curve from 0 to 30 minutes after IMP injection	From 0 to 30 minutes after IMP injection	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). Hypoglycaemic episodes considered treatment emergent will be presented descriptively.

9.3.4 Exploratory analysis

Not applicable for this study.

9.3.5 Other safety analyses

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

9.3.6 Other analyses

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

Efficacy (pharmacodynamic) parameters

Table 9-2 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 9-3 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 $AUMC_{Dasi,0-inf}/AUC_{Dasi,0-inf}$. The numerator is the area under the first moment dasiglucagon concentration-time curve which is calculated as: $AUMC_{Dasi,0-inf}=AUMC_{Dasi,0-tz}+AUMC_{Dasi,tz-inf}$, where $AUMC_{Dasi,0-tz}$ is calculated using the linear trapezoidal technique based on observed values and actual measurement times from 0 to t_z and $AUMC_{Dasi,tz-inf}$ 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 $Dose_{Dasi}/AUC_{Dasi,0-inf}$
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.

9.4 Interim analysis

Not applicable for this study.

9.5 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 9-4 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 9-4 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.

10 Supporting documentation and operational considerations

10.1 Appendix 1: Regulatory, ethical and study oversight considerations

10.1.1 Regulatory and ethical considerations

This study will be conducted in accordance with the protocol and with the following:

- Consensus ethical principles derived from international guidelines including the Declaration of Helsinki[28] and applicable ICH Good Clinical Practice (GCP) Guideline[29].
- Applicable laws and regulations

The protocol, informed consent form, investigator's brochure (as applicable) and other relevant documents (e.g., advertisements) must be submitted to an IRB/IEC and reviewed and approved by the IRB/IEC before the study is initiated.

Regulatory authorities will receive the clinical trial application, protocol amendments, reports on SAEs and the CSR according to national requirements.

Any amendments to the protocol will require IRB/IEC approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate safety hazard to study participants. In case of changes made to eliminate an immediate safety hazard, the actions must be reported to Novo Nordisk immediately (within 24 hours after the action was taken).

Before a site is allowed to start screening participants, written notification from Novo Nordisk must be received.

The investigator will be responsible for:

- providing written summaries of the status of the study annually or more frequently in accordance with the requirements, policies and procedures established by the IRB/IEC and/or regulatory authorities
- notifying the IRB/IEC of SAEs or other significant safety findings as required by IRB/IEC procedures
- providing oversight of the conduct of the study at the site and adherence to requirements of ICH guidelines, the IRB/IEC and all other applicable local regulations
- ensuring submission of the CSR synopsis to the IRB/IEC, as applicable according to local requirements
- reporting any potential serious breaches to the sponsor immediately (within 24 hours after discovery). A serious breach is a breach likely to affect to a significant degree the safety and rights of a participant or the reliability and robustness of data generated in the clinical study. This includes persistent or systematic non-compliance with ICH GCP E6 and/or the protocol.

10.1.2 Financial disclosure

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

10.1.3 Informed consent process

The investigator or his/her representative will explain the nature of the study, including the risks and benefits, to the participant and the participant's parent(s)/participant's LAR (applicable for adolescents) and answer all questions regarding the study. This includes the use of an impartial witness where required according to local requirements.

The investigator must ensure that the participant and/or the participant's parent(s)/participant's LAR (applicable for adolescents) has ample time to read and understand the written information and come to a decision on whether or not to participate in the study.

Participants must be informed that their participation is voluntary. Participants or their parent(s)/LAR (applicable for adolescents) will be required to sign and date a statement of informed consent ('Agreement to take part' form) that meets the requirements of local regulations, ICH GCP[29] guidelines, Declaration of Helsinki,[28] privacy and data protection requirements, where applicable, and the IRB/IEC or site.

The medical record must include a statement that written informed consent was obtained before any study-related activity and the date when the written consent was obtained. The authorised person obtaining the informed consent must also sign and date the informed consent form before any study-related activity.

The responsibility of seeking informed consent must remain with the investigator, but the investigator may delegate the task to a medically qualified person, in accordance with local requirements.

Participants and/or their parent(s)/LAR (applicable for adolescents) must be re-consented to the most current version of the informed consent form(s) during their participation in the study according to sponsor instructions.

A copy of the informed consent form(s) must be provided to the participant or the participant's parent(s)/participant´s LAR (applicable for adolescents).

During the study, the investigator will reassess the assent of a child in recognition of their advancing age, evolving maturity and competency.

10.1.4 Recruitment and information to participants during the study

Recruitment of potential participants to the study can include activities such as review of patient medical records, handing out study information and database review. Such activities are carried out prior to obtaining participant consent to participate, or any screening activities, and should be documented by the investigator.

The site will be offered a communication package for the participant during the conduct of the study. The package content is issued by Novo Nordisk. The communication package will contain written information intended for distribution to the participants. The written information will be translated and adjusted to local requirements and distributed to the participant at the discretion of the investigator. The participant may receive a "thank you for your participation letter" after

completion of the study. Further, the participant may receive other written information during the study.

All written information to participants must be sent to IRB/IEC for approval/favourable opinion and to regulatory authorities for approval or notification according to local regulations.

10.1.5 Data protection

Participants will be assigned a 6-digit subject ID as an identifier. Any participant records or datasets that are transferred to Novo Nordisk will contain the identifier only. No direct identifiers from the participant are transferred to Novo Nordisk.

The participant and any biological material obtained from the participant will be identified by subject ID, visit number and study ID. Appropriate measures such as encryption or leaving out certain identifiers will be enforced to protect the identity of participants as required by local, regional and national requirements.

The participant must be informed about his/her privacy rights, including that his/her personal study-related data will be used by Novo Nordisk in accordance with local data protection law. The sharing and disclosure of the data must also be explained to the participant.

The participant must be informed that his/her medical records may be examined by auditors or other authorised personnel appointed by Novo Nordisk, by appropriate IRB/IEC members and by inspectors from regulatory authorities.

There may be instances when copies of source documents (e.g., medical records) for certain cases are requested by Novo Nordisk. In such cases, all participant identifiers, with the exception of the subject ID, must be redacted on the copies of the source documents before submission to Novo Nordisk.

Personal data may be collected from participants due to process requirements from Novo Nordisk's suppliers. This data is needed to ensure that the relevant data analysis for the study can be performed, but will not be part of the data transferred to Novo Nordisk, the assessment of the study endpoints or the clinical study report. A list of any such data values must be kept as part of the study documentation along with an explanation of why it was required.

The contract between sponsor and study sites specifies responsibilities of the parties related to data protection, including implementation of appropriate security measures, handling of data security breaches and respective communication and cooperation of the parties.

Information technology systems used to collect, process, and store study-related data are secured by technical and organisational security measures designed to protect such data against accidental or unlawful loss, alteration, or unauthorised disclosure or access.

In case of a data security breach, appropriate actions will be taken promptly to mitigate any potential adverse effects, in accordance with the sponsor's procedures on how to respond in the event of unauthorized access, use or disclosure of sponsor information or systems.

10.1.6 Committees structure

10.1.6.1 Novo Nordisk safety committee

Novo Nordisk will perform ongoing safety surveillance. If new safety signals are identified, these will be evaluated by an internal safety committee. The safety committee may recommend unblinding of any data for further analysis. In this case, an internal, study-independent ad hoc group may be established to review the unblinded data and recommend further action to the safety committee, thereby ensuring the maintenance of blinding for the study personnel.

10.1.6.2 Study safety group

Not applicable for this study.

10.1.6.3 Data monitoring committee

Not applicable for this study.

10.1.6.4 Event adjudication committee

Not applicable for this study.

10.1.7 Dissemination of clinical study data

Study information will be disclosed at clinicaltrials.gov and novonordisk-trials.com, if applicable, also on other national or regional study registries. It will be disclosed according to applicable requirements, relevant recommendations or regulations, such as the Declaration of Helsinki,[28] the International Committee of Medical Journal Editors (ICMJE),[30] the Food and Drug Administration Amendment Act (FDAAA),[31] European Commission Requirements[1, 32, 33] and in accordance with Novo Nordisk commitment to clinical transparency. If a participant requests to be included in the study via the Novo Nordisk e-mail contact at these web sites, Novo Nordisk may disclose the investigator's contact details to the participant. As a result of increasing requirements for transparency, some countries require public disclosure of investigator names and their affiliations.

The privacy of each participant providing data for the clinical study, and the confidentiality of his/her information shall be preserved in reports, communications and when publishing any data.

The study will be registered at www.jrct.niph.go.jp.

10.1.8 Data quality assurance

10.1.8.1 Case report forms

Novo Nordisk or designee is responsible for the data management of this study including quality checking of the data.

To demonstrate his/her oversight of the collected data, the investigator will sign the eCRF at the end of the study, as described in the eCRF completion guideline.

All participant data relating to the study will be recorded on eCRFs unless transmitted electronically to Novo Nordisk or designee (e.g., laboratory data). The investigator is responsible for verifying that data entries in CRFs are accurate and correct by physically or electronically signing the eCRF.

CRFs will be provided in an electronic format except the following that are provided as paper CRFs:

- Pregnancy forms
- Safety information forms
- Technical complaint forms

The CRFs for AE forms will be provided as paper backup CRFs to be used when access to the eCRF is revoked or the eCRF is temporarily unavailable.

Corrections to the eCRF data may be made by the investigator or the investigator's delegated staff. An audit trail will be maintained in the eCRF application containing as a minimum: the old and the new data, identification of the person entering the data, date and time of the entry and reason for the correction. If corrections are made by the investigator's delegated staff after the date when the investigator signed the eCRF, the eCRF must be signed and dated again by the investigator.

The investigator must ensure that data is recorded in the eCRF as soon as possible, preferably within 5 working days after the visit. Once data has been entered, it will be available to Novo Nordisk for data verification and validation purposes.

10.1.8.2 Monitoring

The investigator must permit study-related monitoring, audits, IRB/IEC review and regulatory agency inspections and provide direct access to source data documents (original documents, data and records). Remote access to the source data documents by Novo Nordisk monitors and auditors can be agreed in countries where this is acceptable according to regulatory requirements and national legislation. Direct access includes permission to examine, analyse, verify and reproduce any record(s) and report(s) that are important to the evaluation of the study. If the electronic source data does not have a visible audit trail, the investigator must provide the monitor with signed and dated printouts. In addition, the relevant site staff should be available for discussions at monitoring visits and between monitoring visits (e.g., by telephone).

Study monitors will perform ongoing source data verification of critical data points to confirm that data entered into the CRF by authorised site personnel are accurate, complete and verifiable from source documents. Study monitors will perform ongoing source data review to ensure that the study is being conducted in accordance with the current approved protocol and any other study agreements, ICH GCP[29] and all applicable regulatory requirements, evaluating the adequacy of critical processes at site for the execution of the protocol, collection of study data, to ensure that the safety and rights of participants are being protected.

Monitoring will be conducted using a risk-based approach including risk assessment, monitoring plans, centralised monitoring (remote assessment of data by Novo Nordisk) and visits to sites.

Quality tolerance limits (QTLs) will be predefined for relevant study level risks, if any, in the relevant monitoring plan to identify systematic issues that can impact participant safety and/or reliability of study results. Predefined QTLs will be monitored during the study, and important deviations from the QTLs and remedial actions taken will be summarised in the CSR.

10.1.8.3 Protocol compliance

Deviations from the protocol should be avoided. If deviations do occur, the investigator must inform the monitor without delay and the implications of the deviation must be reviewed and discussed.

Deviations must be documented and explained in a protocol deviation by stating the reason, date and the action(s) taken. Some deviations, for which corrections are not possible, can be acknowledged and confirmed via edit checks in the eCRF or via listings from the study database.

Note: Throughout the protocol, the term 'should' indicates an expectation that applies unless an alternative scenario is described.

10.1.9 Source documents and source data

All data entered in the eCRF must be verifiable in source documentation other than the eCRF.

The original of the completed diaries must not be removed from the site, unless they form part of the eCRF, and a copy is kept at the site.

Source documents provide evidence for the existence of the participant and substantiate the integrity of the data collected. Source documents are filed at the site. Any source data generated by investigator's subcontractors must be archived and accessible by the site.

Data that is transcribed into the eCRF from source documents must be consistent with the source documents, or the discrepancies must be explained. The investigator may need to request previous medical records or transfer records. Also, current medical records must be available.

It must be possible to verify participant's medical history in source documents, such as participant's medical record.

The investigator must document any attempt to obtain external medical information by noting the date(s) when information was requested and who was contacted.

Definition of what constitutes source data can be found in a source document agreement at the site. There will only be one source document defined at any time for any data element.

10.1.10 Retention of clinical study documentation

Records and documents, including signed informed consent forms, pertaining to the conduct of this study must be retained by the investigator for 25 years after end of study unless local regulations or institutional policies require a longer retention period.

No records may be destroyed during the retention period without the written approval of Novo Nordisk. No records may be transferred to another location or party without written notification to Novo Nordisk.

The investigator/institution must have control of all essential documents and records generated by the investigator/institution before, during and after the study. The investigator must be able to access his/her study documents without involving Novo Nordisk in any way. If applicable, eCRF

and other participant data will be provided in an electronic readable format to the investigator before access is revoked to the systems supplied by Novo Nordisk. Site-specific CRFs and other participant data (in an electronic readable format or as paper copies or prints) must be retained by the site. A copy of all data will be stored by Novo Nordisk.

Participant's medical records must be kept for the maximum period permitted by the hospital, institution or private practice.

Novo Nordisk will retain study documentation for 25 years or longer as required by local law, or as long as the information contributes to scientific purposes or product safety.

10.1.11 Study and site start and closure

First act of recruitment

The start of study is defined as the date when the clinical study will be open for recruitment of participants, i.e., the 'first act of recruitment.' The first act of recruitment is defined as the first site activation in the study.

First participant's first visit

First participant's first visit in the study is defined as the date the first participant is screened in the study.

Study or site termination

Novo Nordisk reserves the right to close the site or terminate the study at any time for any reason at the sole discretion of Novo Nordisk. If the study is suspended or terminated, the investigator must inform the participants promptly and ensure appropriate therapy and follow-up. Novo Nordisk will take appropriate actions, and the investigator and/or Novo Nordisk must promptly inform the regulatory authorities and IRBs/IECs and provide a detailed written explanation.

Sites will be closed upon study completion. A site is considered closed when all required documents and study supplies have been collected and a site closure visit has been performed.

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

Reasons for the early closure of a site by Novo Nordisk or investigator may include but are not limited to:

- failure of the investigator to comply with the protocol, the requirements of the IRB/IEC or local health authorities, or Novo Nordisk procedures or GCP guidelines
- inadequate recruitment of participants by the investigator
- discontinuation of further study intervention development.

10.1.12 Responsibilities

The investigator is responsible for conducting the study in accordance with the protocol at his/her site and must ensure adequate supervision of the conduct of the study at the site. If any tasks are delegated, the investigator must maintain a log of appropriately qualified persons to whom he/she

has delegated specified study-related duties. The investigator must ensure that there is adequate and documented training for all staff participating in the conduct of the study. It is the investigator's responsibility to supervise the conduct of the study and to protect the rights, safety, dignity and well-being of the participants.

A qualified physician, who is an investigator or a sub investigator for the study, must be responsible for all study-related medical decisions.

The investigator is responsible for filing essential documents (i.e., those documents which individually and collectively permit evaluation of the conduct of a study and the quality of the data produced) in the investigator trial master file. The documents, including the participant identification code list must be kept in a secure locked facility so that no unauthorised persons can get access to the data.

The investigator will take all necessary technical and organisational safety measures to prevent accidental or wrongful destruction, loss or deterioration of data. The investigator will prevent any unauthorised access to data or any other processing of data against applicable law. This also includes ensuring that no indirect sharing of user credentials for IT systems used in this study takes place (e.g., by not sharing IT equipment with others in a way where user credentials have the possibility of being shared). The investigator must be able to provide the necessary information or otherwise demonstrate to Novo Nordisk that such technical and organisational safety measures have been taken.

During any period of unavailability, the investigator must delegate responsibility for medical care of participants to a specific qualified physician who will be readily available to participants during that time.

If the investigator is no longer able to fulfil the role as investigator (e.g., if he/she moves or retires), a new investigator will be appointed in consultation with Novo Nordisk.

The investigator and other site personnel must have sufficient English skills according to their assigned task(s).

10.1.13 Indemnity statement

Novo Nordisk carries product liability for its products, and liability as assumed under the special laws, acts and/or guidelines for conducting clinical studies in any country, unless others have shown negligence.

Novo Nordisk assumes no liability in the event of negligence or any other liability of the sites or investigators conducting the study or by persons for whom the said site or investigator are responsible.

Novo Nordisk accepts liability in accordance with local laws/acts/guidelines in Japan.

10.1.14 Publication policy

The information obtained during the conduct of this study is considered confidential and may be used by or on behalf of Novo Nordisk for regulatory purposes as well as for the general

development of the study intervention. All information supplied by Novo Nordisk in connection with this study shall remain the sole property of Novo Nordisk and is to be considered confidential information.

No confidential information shall be disclosed to others without prior written consent from Novo Nordisk. Such information shall not be used except in the performance of this study.

The information obtained during this study may be made available to other investigators who are conducting other clinical studies with the study intervention, if deemed necessary by Novo Nordisk. Provided that certain conditions are fulfilled, Novo Nordisk may grant access to information obtained during this study to researchers who require access for research projects studying the same or related diseases and/or study intervention studied in this study.

Novo Nordisk may publish on its clinical studies website a redacted CSR for this study.

10.1.14.1 Communication of results

Novo Nordisk commits to communicate and disclose results of studies regardless of outcome. Disclosure includes publication of a manuscript in a peer-reviewed scientific journal, abstract submission with a poster or oral presentation at a scientific meeting or disclosure by other means.

The results of this study will be subject to public disclosure on external web sites according to international and national regulations. Novo Nordisk reserves the right to defer the release of data until specified milestones are reached, for example when the CSR is available. This includes the right not to release the results of interim analyses, because the release of such information may influence the results of the entire study.

At the end of the study, one or more scientific publications may be prepared collaboratively by the investigator(s) and Novo Nordisk. Novo Nordisk reserves the right to postpone publication and/or communication for up to 60 days to protect intellectual property.

In all cases, the study results will be reported in an objective, accurate, balanced and complete manner, with a discussion of the strengths and limitations. In the event of any disagreement on the content of any publication, both the investigators' and Novo Nordisk opinions will be fairly and sufficiently represented in the publication.

10.1.14.2 Authorship

Novo Nordisk will work with one or more investigator(s) and other experts who have contributed to the study concept or design, acquisition, analysis or interpretation of data to report the results in one or more publications.

Authorship of publications will be in accordance with the Recommendations for the Conduct, Reporting, Editing and Publication of Scholarly Work in Medical Journals by the International Committee of Medical Journal Editors.[34]

All authors will be provided with the relevant statistical tables, figures and reports needed to evaluate the planned publication.

Where required by the journal, the investigator from each site will be named in an acknowledgement or in the supplementary material, as specified by the journal.

10.1.14.3 Site-specific publication(s) by investigator(s)

Not applicable for this study.

10.1.14.4 Investigator access to data and review of results

As owner of the study database, Novo Nordisk has the discretion to determine who will have access to the database.

Individual investigator will have their own research participants' data and will be provided with the randomisation code after results are available.

10.2 Appendix 2: Clinical laboratory tests

The tests detailed in Table 10-1 and Table 10-2 will be performed by the local laboratory at the site or subcontracted by the site except for safety PG measurements during the clamp procedure as described in Section 8.

Dasiglucagon PK, glucagon PK and anti-dasiglucagon antibody analysis will be performed by special laboratories (see Attachment I). Samples (including back-up samples) for PK and PD analysis will be collected at sampling timepoints presented in Table 1-1 and Table 1-2.

Additional tests may be performed at any time during the study as deemed necessary by the investigator or required by local regulations e.g., to follow up on AEs. Only laboratory samples specified in the protocol should be sent to the local laboratory at the site or subcontracted by the site for analysis; if additional laboratory sampling is needed, e.g., to follow-up on AEs, this must be done at a local laboratory at the site or subcontracted by the site.

COVID-19 test may be performed at any time during the trial as required by local regulations or deemed necessary by the investigator or sponsor. Laboratory results will not be transferred to the trial database, but COVID-19 infection should be reported as concomitant illness or AEs as described in Sections 8.1 and 8.5.

The local laboratory at the site or subcontracted by the site used for study related analyses may include testing of additional parameters not requested in the protocol due to their internal laboratory equipment and/or processes. These data results will not be transferred to the study database, but any abnormal values should be communicated to the investigator. The investigator should review such values for AEs and report these according to this protocol.

The investigator must review all laboratory results for concomitant illnesses and AEs.

The investigator must keep an overview, e.g., a log, of laboratory samples not handled according to the laboratory manual. In addition, the investigator must keep an overview, e.g., a log, of laboratory samples stored at site.

Clinical laboratory samples will be destroyed no later than at end of study or no later than at finalisation of the CSR.

Residual samples collected for PD, PK and immunogenicity will be stored as described in the laboratory manual.

Safety PG measurements during the clamp procedure will be collected in the source data.

Table 10-1 Protocol-required efficacy laboratory assessments

Laboratory assessments	Parameters
Pharmacodynamic	• Plasma glucose

Table 10-2 Protocol-required safety laboratory assessments

Laboratory assessments	Parameters
Haematology	• Erythrocytes • Haematocrit • Haemoglobin • Reticulocytes • Thrombocytes • Total leucocytes • Differential leucocyte count (neutrophils, lymphocytes, monocytes, eosinophils, basophils) • Mean corpuscular volume (MCV)
Coagulation parameters[a]	• Activated partial thromboplastin time (APTT) • Fibrinogen • International normalized ratio (INR) of prothrombin time (PT; PT-INR)
Biochemistry	• Alanine Aminotransferase (ALT) • Alkaline phosphatase • Aspartate Aminotransferase (AST) • Calcium • C-reactive protein • Blood urea nitrogen (BUN) • Creatinine • Total bilirubin • Potassium • Sodium
Lipids	• Cholesterol • High density lipoprotein (HDL) cholesterol • Low density lipoprotein (LDL) cholesterol • Triglycerides
Urine drug screen[b]	• Amphetamines • Barbiturates • Benzodiazepines • Cocaine • Opiates • Cannabinoids • Methamphetamine • Methadone • Tricyclic antidepressants • Cannabis • Phencyclidine
Serology[a,b]	• HIV-1 and HIV-2 antibodies and HIV-1 antigen • Hepatitis B surface antigen (HBsAg) • Hepatitis C virus antibody • Syphilis

Laboratory assessments	Parameters
Pregnancy Testing[c]	• Highly sensitive serum human chorionic gonadotropin (hCG) pregnancy test • Urine pregnancy test
Urinalysis	• pH, glucose, protein, blood (leukocytes and erythrocytes), ketones, and nitrite by dipstick
Glucose metabolism[a]	• HbA1c
Alcohol breath test[b]	• Alcohol breath test
Other tests	• eGFR calculated by the local laboratory at the site based on the creatinine value using the 2009 CKD-EPI creatinine equation • COVID-19 test[d]

Notes:
[a] For screening purpose only.
[b] Test results will be noted in the participant's source data but will not be transferred to the eCRF or study database.
[c] For women of childbearing potential. Serum pregnancy testing to be performed at screening and urine pregnancy testing will be performed at visits 2, 3 and 3a. Data will not be collected in the eCRF, but only in the source data.
[d] COVID-19 test may be performed at any time during the study as required by local requirements or deemed necessary by the investigator or by sponsor. Test results will be noted in the participant's source data but will not be transferred to the eCRF or study database, but COVID-19 infection should be registered as a concomitant illness or AE.

10.3 Appendix 3: Adverse Events and Serious Adverse Events: Definitions and procedures for recording, evaluating, follow-up and reporting

10.3.1 Definition of AE

An AE is any untoward medical occurrence in a clinical study participant that is temporally associated with the use of IMP, whether or not considered related to the IMP. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease (new or exacerbated) temporally associated with the use of an IMP.

Events to be reported as AEs

In addition to events falling under the AE definition above, the following are examples of events that also are to be reported as AEs:

- Any abnormal laboratory test results or safety assessments considered clinically significant in the medical and scientific judgment of the investigator, including events that have worsened from prior to the time point from which AEs are collected.
- Conditions detected or diagnosed after IMP administration even though it may have been present prior to the time point from which AEs are collected.
- Exacerbation/worsening of a chronic or intermittent condition including either an increase in frequency and/or intensity of the condition.
- Signs, symptoms or the clinical sequelae of a suspected drug–drug interaction.
- Signs, symptoms or the clinical sequelae of a suspected overdose of IMP regardless of intent.

A 'lack of efficacy' or 'failure of expected pharmacological action' per se will not be reported as an AE or SAE. Such instances will be captured in the efficacy assessments. However, the signs, symptoms and/or clinical sequelae resulting from lack of efficacy will be reported as AE or SAE if they fulfil the definition.

Events NOT to be reported as AEs:

- Conditions present prior to the time point from which AEs are collected and anticipated day-to-day fluctuations of these conditions. Record such conditions as concomitant illness/medical history. This includes those conditions identified during screening or identified during other study procedures performed before exposure to IMP.
- Medical or surgical procedures (e.g., endoscopy, appendectomy). The condition that leads to the procedure is the AE.
- Medical or surgical procedures not preceded by an AE or worsening of a known condition.

10.3.2 Definition of an SAE

An SAE is an AE that fulfils at least one of the following criteria:

- **Results in death**
- **Is life-threatening**
  - The term 'life-threatening' refers to an event in which the participant was at risk of death at the time of the event. It does not refer to an event which hypothetically might have caused death, if it were more severe.
- **Requires inpatient hospitalisation or prolongation of existing hospitalisation**

- Hospitalisation signifies that the participant has been admitted at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalisation are AEs. If a complication prolongs hospitalisation or fulfils any other seriousness criteria, the event is serious. When in doubt as to whether 'hospitalisation' occurred or was necessary, the AE should be considered serious.
- Hospitalisation for elective treatment (e.g., elective medical or surgical procedures) of a condition that was present prior to the time point from which AEs are collected and that did not worsen, is not considered an AE.
  Note: Hospitalisations for administrative, study-related, social and convenience reasons do not constitute AEs and should therefore not be reported as AEs or SAEs. Hospital admissions for medical or surgical procedures, planned before study inclusion, are not considered AEs or SAEs.

- **Results in persistent or significant disability/incapacity**
  - The term 'disability' means a substantial disruption of a person's ability to conduct normal life functions. This definition is not intended to include experience of relatively minor medical significance, such as uncomplicated headache, nausea, vomiting, diarrhoea, influenza, and accidental trauma (e.g., sprained ankle), that may interfere with or prevent everyday life functions but do not constitute a substantial disruption.
- **Is a congenital anomaly/birth defect**
- **Important medical event:**
  - Medical or scientific judgment should be exercised by the investigator in deciding whether SAE reporting is appropriate in other situations. This includes important medical events that may not be immediately life-threatening or result in death or hospitalisation but may jeopardise the participant or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These events should usually be considered serious and reported as SAEs using the important medical event criterion.
  - The following must be reported as an SAE using the important medical event criterion if no other seriousness criteria are applicable:
    - Suspicion of transmission of infectious agents via IMP
    - Risk of liver injury defined as alanine aminotransferase (ALT) or aspartate aminotransferase (AST) >3x ULN and total bilirubin >2x ULN where no alternative aetiology exists (Hy's law)

10.3.3 Description of AEs requiring additional data collection and other events requiring collection of additional information

Adverse events requiring additional data collection

An AE requiring additional data collection is an AE where Novo Nordisk has evaluated that additional data is needed in the evaluation of safety. A specific event form needs to be completed for the following events.

Medication error:

- A medication error is an unintended failure in the IMP treatment process that leads to, or has the potential to lead to, harm to the participant, such as:

- administration of wrong drug
  Note: Use of wrong Kit ID/DUN is not considered a medication error unless it results in administration of wrong drug.
- wrong route of administration, such as s.c. instead of i.m.
- accidental administration of a lower or higher dose than intended. The administered dose must deviate from the intended dose to an extent where clinical consequences for the study participant were likely to happen as judged by the investigator, although they did not necessarily occur.

Misuse and abuse:

- Situations where the IMP is intentionally and inappropriately used not in accordance with the protocol (e.g., overdose to maximise effect)
- Persistent or sporadic, intentional excessive use of an IMP which is accompanied by harmful physical or psychological effects (e.g., overdose with the intention to cause harm)

Note: Medication error, misuse and abuse must always be reported on an AE form and the specific event form must be completed. The AE diagnosis on the AE form must reflect what occurred (e.g., accidental overdose, intentional overdose or other). If the medication error and/or misuse and abuse resulted in a clinical consequence, this must be reported on an additional AE form.

Other events requiring collection of additional information

Hypoglycaemic episodes
All hypoglycaemic episodes must be recorded on a hypoglycaemic episode form (see Appendix 6 (Section 10.6)). If the hypoglycaemic episode fulfils the criteria for an SAE, then in addition to the hypoglycaemic episode form, an AE form and a safety information form must be filled in. One AE form and safety information form can cover several hypoglycaemic episode forms, if the participant has not recovered between the episodes.

Hyperglycaemic episodes
All hyperglycaemic episodes must be recorded on a hyperglycaemic episode form (see Appendix 7 (Section 10.7)). If the hyperglycaemic episode fulfils the criteria for an SAE, then in addition to the hyperglycaemic episode form, an AE form and a safety information form must be filled in. One AE form and safety information form can cover several hyperglycaemic episode forms, if the participant has not recovered between the episodes.

10.3.4 Recording and follow-up of AEs and/or SAEs

10.3.4.1 AE and SAE recording

The investigator will record all relevant information about AEs/SAEs in the eCRF.

The investigator will attempt to establish a diagnosis of the event based on signs, symptoms and/or other clinical information. In such cases, the diagnosis (not the individual signs/symptoms) will be documented as the AE/SAE.

When an AE/SAE occurs, it is the responsibility of the investigator to review all documentation (e.g., hospital progress notes, laboratory and diagnostics reports) related to the event.

There may be instances when copies of source documents (e.g., medical records) for certain cases are requested by Novo Nordisk. In such cases, all participant identifiers, with the exception of the subject ID, must be redacted on the copies of the source documents before submission to Novo Nordisk, see Section 10.1.5.

Please refer to Figure 10-1 for reporting of non-serious AEs. For all non-serious AEs, the applicable forms should be signed when the event is resolved or at the end of the study at the latest. For sign-off of SAE-related forms, refer to Section 10.3.5.

If an SAE is considered to have a causal relationship with a concomitant medication or AxMP, it is important that the suspected relationship is reported to Novo Nordisk in e.g., the alternative aetiology section on the safety information form. Novo Nordisk may need to report it to relevant regulatory authorities.

If an SAE is a suspected drug – drug interaction with the IMP, the investigator should report this to Novo Nordisk, e.g., in the alternative aetiology section on the safety information form.

10.3.4.2 Assessment of severity

The investigator will assess severity for each event reported during the study and assign it to one of the following categories:

- **Mild**: A type of adverse event that is usually transient and may require only minimal treatment or therapeutic intervention. The event does not generally interfere with usual activities of daily living.
- **Moderate**: A type of adverse event that is usually alleviated with additional specific therapeutic intervention. event interferes with usual activities of daily living, causing discomfort but poses no significant or permanent risk of harm to the research participant.
- **Severe**: A type of adverse event that interrupts usual activities of daily living, or significantly affects clinical status, or may require intensive therapeutic intervention.
  Note: An AE that is assessed as severe should not be confused with an SAE. Both AEs and SAEs can be assessed as severe.

10.3.4.3 Assessment of causality

The investigator is obligated to assess the relationship between IMP and the occurrence of each AE/SAE. The investigator will use clinical judgment to determine the relationship.

Relationship between an AE/SAE and the relevant IMP should be assessed as:

- **Probable** - Good reason and sufficient documentation to assume a causal relationship.
- **Possible** - A causal relationship is conceivable and cannot be dismissed.
- **Unlikely** - The event is most likely related to aetiology other than the IMP.

Alternative aetiology, such as underlying disease(s), concomitant medication and other risk factors, as well as the temporal relationship of the event to IMP administration, should be considered and investigated.

The investigator should consult the IB and/or product information, for marketed products, when making the causality assessment. For each AE/SAE, the investigator must document in the medical records that he/she has reviewed the AE/SAE and has provided an assessment of causality.

There may be situations in which an SAE has occurred, and the investigator has minimal information to include in the initial report. However, **it is important that the investigator always makes an assessment of causality for every event before the initial transmission of the SAE data**.

The investigator may change his/her opinion of causality, in light of follow-up information, and update the causality assessment in the eCRF.

The causality assessment is one of the criteria used when determining regulatory reporting requirements.

10.3.4.4 Outcome of AEs

The investigator will select the most appropriate outcome that reflects the event outcome status at the time of reporting:

- **Recovered/resolved**: The participant has fully recovered from the event, or by medical or surgical treatment the condition has returned to the level observed when first documented.
- **Recovering/resolving**: The condition is improving, and the participant is expected to recover from the event. This term may also be applicable for AEs ongoing at the time of death (where death is due to another AE).
  Note: SAEs can be closed with the outcome 'recovering/resolving' only if the participant has withdrawn or completed the study, or end of study is near, and the investigator confirms that the participant is expected to fully recover from the event.
- **Recovered/resolved with sequelae**: The participant has recovered from the event but with long-lasting or permanent effect(s) due to a disease, injury, treatment or procedure. If a sequela meets an SAE criterion, the AE must be reported as an SAE.
- **Not recovered/not resolved**: The condition has not improved. This term may be applicable in cases of chronic conditions, cancer or AEs ongoing at time of death (where death is due to another AE).
- **Fatal**: This term is only applicable if the participant died from a condition related to the reported AE. Outcomes of other reported AEs that occurred before the participant died should be assessed as 'recovered/resolved', 'recovering/resolving', 'recovered/resolved with sequelae' or 'not recovered/not resolved'. An AE with a fatal outcome must be reported as an SAE.
- **Unknown**: The outcome is unknown.
  Note: AEs can be closed with the outcome 'unknown' only if the participant is lost to follow-up.

10.3.4.5 Follow-up of AE and SAE

The investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by Novo Nordisk to elucidate the nature and/or causality of the AE or SAE as fully as possible (e.g., severe hypersensitivity reactions, Hy's law). This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.

If a participant dies during participation in the study or during a recognised follow-up period, the investigator should, upon request, provide Novo Nordisk with the autopsy results including histopathology.

New or updated information will be recorded in the eCRF.

The investigator will submit any updated SAE data to Novo Nordisk without undue delay, but not later than within 24 hours of receipt of the information.

10.3.5 Reporting of SAEs

SAE reporting via eCRF

Relevant forms must be completed in the eCRF.

For SAEs, initial notification via telephone is acceptable, although it does not replace the need for the investigator to complete the AE forms and safety information forms within the designated reporting timelines (see Figure 10-1):

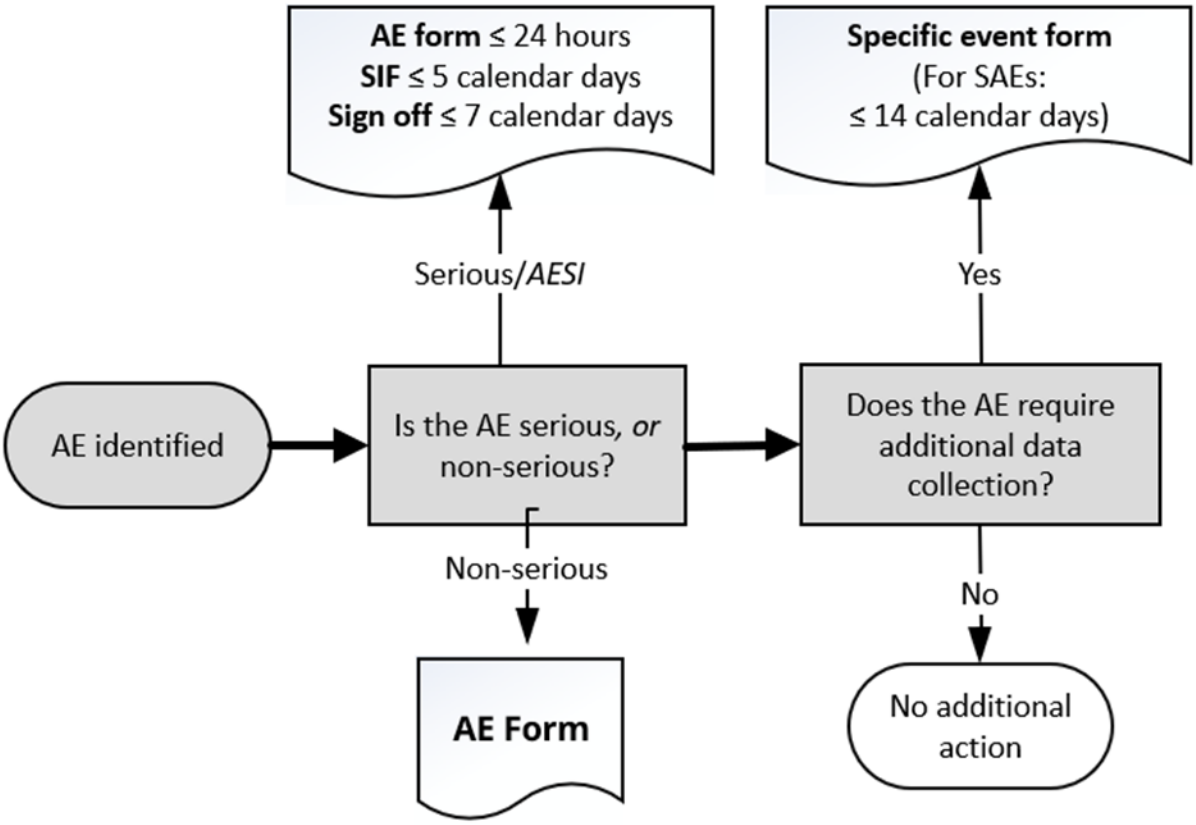
- AE form without undue delay, but not later than within 24 hours.
- Safety information form within 5 calendar days.
- Both the AE form and the safety information form must be signed within 7 calendar days after first knowledge by the investigator.
- Specific event form within 14 calendar days.

If the eCRF is unavailable for more than 24 hours, then the sites will use the paper backup AE form for SAE reporting. The information from the paper backup AE form will be entered in the eCRF as soon as it becomes available.

All CRFs that are sent to Novo Nordisk must be forwarded in accordance with Section 10.1.5. Contact details for SAE reporting can be found in the investigator trial master file and in Attachment I of the protocol.

After the study is completed, the study database will be locked, and the eCRF will be decommissioned to prevent the entry of new data or changes to existing data. If a new SAE from a participant or updated information on a previously reported SAE needs to be reported after eCRF decommission, a paper AE form and safety information form will be used to notify Novo Nordisk.

Figure 10-1 Decision tree for determining the event type and the respective forms to complete with associated timelines



- **Timelines** are from the awareness of an AE.
- Hypoglycaemic episodes should be reported on the hypoglycaemic episodes form. If the hypoglycaemic episode fulfils the criteria for an SAE then an AE form and a safety information form must also be filled in.
- **Queries and follow-up** requests to be resolved ≤ 14 calendar days.
- In general data must be recorded in the eCRF as soon as possible, preferably within 5 working days (see Appendix 1)

AE: Adverse Events, SAE: Serious Adverse Events, SIF: Safety Information Form

10.4 Appendix 4: Contraceptive guidance and collection of pregnancy information

10.4.1 Definitions

Woman of childbearing potential (WOCBP)

A woman is considered fertile following menarche and until becoming postmenopausal unless permanently sterile.

If fertility is unclear (e.g., amenorrhea in adolescents or athletes) and a menstrual cycle cannot be confirmed before first dose of study intervention, additional evaluation should be considered.

Females in the following categories are not considered WOCBP

1. Premenarcheal
2. Females with one or more of the following:
   - Documented hysterectomy
   - Documented bilateral salpingectomy
   - Documented bilateral oophorectomy

   For females with permanent infertility due to an alternate medical cause other than the above (e.g., Müllerian agenesis, androgen insensitivity), investigator discretion should be applied in determining study enrolment.
3. Postmenopausal female:
   - A postmenopausal state is defined as amenorrhoea for at least 12 months without an alternative medical cause in a female > 45 years of age. Alternative medical causes for amenorrhoea include, but are not limited to, hormonal contraception or hormonal replacement therapy.
   - Females ≥ 60 years of age can be considered postmenopausal.

Females on HRT and whose menopausal status is in doubt are considered of childbearing potential and will be required to use one of the highly effective contraception methods.

Note: Documentation regarding categories 1-3 can come from the site staff's review of participant's medical records, medical examination or medical history interview.

10.4.2 Contraceptive guidance

Male participants

A male adult participant must meet the following criteria: Surgically sterilized or willing to refrain from heterosexual intercourse from screening and until end of study visit or, if sexually active, uses condom and partner practices highly effective contraception (Table 10-3) during the study from screening and until end of study.

Male adolescent participants becoming sexually active during the study will receive age-appropriate guidance regarding contraceptives.

Female participants

Female participants of childbearing potential are eligible to participate if they agree to use methods of contraception consistently and correctly. Table 10-3 lists the highly effective methods of contraception allowed.

Female participants having first menstrual period during the study will receive age-appropriate guidance regarding contraception if sexually active.

Highly effective or acceptable contraception should be utilised from screening visit (visit 1) until the end of study visit (end of study, visit 3a).

Table 10-3 Highly effective and acceptable contraceptive methods allowed[35]

Highly effective methods[a] (Failure rate of <1% per year when used consistently and correctly):

- Combined (estrogen- and progestogen-containing) hormonal contraception associated with inhibition of ovulation[b]
  - oral
  - intravaginal
  - transdermal
- Progestogen-only hormone contraception associated with inhibition of ovulation
  - oral
  - injectable
  - implantable
- Intrauterine device (IUD)
- Intrauterine hormone-releasing system (IUS)
- Bilateral tubal occlusion (including bilateral tubal ligation)
- Vasectomised partner
  Vasectomised partner is a highly effective contraceptive method provided that the partner is the sole sexual partner of the woman of childbearing potential and the absence of sperm has been confirmed. If not, an additional highly effective method of contraception should be used. Spermatogenesis cycle is approximately 90 days.
- Sexual abstinence
  Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study intervention. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the study and the preferred and usual lifestyle of the participant.

Acceptable methods[c]

- Progestogen-only oral hormonal contraception where inhibition of ovulation is not the primary mode of action
- Male or female condom with or without spermicide[d]
- Cervical cap, diaphragm, or sponge with spermicide
- A combination of male condom with either cervical cap, diaphragm, or sponge with spermicide (double-barrier methods)

Notes:
[a]Contraceptive use by men or women should comply with local regulations regarding the use of contraceptive methods for those participating in clinical studies.
[b]If locally required, in accordance with Clinical Trial Facilitation Group (CTFG) guidelines, acceptable contraceptive methods are limited to those which inhibit ovulation as the primary mode of action.
[c]Considered effective, but not highly effective - failure rate of ≥1% per year.
[d]Male condom and female condom should not be used together (due to risk of failure from friction).

The following methods are not acceptable methods of contraception: Periodic abstinence (calendar, symptothermal, post-ovulation methods), withdrawal (coitus interruptus), spermicides only and lactational amenorrhoea method (LAM).

10.4.3 Collection of pregnancy information

Female participants who become pregnant

Investigator will collect pregnancy information on any female participant who becomes pregnant while participating in this study.

Information will be recorded on the appropriate form and submitted to Novo Nordisk within 14 calendar days of learning of a participant's pregnancy (see Figure 10-2).

The participant will be followed to determine the outcome of the pregnancy. The investigator will collect follow-up information on participant and neonate which will be forwarded to Novo Nordisk within 14 calendar days. Generally, follow-up will not be required for longer than 1 month beyond the delivery date.

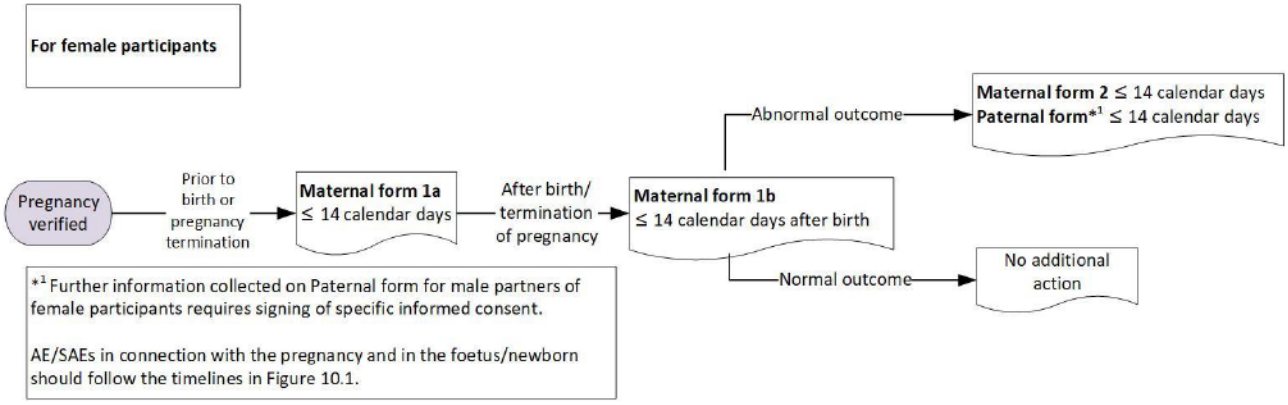
Any termination of pregnancy will be reported, regardless of foetal status (presence or absence of anomalies) or indication for procedure.

While pregnancy itself is not considered to be an AE or SAE, any adverse event in connection with pregnancy or elective termination of a pregnancy for medical reasons will be reported as an AE or SAE. If relevant, consider adding 'gestational', 'pregnancy-related' or a similar term when reporting the AE/SAE.

Pregnancy outcome will be documented in the participant's medical record. Abnormal pregnancy outcome (e.g., spontaneous abortion, foetal death, stillbirth, congenital anomalies and ectopic pregnancy) is considered an SAE. In case of abnormal pregnancy outcome, paternal information will be recorded in the appropriate form after obtaining the necessary signed paternal informed consent.

If the investigator learns of an SAE occurring as a result of a post-study pregnancy which is considered related to the IMP by the investigator, the SAE should be reported to Novo Nordisk as described in Appendix 3 (Section 10.3).

Figure 10-2 Decision tree for determining the forms to complete for collection of pregnancy information and timelines for reporting – For female participants



Any female participant who becomes pregnant while participating in the study will discontinue study intervention.

10.5 Appendix 5: Technical complaints: Definition and procedures for recording, evaluation, follow-up and reporting

10.5.1 Definition of technical complaint

A technical complaint is any written, electronic or oral communication that alleges inadequacy related to any of the following for a product (drug or device): identity, quality, labelling, durability, reliability, usability, safety, performance or malfunction.

The technical complaint may be associated with an AE but does not concern the AE itself.

Examples of technical complaints include:

- Problems with the physical or chemical appearance of study interventions (e.g., discoloration, particles or contamination).
- Problems with packaging material including labelling.
- Problems related to devices (e.g., problems with the prefilled syringe).

Time period for detecting technical complaints

All technical complaints which occur from the time of receipt of the product at site until the time of the last usage of the product must be collected for products predefined on the technical complaint form.

10.5.2 Recording and follow-up of technical complaints

Reporting of technical complaints to Novo Nordisk

For contact details for Customer Complaint Center, please refer to Attachment I.

Technical complaints on products allocated to a participant must be reported on a separate technical complaint form:

1. For products with Kit ID/DUN: One technical complaint form must be completed for each affected Kit ID/DUN.
2. For products without Kit ID/DUN: One technical complaint form must be completed for each batch, code or lot number.

Timelines for reporting technical complaints to Novo Nordisk

The investigator must complete and forward the technical complaint form to Customer Complaint Center, Novo Nordisk, within the following timelines of obtaining knowledge of the technical complaint:

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

Follow-up of technical complaints

The investigator is responsible for ensuring that new or updated information will be recorded on the originally completed form.

Collection, storage and shipment of technical complaint samples

The investigator must collect the technical complaint sample and all associated parts and notify the monitor within 5 calendar days of obtaining the sample at site. The sample and all associated parts must be sent as soon as possible to Customer Complaint Center, Novo Nordisk, together with a copy of the completed technical complaint form. The technical complaint sample should contain the batch, code or lot number and, if available, the Kit ID/DUN. If the technical complaint sample is unobtainable, the reason must be stated on the technical complaint form. If several samples are shipped in one shipment, the sample and the corresponding technical complaint form should be kept together.

Storage of the technical complaint sample must be done in accordance with the conditions prescribed for the product.

10.5.3 Reporting of technical complaints for products not included in the technical complaint form

Technical complaints on products not included in the technical complaint form should be reported to manufacturing holder.

10.6 Appendix 6: Hypoglycaemic episodes

Table 10-4 Classification of hypoglycaemia

Classification of hypoglycaemia		
Level	Glycaemic criteria	Description
Hypoglycaemia alert value (level 1)	< 3.9 mmol/L (70 mg/dL) and ≥ 3.0 mmol/L (54 mg/dL)	Sufficiently low for treatment with fast-acting carbohydrate and dose adjustment of glucose-lowering therapy
Clinically significant hypoglycaemia (level 2)	< 3.0 mmol/L (54 mg/dL)	Sufficiently low to indicate serious, clinically important hypoglycaemia
Severe hypoglycaemia (level 3)[a]	No specific glucose threshold	[a]Hypoglycaemia associated with severe cognitive impairment requiring external assistance for recovery
Notes: The Novo Nordisk terms are adapted from IHSG,[16] ADA,[36] ISPAD,[37] type 1 diabetes outcomes program,[38] ATTD.[39] Severe hypoglycaemia as defined by Seaquist[3] and ISPAD.[37]		

Severe hypoglycaemia

[a] **For adults:** Severe hypoglycaemia is an event requiring assistance of another person to actively administer carbohydrates, glucagon, or take other corrective actions. Plasma glucose concentrations may not be available during an event, but neurological recovery following the return of plasma glucose to normal is considered sufficient evidence that the event was induced by a low plasma glucose concentration.[3]
For adolescents: Severe hypoglycaemia is defined as an event associated with severe cognitive impairment (including coma and convulsions) requiring external assistance of another person to actively administer carbohydrates, glucagon, or take other corrective actions.[37]

Nocturnal hypoglycaemia

Nocturnal hypoglycaemic episodes for adults: episodes occurring between 00:01 and 05:59 both inclusive.

Nocturnal hypoglycaemic episodes for adolescents: episodes occurring between 23:00 and 07:00 both inclusive.

Reporting of hypoglycaemic episodes

Hypoglycaemia is defined as a decline in PG to below 70 mg/dL (3.9 mmol/L). However, in the time period from initiation of the hypoglycaemic clamp procedure (Section 8.2.1) until 45 minutes after dosing, hypoglycaemia is defined as a decline in PG to below 45 mg/dL (2.5 mmol/L).

During the dosing visits (visits 2 and 3 [only for adults]), prior to administration of the IMP, a PG value of <45 mg/dL (2.5 mmol/L) will be treated by an IV glucose solution. After administration of the IMP in the period from 8 minutes until 44 minutes after dosing, a PG value of <45 mg/dL (2.5 mmol/L) will be treated by an IV glucose solution, and if a plasma glucose value of ≥70 mg/dL (3.9 mmol/L) is not achieved within the 45 minutes after IMP administration, IV glucose infusion will also be initiated.

If the participant experiences symptoms of hypoglycaemia, a PG measurement should be taken in order to classify the event.

Reporting of hypoglycaemic episodes by BG meters:
Plasma glucose (PG) should always be recorded in the diary/eCRF when a hypoglycaemic episode is suspected.

When a participant experiences a hypoglycaemic episode, the participant should record the general information in relation to the hypoglycaemia (timing, PG measurements, symptoms, etc.) as described in the diary. The investigator should ensure correct reporting of the hypoglycaemic episode and report the hypoglycaemic episode to the eCRF.

If a participant is not able to fill in the diary at the time of the hypoglycaemic episode (e.g., in case of hospitalisation), the investigator should still report the hypoglycaemic episode on the hypoglycaemic episodes form. The event should be reported as an SAE by the investigator, if applicable according to Appendix 3 (Section 10.3.3) by completing an AE form and a safety information form. One AE form and safety information form can cover several hypoglycaemic episode forms, if the participant has not recovered between the episodes, see Appendix 3 (Section 10.3.3).

Upon onset of a hypoglycaemic episode the participant is recommended to measure PG every 15 minutes until the PG value is ≥3.9 mmol/L (70 mg/dL) and/or symptoms have been resolved in accordance with current guidelines.[3]

Repeated PG measurements and/or symptoms will by default be considered as one hypoglycaemic episode until a succeeding PG value is ≥ 3.9 mmol/L (70 mg/dL) and/or symptoms have been resolved and should be reported as only one hypoglycaemic episode. In case of several low PG values within the hypoglycaemic episode, the lowest value is the one that will be reported as the PG value for the hypoglycaemic episode, but the start time of the episode will remain as the time for the first low PG value and/or symptom. The remaining values will be kept as source data.

If the severity of a hypoglycaemic episode changes, only one hypoglycaemic episode will be reported, reflecting the most severe degree of hypoglycaemia.

Regarding the question: "To feel better, did you need help to get a sugary drink, food, or medicine?" the investigator must instruct the participants to answer "Yes", if the episode was an event that required assistance of another person to actively administer carbohydrate, glucagon, or take other corrective actions. PG concentrations may not be available during an event, but neurological recovery following the return of PG to normal is considered sufficient evidence that the event was induced by a low PG concentration.[3]

Additional information (e.g., description of symptoms, alleviation of symptoms, seizure or coma) in relation to severe hypoglycaemic episodes must be recorded in the hypoglycaemic episode eCRF.

Diary review

The investigator must review the diary data for correct reporting of PG values related to hypoglycaemic episodes according to the SoA (Section 1.2). In case of incomplete or incorrect data in the diary, the participant must be questioned whether there have been any severe hypoglycaemic episodes since the last visit and report accordingly.

For low PG values for hypoglycaemic episodes with incomplete reporting information:

- If a hypoglycaemic episode form in the diary is not completed by the participant within 7 calendar days of the PG measurement, the episode should be described in the source documents and reported by the investigator on a hypoglycaemic episode (e)CRF with as much information as possible. If the participant did not need help to get a sugary drink, food, or medicine, Novo Nordisk will only ask for start date due to recall bias.[40, 41]

Re-training of participants

The participant must be re-trained in how to report hypoglycaemic episodes if the investigator identifies low PG values not reported as hypoglycaemic episodes. The training should be documented by the investigator in source documents.

10.7 Appendix 7: Hyperglycaemic episodes

Plasma glucose (PG) should always be measured and recorded when a hyperglycaemic episode is suspected.

For Adults

A hyperglycaemic episode is defined as PG values >16.7 mmol/L (300 mg/dL). Blood or urine ketones should be measured if the PG value is >16.7 mmol/L (300 mg/dL).

All hyperglycaemic episodes will be reported according to instruction below. When a participant experiences a hyperglycaemic episode, investigator should record the general information in relation to the hyperglycaemia (timing, PG and ketone measurements, symptoms, etc., as described in the diary/eCRF). In case a participant is not able to fill in the diary (e.g., in case of hospitalisation or at the 'follow-up phone contact'), then investigator will report the hyperglycaemic episode in the diary/eCRF.

Repeated PG measurements occurring within a period of 24 hours after onset of a hyperglycaemic episode will per default be considered as one hyperglycaemic episode until a succeeding PG value is ≤16.7 mmol/L (300 mg/dL) and should be reported on one hyperglycaemic episode form. PG measurements >16.7 mmol/L (300 mg/dL) after the 24 hours period shall trigger reporting of a new hyperglycaemia episode. One AE form and safety information form can cover several hyperglycaemic episode forms if the participant has not recovered between the episodes.

In case of several high PG and ketone values within the 24 hours interval, the highest value for PG and ketone, respectively, is the one that will be reported as the PG and ketone value for the hyperglycaemic episode; but the start time of the episode will remain as the time for the first high PG value.

The investigator must review the diary data for correct reporting of hyperglycaemic episodes. High PG values and/or ketones not having a hyperglycaemic episode form completed within 7 calendar days since the PG and/or ketone measurement should be reported on a hyperglycaemic episode form with as much information as possible. Novo Nordisk will not query for additional data, except for the date and PG value due to decreased validity of such data.[40, 41]

The participant must be re-trained in how to report hyperglycaemic episodes if the investigator identifies high PG and/or ketone values not reported as part of a hyperglycaemic episode.

For Adolescents

Blood or urine ketones should be measured if the PG value is >14.0 mmol/L (250 mg/dL) and the participant looks/feels ill.

A hyperglycaemic episode is defined as PG values >14.0 mmol/L (250 mg/dL) AND ketones in urine >moderate or ketones in blood >1.5 mmol/L; which should be reported in the diary according to the SoA and instruction below.

Repeated PG measurements and blood or urine ketone measurements occurring within a period of 24 hours after onset of a hyperglycaemic episode will per default be considered as one

hyperglycaemic episode, until a succeeding PG value is ≤14.0 mmol/L (250 mg/dL), or ketones in the urine are ≤moderate, or ketones in the blood are ≤1.5 mmol/L and should be reported on one hyperglycaemic episode form. PG measurements >14.0 mmol/L (250 mg/dL) AND ketones in urine >moderate or ketones in blood >1.5 mmol/L after the 24 hours period shall trigger reporting of a new hyperglycaemia episode. One AE form and safety information form can cover several hyperglycaemic episode forms if the participant has not recovered between the episodes.

In case of several high PG and ketone values within the 24 hours interval, the highest value for PG and ketone, respectively, is the one that will be reported as the PG and ketone value for the hyperglycaemic episode; but the start time of the episode will remain as the time for the first high PG value.

The investigator must review the diary data at each contact for correct reporting of hyperglycaemic episodes. High PG values where participant looked/felt ill and had elevated ketones, but not having a hyperglycaemic episode form completed in the eCRF within 7 calendar days since the PG measurement, should be reported on a hyperglycaemic episode form in the eCRF with as much information as possible. Novo Nordisk will not query for additional data, except for the date, PG value, ketone value and looking/feeling ill due to decreased validity of such data.[40, 41]

The participant must be re-trained in how to report hyperglycaemic episodes if the investigator identifies high PG and/or ketone values not reported as part of a hyperglycaemic episode.

10.8 Appendix 8: Mitigations to ensure participant safety and data integrity during a crisis

10.8.1 Definition and scope of appendix

A crisis is defined as a situation that causes substantial restrictions to study site access for participants and/or sponsor representatives.

The mitigations mentioned in this appendix must only be used after approval by Novo Nordisk.

According to local regulation, health authorities and independent ethics committees will be notified in case elements of the crisis appendix are activated.

Sites must comply with local regulations, requirements and/or guidelines for the particular crisis if they are issued.

10.8.2 Visits

All visits (per Section 1.2) must be conducted on-site, since they entail ECG measurements and blood sample collection. Therefore, these visits cannot be substituted by remote or home visits. In the event that the site is incapable of performing on-site visits, it should suspend the screening and randomisation of new participants and scheduled visits of randomised/enrolled participants until on-site visits are feasible.

10.9 Appendix 9: Abbreviations

ADA	American Diabetes Association
AE	adverse event
ALT	alanine aminotransferase
AST	aspartate aminotransferase
ATTD	Advanced Technologies & Treatments for Diabetes
AUC	area under the plasma drug concentration time curve
AUE	area under the baseline-adjusted effect curve
AUMC	area under the first moment dasiglucagon concentration-time curve
AxMP	auxiliary medicinal product
BG	blood glucose
BMI	body mass index
CE_{max}	maximum observed baseline adjusted plasma glucose level
CI	confidence interval
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
CL	clearance
COVID-19	Coronavirus disease 2019
CRF	case report form
CSR	clinical study report
CTFG	clinical trial facilitation group
DBL	data base lock
DKA	diabetic ketoacidosis
DRE	disease related event
DUN	dispensing unit number
ECG	electrocardiogram
eCRF	electronic case report form
EU	European Union
EU CTR	European Union Clinical Trial Regulation
FDA	U.S. Food and Drug Administration
FDAAA	FDA Amendments Act
GCP	Good Clinical Practice
eGFR	estimated glomerular filtration rate
H_A	alternative hypothesis
$HbA1_c$	glycated haemoglobin
hCG	human chorionic gonadotropin
HIV	Human Immunodeficiency Virus

HRT	hormone replacement therapy
IB	investigator's brochure
ICH	International Council for Harmonisation
IEC	independent ethics committee
IHSG	The International Hypoglycaemia Study Group
i.m.	intramuscular
IMP	investigational medicinal product
IRB	institutional review board
ISPAD	International Society for Paediatric and Adolescent Diabetes
IUD	intrauterine device
IV	intravenous
IVD	In vitro diagnostic
LAR	legally acceptable representative
LDL	low-density lipoprotein
MAO	monoamine oxidase
MRT	mean residence time
NPH	Neutral Protamine Hagedorn
PCD	primary completion date
PD	pharamcodynamic(s)
PG	plasma glucose
PK	pharmacokinetic(s)
QTL	quality tolerance limit
RMP	Risk management plan
SAE	serious adverse event
SAP	Statistical Analysis Plan
s.c.	subcutaneous
SD	standard deviation
SMPG	self-measured plasma glucose
SoA	schedule of activities
SOP	standard operating procedure
SUSAR	suspected unexpected serious adverse reaction
SWFI	Sterile Water for Injection
T1D	type 1 diabetes mellitus
T2D	type 2 diabetes mellitus
TMM	Trial Materials Manual
UNL	upper normal limit
US	United States

WOCBP	woman of childbearing potential

10.10 Appendix 10: Protocol amendment history

The Protocol amendment summary of changes table for the current protocol version is located directly before the table of contents.

Protocol version 3.0 (24 September 2024)

This amendment is considered to be non-substantial based on the criteria set forth in Article 2(13) of Regulation (EU) No 536/2014 of the European Parliament and of the Council of 16 April 2014;[1] because it neither substantially impacts the safety or rights of the participants nor the reliability or robustness of the data generated in the study.

Overall rationale for preparing protocol, version 3.0:

Section # and name	Description of change	Brief rationale
Section 4.1, Figure 4-2 Clamp Design	Figure was updated to include PK timepoint at 15 minutes.	Updated for alignment with Table 1-1.
Section 6.1 Study intervention(s) administered	Added 'or FreeStyle Precision Neo/Abbott or equivalent' as alternative BG meter or equivalent.	Updated to provide flexibility with use of alternative BG meter or equivalent.
Section 8.2 Dose adjustment guidelines	Added '(if using NovoRapid)' to clarify applicability of discontinuation of the basal rate of insulin pumps on the morning of the dosing day. Added 'and/or glucose solution (10%)' to describe possible use of glucose solution (10%) to achieve targeted glucose level.	Updated for clarity.
Section 8.2.1 Hypoglycaemic clamp procedure	Under 'Dosing day – hypoglycaemic clamp procedure' • deleted 'in the morning' from sentence regarding placement of catheter. • added description for calculation of basal rate and target glucose levels for adults and adolescents to stop insulin infusion.	Updated for clarity and feasibility of placement of catheter at site.
Section 8.10 Immunogenicity assessments	Added inclusion of photograph as a part of relevant exploratory tests for follow-up on the hypersensitivity reactions.	Updated for clarity and to align with ICFs.
Section 10.2 Appendix 2: Clinical laboratory tests	Added text "at the site or subcontracted by the site" to describe local laboratory. Deleted Table 10-2 footnote 'b' and replaced with footnote 'c' as applicable to align with SoA.	Updated for clarity and to align with other text in this section. Updated for clarity and to align with the SoA.

Protocol version 2: (27 June 2024**), global**

This amendment is considered to be non-substantial based on the criteria set forth in Article 2(13) of Regulation (EU) No 536/2014 of the European Parliament and of the Council of 16 April 2014; because it neither substantially impacts the safety or rights of the participants nor the reliability or robustness of the data generated in the study.

Overall rationale for preparing protocol version 2.0

Section # and name	**Description of change**	**Brief rationale**
Section 1.1 Synopsis	Specified description of study intervention related products as 'glucagon or its derivatives' in the key exclusion criterion.	Updated in response to PMDA enquiry for clarity.
Section 5.2 Exclusion criteria	Specified description of study intervention related products as 'glucagon or its derivatives'.	Updated in response to PMDA enquiry for clarity.
Section 6.8.1 Rescue medicine	Added description to clarify dosing condition for rescue medicine for adolescents.	Updated in response to PMDA enquiry for clarity.
Section 10.4.2 Contraceptive guidance	Clarified text to describe the requirement of use of contraceptives during the study from screening till end of study.	Updated in response to PMDA enquiry for clarity.

11 References

1. The European Parliament and the Council of the European Council of the European Union. Regulation (EU) No 536/2014 of the European Parliament and of the Council of 16 April 2014 on clinical trials on medicinal products for human use, and repealing Directive 2001/20/EC. 27 May 2014.
2. Clarke WL, Cox DJ, Gonder-Frederick LA, Julian D, Schlundt D, Polonsky W. Reduced awareness of hypoglycemia in adults with IDDM. A prospective study of hypoglycemic frequency and associated symptoms. Diabetes Care. 1995;18(4):517-22.
3. Seaquist ER, Anderson J, Childs B, Cryer P, Dagogo-Jack S, Fish L, et al. Hypoglycemia and diabetes: a report of a workgroup of the American Diabetes Association and the Endocrine Society. Diabetes Care. 2013;36(5):1384-95.
4. Cryer PE. The barrier of hypoglycemia in diabetes. Diabetes. 2008;57(12):3169-76.
5. Committee ADAPP. 6. Glycemic Goals and Hypoglycemia: Standards of Care in Diabetes-2024. Diabetes Care. 2024;47(Suppl 1):S111-S25.
6. Davies MJ, Gagliardino JJ, Gray LJ, Khunti K, Mohan V, Hughes R. Real-world factors affecting adherence to insulin therapy in patients with Type 1 or Type 2 diabetes mellitus: a systematic review. Diabet Med. 2013;30(5):512-24.
7. Pedersen-Bjergaard U, Pramming S, Heller SR, Wallace TM, Rasmussen AK, Jørgensen HV, et al. Severe hypoglycaemia in 1076 adult patients with type 1 diabetes: influence of risk markers and selection. Diabetes Metab Res Rev. 2004;20(6):479-86.
8. Khunti K, Alsifri S, Aronson R, Berković MC, Enters-Weijnen C, Forsén T, et al. Rates and predictors of hypoglycaemia in 27,585 people from 24 countries with insulin-treated type 1 and type 2 diabetes: the global HAT study. Diabetes Obes Metab. 2016.
9. UK Hypoglycaemia Study Group. Risk of hypoglycaemia in types 1 and 2 diabetes: effects of treatment modalities and their duration. Diabetologia. 2007;50(6):1140-7.
10. Iwahori TS, Frank. Nagai, Yukiko. Spaepen, Erik. Mitchell, Beth D. Peyrot, Mark. Conversations and Reactions Around Severe Hypoglycemia (CRASH): Japan Results From a Global Survey of People with T1DM or Insulin-Treated T2DM and Caregivers. Diabetes Ther. 2022;13:517–33.
11. Cryer PE. Hypoglycaemia: the limiting factor in the glycaemic management of Type I and Type II diabetes. Diabetologia. 2002;45(7):937-48.
12. Frier BM. How hypoglycaemia can affect the life of a person with diabetes. Diabetes-Metabolism Research and Reviews. 2008;24(2):87-92.
13. ElSayed NA, Aleppo G, Aroda VR, Bannuru RR, Brown FM, Bruemmer D, et al. 6. Glycemic Targets: Standards of Care in Diabetes-2023. Diabetes Care. 2023;46(Suppl 1):S97-S110.
14. Araki E, Goto A, Kondo T, Noda M, Noto H, Origasa H, et al. Japanese Clinical Practice Guideline for Diabetes 2019. Diabetol Int. 2020;11(3):165-223.
15. American Diabetes Association. Standards of medical care in diabetes - 2017. Diabetes Care. 2017;40(Suppl 1):S1-135.
16. International Hypoglycaemia Study Group. Glucose Concentrations of Less Than 3.0 mmol/L (54 mg/dL) Should Be Reported in Clinical Trials: A Joint Position Statement of the American Diabetes Association and the European Association for the Study of Diabetes. Diabetes Care. 2017;40(1):155-7.
17. Christensen M, Bagger JI, Vilsbøll T, Knop FK. The alpha-cell as target for type 2 diabetes therapy. Rev Diabet Stud. 2011;8(3):369-81.
18. Lund A, Bagger JI, Christensen M, Knop FK, Vilsbøll T. Glucagon and type 2 diabetes: the return of the alpha cell. Curr Diab Rep. 2014;14(12):555.
19. Zealand Pharma A/S. Dasiglucagon NDA 214231 Summary Review. 2021.

20. Novo Nordisk A/S. Zegalogue® (dasiglucagon), US Prescribing Information (PI). Jun 2023.
21. Zealand Pharma A/S. Modeling and Simulation Report (20-046). Pharmacokinetic/Pharmacodynamic Modeling of Dasiglucagon in Subjects with Type 1 Diabetes Mellitus. 27 Apr 2021.
22. The European Parliament and the Council of the European Council of the European Union. Regulation (EU) No 536/2014 of the European Parliament and of the Council of 16 April 2014 on clinical trials on medicinal products for human use, and repealing Directive 2001/20/EC. Annex VI - Labelling of Investigational medicinal products and auxiliary medicinal products. 27 May 2014.
23. European Commission. Ethical considerations for clinical trials on medicinal products conducted with minors. Recommendations of the expert group on clinical trials for the implementation of Regulation (EU) No 536/2014 on clinical trials on medicinal products for human use. 2017.
24. Liu D, Moberg E, Kollind M, Lins PE, Adamson U, Macdonald IA. Arterial, arterialized venous, venous and capillary blood glucose measurements in normal man during hyperinsulinaemic euglycaemia and hypoglycaemia. Diabetologia. 1992;35(3):287-90.
25. McGuire EA, Helderman JH, Tobin JD, Andres R, Berman M. Effects of arterial versus venous sampling on analysis of glucose kinetics in man. J Appl Physiol. 1976;41(4):565-73.
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.
28. World Medical Association. WMA Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects. Last amended by the 64th WMA General Assembly, Fortaleza, Brazil. October 2013.
29. ICH Harmonised Tripartite Guideline. Guideline for Good Clinical Practice E6(R2), Current step 4 version. 09 Nov 2016.
30. De Angelis C, Drazen JM, Frizelle FA, Haug C, Hoey J, Horton R, et al. Clinical trial registration: a statement from the International Committee of Medical Journal Editors. N Engl J Med. 2004;351(12):1250-1.
31. U.S. Department of Health and Human Services, Food and Drug Administration. Food and Drug Administration Amendments Act of 2007 as amended by the Final Rule "Clinical Trials Registration and Results Information Submission". 21 September 2016.
32. The European Parliament and the Council of the European Council. Regulation (EC) No 726/2004 of the European Parliament and of the Council of 31 March 2004 laying down Community procedures for the authorisation and supervision of medicinal products for human and veterinary use and establishing a European Medicines Agency, article 57. 30 April 2004.
33. The European Parliament and the Council of the European Council. Regulation (EC) No 1901/2006 of the European Parliament and of the Council of 12 December 2006 on medicinal products for paediatric use and amending Regulation (EEC) No 1768/92, Directive 2001/20/EC, Directive 2001/83/EC and Regulation (EC) No 726/2004, article 41. Official Journal of the European Communities. 27 Dec 2006.
34. International Committee of Medical Journal Editors. Recommendations for the Conduct, Reporting, Editing and Publication of Scholarly Work in Medical Journals; current version available at www.icmje.org.

35. Clinical Trial Facilitation Group (CTFG), Heads of Medicines Agency. Recommendations related to contraception and pregnancy testing in clinical trials. 21 Sep 2020.
36. American Diabetes Association. 6. Glycemic Targets: Standards of Medical Care in Diabetes-2018. Diabetes Care. 2018;41(Suppl 1):S55-S64.
37. Abraham MB, Jones TW, Naranjo D, Karges B, Oduwole A, Tauschmann M, et al. ISPAD Clinical Practice Consensus Guidelines 2018: Assessment and management of hypoglycemia in children and adolescents with diabetes. Pediatr Diabetes. 2018;19 Suppl 27:178-92.
38. Agiostratidou G, Anhalt H, Ball D, Blonde L, Gourgari E, Harriman KN, et al. Standardizing Clinically Meaningful Outcome Measures Beyond HbA1c for Type 1 Diabetes: A Consensus Report of the American Association of Clinical Endocrinologists, the American Association of Diabetes Educators, the American Diabetes Association, the Endocrine Society, JDRF International, The Leona M. and Harry B. Helmsley Charitable Trust, the Pediatric Endocrine Society, and the T1D Exchange. Diabetes Care. 2017;40(12):1622-30.
39. Danne T, Nimri R, Battelino T, Bergenstal RM, Close KL, DeVries JH, et al. International Consensus on Use of Continuous Glucose Monitoring. Diabetes Care. 2017;40(12):1631-40.
40. US Food and Drug Administration. Guidance for Industry. Patient-Reported Outcome Measures: Use in Medical Product Development to Support Labeling Claims. December 2009.
41. Stull DE, Leidy NK, Parasuraman B, Chassany O. Optimal recall periods for patient-reported outcomes: challenges and potential solutions. Curr Med Res Opin. 2009;25(4):929-42.