30 May 2024
EMA/281112/2024
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Zegalogue

International non-proprietary name: dasiglucagon

Procedure No. EMEA/H/C/006214/0000

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

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List of abbreviations

ADA	Anti-drug antibody
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
AUC	Area under the concentration-time curve
Cl	Clearance
Cmax	Maximum observed concentration
CYP	Cytochrome P450
DRF	Dose-range finding
DHAP	Dual hormone artificial pancreas
EC50	Half-maximal effective concentration
EDTA	Ethylenediaminetetraacetic acid
FAS	Full analysis set
G	Gestation day
GCGR	Glucagon receptor
GFR	Glomerular filtration rate
GLP	Good Laboratory Practice
hERG	Human ether-a-go-go-related gene
KM	Kaplan-Meyer
ICH	International Conference on Harmonisation
Ig	Immunoglobulin
IM	Intramuscular
IMP	Investigational medicinal product
IND	Investigational New Drug
IV	Intravenous
LC	Liquid chromatography
LC-MS/MS	Liquid chromatography coupled to tandem mass spectrometry
MRM	Multiple reaction monitoring
MS	Mass spectrometry
MTD	Maximum tolerated dose
Nab	Neutralizing antibody
NOAEL	No observed adverse effect level
PD	Pharmacodynamic(s)
PG	Plasma glucose
PK	Pharmacokinetic(s)
PP	Per protocol
PPS	Per protocol set
SC	Subcutaneous
SPE	Solid phase extraction
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
t1/2	Elimination half-life
TK	Toxicokinetic(s)
Vss	Volume of distribution at steady state

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Zealand Pharma A/S submitted on 23 June 2023 an application for a marketing authorisation to the European Medicines Agency (EMA) for Zegalogue, through the centralised procedure under Article 3 (2)(a) of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 13 October 2022.

The applicant applied for the following indication:

Dasiglucagon is indicated for the treatment of severe hypoglycaemia in adults, adolescents, and children aged 6 years and over with diabetes mellitus.

1.2. Legal basis, dossier content

The legal basis for this application refers to Article 8.3 of Directive 2001/83/EC - complete and independent application.

The application submitted is composed of administrative information, complete quality data, non-clinical and clinical data based on applicants' own tests and studies and/or bibliographic literature substituting/supporting certain test(s) or study(ies).

1.3. Information on paediatric requirements

At the time of submission of the application, the PIP (EMEA- 002233-PIP01-17-M02, decision number P/0176/2023) was not yet completed as some measures were deferred.

1.4. Information relating to orphan market exclusivity

1.4.1. Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant did not submit a critical report addressing the possible similarity with authorised orphan medicinal products because there is no authorised orphan medicinal product for a condition related to the proposed indication.

1.5. Applicant's request(s) for consideration

1.5.1. New active substance status

The applicant requested the active substance dasiglucagon contained in the above medicinal product to be considered as a new active substance, as the applicant claims that it is not a constituent of a medicinal product previously authorised within the European Union.

1.6. Steps taken for the assessment of the product

The Rapporteur and Co-Rapporteur appointed by the CHMP were:

Rapporteur: Paolo Gasparini Co-Rapporteur: Hjalti Kristinsson

The application was received by the EMA on	23 June 2023
The procedure started on	13 July 2023
The CHMP Rapporteur's first Assessment Report was circulated to all CHMP and PRAC members on	9 October 2023
The PRAC Rapporteur's first Assessment Report was circulated to all PRAC and CHMP members on	18 October 2023
The CHMP agreed on the consolidated List of Questions to be sent to the applicant during the meeting on	9 November 2023
The applicant submitted the responses to the CHMP consolidated List of Questions on	21 January 2024
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Questions to all CHMP and PRAC members on	26 February 2024
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	7 March 2024
The CHMP agreed on a list of outstanding issues to be sent to the applicant on	21 March 2024
The applicant submitted the responses to the CHMP List of Outstanding Issues on	30 April 2024
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP and PRAC members on	17 March 2024
The CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a marketing authorisation to Zegalogue on	30 May 2024
Furthermore, the CHMP adopted a report on New Active Substance (NAS) status of the active substance contained in the medicinal product (see Appendix on NAS)	30 May 2024

2. Scientific discussion

2.1. Problem statement

2.1.1. Disease or condition

The claimed indication for dasiglucagon is “the treatment of severe hypoglycaemia in adults, adolescents, and children aged 6 years and over with diabetes mellitus."

Recurrent hypoglycemia and fear of hypoglycemia remain some of the greatest barriers for achieving optimal glycemic control in patients with diabetes, especially treated with insulin [1, 2]. Severe hypoglycemia, characterized by severe cognitive impairment requiring assistance from another person for recovery, is feared by patients and their relatives and may discourage patients and caregivers from pursuing appropriate glycemic targets. A severe hypoglycemic episode may also in itself have serious medical consequences. In addition to the well-known associations with cardiac arrhythmias and increased risk of accidents due to impaired consciousness, severe hypoglycemia has been associated with increased risks of several adverse, long-term clinical outcomes including cardiovascular events and death [3, 4].

Whether symptomatic or not, hypoglycemia can lead to or worsen the so-called "impaired awareness of hypoglycaemia", that is the impairment in the patient subjective perception of its own hypoglycemic state and thus the impossibility to take appropriate corrective measures. This leads to a vicious cycle that can result in increasingly worse hypoglycemic episodes.

Ref.
1. Cryer PE. Hypoglycaemia: the limiting factor in the glycaemic management of type I and type II diabetes. Diabetologia 2002;45(7):937-48
2. Frier BM. How hypoglycaemia can affect the life of a person with diabetes. Diabetes Metab Res Rev 2008;24(2):87-92. D
3. Zoungas S, Patel A, Chalmers J, et al. Severe hypoglycemia and risks of vascular events and death. N Engl J Med 2010;363(15):1410-8.
4. McCoy RG, Van Houten HK, Ziegenfuss JY, Shah ND, Wermers RA, Smith SA. Increased mortality of patients with diabetes reporting severe hypoglycemia. Diabetes Care 2012;35(9):1897-901.

2.1.2. Epidemiology

In spite of advances in diabetes treatment, severe hypoglycemia remains a relatively frequent complication. Event rates are not precisely established, but a Danish-British multicenter survey of 1076 consecutive adult patients with type 1 diabetes mellitus (T1DM) who completed a detailed questionnaire on hypoglycemia and related issues determined that the overall rate of severe hypoglycemia in the preceding year was 1.3 episodes/patient-year, with episodes being reported for 37% of patients [6].

In a 6-month retrospective and 4-week prospective global study in 27,585 people from 24 countries with insulin-treated diabetes, the rate of severe hypoglycemia in patients with T1DM was 4.9 episodes/patient-year [7]. The incidence of severe hypoglycemia in T2DM patients receiving insulin has been estimated to be about third to half of that observed in patients with T1DM [7,8].

In children with diabetes the incidence of severe hypoglycemia (comprising hypoglycemic) has fallen during the last decades but nonetheless remains a clinical challenge, with a current rate of 3-7 episodes/100 patient-years [9].

Ref.
6. Pedersen-Bjergaard U, Pramming S, Heller SR, 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.
7. Khunti K, Alsifri S, Aronson R, 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;18(9):907-15.
8. U. K. 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.

9. Abraham MB, Jones TW, Naranjo D, et al. ISPAD Clinical Practice Consensus Guidelines 2018: Assessment and management of hypoglycemia in children and adolescents with diabetes. Pediatr Diabetes 2018;19 Suppl 27:178-192.

2.1.3. Aetiology and pathogenesis

Hypoglycemia develops when the rate at which blood glucose is used exceeds the rate at which it enters the bloodstream. The most common mechanism for this unbalance is a lower than normal influx of glucose into the blood.

In normal conditions, when blood glucose decreases a parallel decrease in insulin level is observed; if glucose decreases further, an increase of counterregulatory hormones (mainly glucagon) is elicited. However, in patients with diabetes mellitus in therapy with insulin (when there is an absolute deficit of endogenous insulin) these mechanisms can fail; for instance, insulin cannot decrease, being exogenous, in response to falling blood glucose levels. The increase in endogenous glucagon is impaired as well, since the main signal for its secretion is the reduction in insulin levels. Moreover, behavioural countermeasures (mainly intake of carbohydrates) can be defective because of an impairment of the sympathetic system (due to the underlying disorder and to recurrent hypoglycemic episodes) that is involved in producing the symptoms eliciting the behavioural response.

Therefore, the main physiologic measures against hypoglycemia in patients with T1DM or advanced T2DM are defective or lost. The pathogenesis of hypoglycemia is the same on both types of diabetes mellitus; the only difference is the temporal pattern due to the time course of onset of absolute insulin deficiency. This occurs very early in T1DM whereas it develops slowly over many years in T2DM.

Hypoglycemia is an important aspect of diabetes mellitus since it is the main factor limiting the use and dosage of anti-diabetic treatments and, thus, it precludes reaching and maintaining normal blood glucose levels in the long-term treatment.

2.1.4. Clinical presentation, diagnosis

The symptoms and signs of hypoglycemia are non-specific. Symptomatic (i.e. clinical) hypoglycemia can be documented by Whipple triad: (1) symptoms or signs consistent with hypoglycemia; (2) a low reliably measured plasma glucose concentration; and (3) resolution of those symptoms and signs after the plasma glucose concentration is raised.

Hypoglycemia symptoms can be classified in two groups according to their pathogenesis:

- neurogenic (or autonomic) symptoms, due the perception of physiologic changes caused by the sympathoadrenal discharge triggered by hypoglycemia; they include adrenergic (catecholamine-mediated) symptoms such as palpitations, tremor, and anxiety/arousal and cholinergic (acetylcholine-mediated) symptoms such as sweating, hunger, and paresthesias.
- neuroglycopenic symptoms, a direct result of brain glucose deprivation; they include cognitive impairments, behavioral changes, psychomotor abnormalities, and at lower glucose levels, seizure and coma.

Neurogenic symptoms are important since the subjective awareness of hypoglycemia is mainly due to their perception by the patient.

Severe hypoglycemia is defined as a hypoglycemic episode of such severity as to require the assistance of another person (e.g. to actively administer carbohydrate, glucagon, or other resuscitative actions). The latter type of episodes is the intended use for the current product indication.

2.1.5. Management

Mild cases of hypoglycemia can be treated by the patient her/himself with oral intake of carbohydrates.

Severe hypoglycemia requires a more complex approach with parental administration of glucagon (via subcutaneous or intramuscular injection) usually at the dose of 1.0 mg. Recovery usually occurs within

15-20 minutes. In medical settings, for the treatment of severe hypoglycemia, intravenous glucose is usually considered the standard therapy.

The intended use for dasiglucagon is the treatment of severe hypoglycemia; it is expected to be administered mostly by caregivers (i.e. people who are not healthcare professionals).

Currently, different glucagon formulations are available in EU. Some require constitution before being administered to the patient; this poses some problems since glucagon is usually administered by caregivers that are not healthcare professionals and thus might be uncomfortable to prepare and perform injections. Moreover, time is required to reconstitute the glucagon formulation, potentially causing treatment delay.

There are also 2 available ready to use formulations: Ogluo that is already reconstituted, and Baqsimi, an intranasal formulation; both are centrally approved. Some available ready-to-use formulations seem to have a slightly longer "time to patient recovery" with respect to comparators (i.e. glucagon formulations requiring reconstitution) when "time from administration" to patient recovery is considered an outcome measure; in some cases this small delay might persist even when "time from decision" is considered instead (that is, the shorter time required for administration might be unable to compensate for the observed longer time required for the onset of the effect).

2.2. About the product

Dasiglucagon is a synthetic glucagon analogue peptide comprised of 29 amino acids, with 7 amino acid substitutions compared to glucagon, which have been introduced to improve physical and chemical stability in aqueous media.

Dasiglucagon increases blood glucose concentration by activating hepatic glucagon receptors, thereby stimulating glycogen breakdown and release of glucose from the liver. Hepatic stores of glycogen are necessary for dasiglucagon to produce an antihypoglycaemic effect.

The claimed indication for dasiglucagon is "the treatment of severe hypoglycaemia in adults, adolescents, and children aged 6 years and over with diabetes mellitus."

The finished product is presented as solution for injection in single-dose pre-filled pen and syringe containing 0.6 mg dasiglucagon (hydrochloride salt) as active substance. The proposed posology is 0.6 mg administered by a single subcutaneous injection.

2.3. Quality aspects

2.3.1. Introduction

The finished product is presented as solution for injection in pre-filled pen and pre-filled syringe, each pre-filled pen and pre-filled syringe contain 0.6 mg dasiglucagon (as a hydrochloride salt) as active substance.

Other ingredients are: trometamol, sodium chloride, water for injections, hydrochloric acid (for pH adjustment), and sodium hydroxide (for pH adjustment)
The pre-filled pen (hereafter referred to as auto-injector) is available in glass (type I) pre-filled syringe with staked stainless steel needle, rigid needle shield (polypropylene and natural rubber), and rubber plunger (bromobutyl). The pre-filled syringe is assembled into a disposable pre-filled pen with a grey cap as described in section 6.5 of the SmPC.

The pre-filled syringe (hereafter referred to as ready-to-use (RtU) pre-filled syringe) is available in glass (type I) pre-filled syringe with staked stainless steel needle, rigid needle shield/grey needle cover, rubber plunger (bromobutyl) and red plunger rod (polypropylene) as described in section 6.5 of the SmPC.

2.3.2. Active substance

General information

The active substance is a synthetic linear 29-amino acid peptide with a free amino group at the N-terminus and a carboxylic acid at the C-terminus. The active substance is isolated as a hydrochloride salt. The counterion forms ion pairs with positively charged functional groups, e.g. amines (N-terminus, lysine side chains) or guanidines (arginine side chains)

The chemical name of the active substance is L-Histidyl-L-seryl-L-glutaminylglycyl-L-threonyl-L-phenylalanyl-L-threonyl-L-seryl-L-aspartyl-L-tyrosyl-L-seryl-L-lysyl-L-tyrosyl-L-leucyl-L-aspartyl-α-aminoisobutyryl-L-alanyl-L-arginyl-L-alanyl-L-glutamyl-L-glutamyl-L-phenylalanyl-L-valyl-L-lysyl-L-tryptophyl-L-leucyl-L-glutamyl-L-seryl-L-threonine, hydrochloride salt corresponding to the molecular formula $C_{152}H_{222}N_{38}O_{50}$. It has a relative molecular mass of 3381.7 and the following structure:

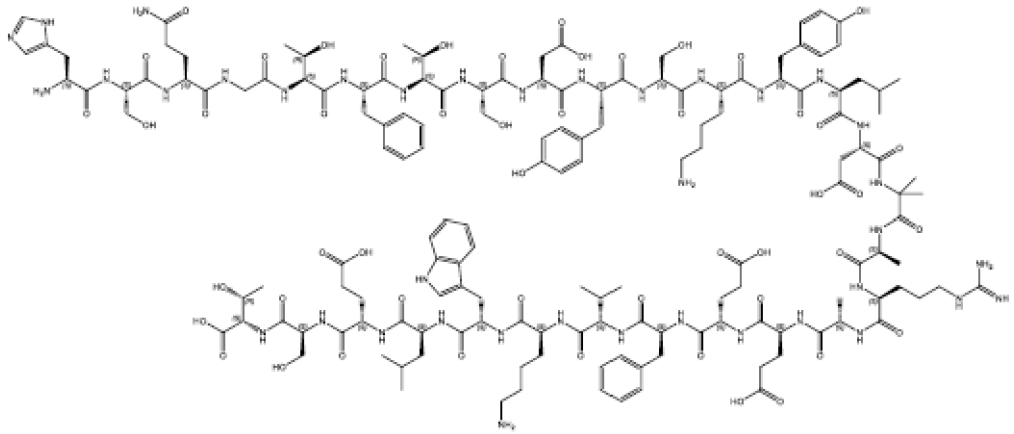


Figure 1: Active substance structure

The chemical structure of the active substance was elucidated by a combination of evidence from route of synthesis, aminoacid analysis, gas chromatography - mass spectrometry (GC-MS), mass spectrometry (ESI-MS and ESI-MS/MS), and evidence from circular dichroism.

The active substance is a very hygroscopic white to off-white powder freely soluble in water and soluble at the pH and the concentration used in the finished product.

All optically active amino acid residues are in L-configuration.

The active substance is an amorphous powder, no crystalline or polymorphic forms have been identified

Manufacture, characterisation and process controls

The active substance is manufactured by one manufacturing site.

Detailed information on the manufacturing of the active substance has been provided in the restricted part of the ASMF and it was considered satisfactory.

The active substance is synthesized in 4 main steps: solid phase peptide synthesis (SPPS), cleavage from the resin including cleavage of the side-chain protecting groups, purification by preparative HPLC, and using commercially available well defined starting materials with acceptable specifications.

Both potential and actual impurities have been explored. The formation of impurities may arise from starting materials manufacturing process (insertion/deletion of sequence, cleavage), degradation, process reagents, solvents etc.

Genotoxic impurities have been assessed with negative outcome., potential impurities of isopropanol and ACN (acetonitrile), respectively, have been tested negative in the three validation batches and will not be tested at release. The approach is deemed acceptable.

Additional risk assessment which takes into consideration the active substance manufacturing process was provided, herewith including the evaluation of the impact of reagents and solvents. In this risk assessment the potential presence of nitrites have been identified and analytical tests have been carried out in order to determine the nitrites content and eventual carry-on into dasiglucagon active substance. The risk is considered negligible, but as additional measure, the manufacturer will test for nitrites all incoming batches in the future. The issue was deemed acceptable.

Adequate in-process controls are applied during the synthesis. The specifications and control methods for intermediate products, starting materials and reagents have been presented.

The characterisation of the active substance and its impurities are in accordance with the EU guideline on chemistry of new active substances.

Potential and actual impurities were well discussed with regards to their origin and characterised.

The active substance is packaged in bottles which complies with Commission Regulation (EU) 10/2011, as amended.

Specification

The active substance specification includes tests for appearance (visual), appearance of solution (visual), identification (MS, amino acid analysis), identification of chloride (Ph. Eur.), purity (HPLC), related impurities (HPLC), assay (Elemental analysis), water content (KF), chloride content (Potentiometric titration), residual organic solvents (GC), mass balance (calculation), bacterial endotoxins (Ph. Eur.), and microbiological limit test (Ph. Eur.)

The proposed specification was established using an integrated control strategy based on product and process knowledge gained through process development, characterization studies, manufacturing experience, release data and stability data. The specification was also developed by following the recommendations of the ICH guidelines Q3C (1), Q6A (2), M7 (3), and the Ph. Eur. general monograph 2034, taking the general and product-specific characteristics into account.

Impurities present at higher than the qualification threshold according to ICH Q3A were qualified by toxicological and clinical studies and appropriate specifications have been set.

The analytical methods used have been adequately described and (non-compendial methods) appropriately validated in accordance with the ICH guidelines. Satisfactory information regarding the reference standards used for assay and impurities testing has been presented.

Batch analysis data (4 commercial scale batches) of the active substance are provided. The results are within the specifications and consistent from batch to batch.

Stability

Stability data from 3 commercial scale batches of active substance from the proposed manufacturer stored in the intended commercial package for up to 60 months under long term conditions (-20 °C and 5 °C) and for up to 6 months under accelerated conditions (25 °C/60% RH and 40 ºC / 75% RH) according to the ICH guidelines were provided.

The following parameters were tested: appearance, assay, purity, related impurities, water content, chloride content and mass increase.

The total related impurities and consequently the purity remained unchanged during storage at -20 °C and showed a slight purity decrease at 5 °C within the requirement for up to 60 months. A clear increase in total related impurities and a decrease of the purity was observed after storage of all batches at 25 °C/60% RH.

One batch of the active substance was exposed to stress testing. Based on the results obtained, it can be concluded that the active is stable when stored in a closed container.

The stability results indicate that the active substance manufactured by the proposed supplier is sufficiently stable. The stability results justify the proposed retest period of 60 months stored in a freezer (< -15 °C) in the proposed container.

2.3.3. Finished Medicinal Product

Description of the product and pharmaceutical development

The finished product is presented as clear, colourless solution, pH 6.5 and osmolality of 330-490 mOsm/kg.

The finished product is an unpreserved, sterile, clear and colourless solution for subcutaneous injection filled into single-dose pre-filled syringes (PFS). The PFS constitutes the finished product in its primary container closure system and is subsequently final assembled into an auto-injector (pre-filled pen) or mounted with a plunger rod, resulting in two integral combination product presentations, i.e. dasiglucagon auto-injector and dasiglucagon ready-to-use pre-filled syringe (pre-filled syringe), respectively.

The formulation development work was focused on obtaining the best chemical stability for the active substance, without compromising its physical stability. Dasiglucagon is a physically stable glucagon analogue and therefore the main focus of the formulation development work was to obtain the best chemical stability.

All excipients are well known pharmaceutical ingredients and their quality is compliant with Ph. Eur. standards. There are no novel excipients used in the finished product formulation. The list of excipients is included in section 6.1 of the SmPC.

During development, a quality target product profile (QTPP) was established for the finished product and regularly revised to guide the development process.

During formulation development, Design of experiments (DoE) screening of excipients were performed in order to select the excipients and tonicity agents that could best contribute to the stability of the formulation. However, no design space was claimed the applicant.

The finished product manufacturing process is common process for solutions for injections using standard equipment and was developed to achieve optimal product quality and process consistency. The process consists of the following steps: compounding of the drug product bulk solution, sterilization by filtration, aseptic filling, stoppering and visual inspection. No design spaces were claimed for the manufacturing process of the finished product.

A scientific and risk-based approach, which included laboratory and full scale manufacturing characterisation studies and process risk assessments, was applied during manufacturing process development, to establish the critical manufacturing process steps, critical process parameters as well

as in-process controls for the commercial process in accordance with the requirements of ICH Q8, ICH Q9 and ICH Q10. The outcome of the process development is a robust manufacturing process that consistently produces drug product of the desired quality when operated within the process parameter ranges established for the commercial process. Detailed data and rationales for the established process parameters (critical and non-critical) for each process step, as well as identification of critical steps were provided.

The principles for the choice of sterilisation process for finished products and containers are presented in the form of decision trees in the 'Guideline on the Sterilisation of the Medicinal Product, Active Substance, Excipient and Primary Container' are also relevant for synthetic peptides. Sterilisation by sterile filtration combined with aseptic filling into pre-sterilized container closure system was selected due to the intrinsic nature of dasiglucagon (heat sensitive peptide) which makes terminal sterilization of the finished product impossible. This was considered acceptable.

Furthermore, the finished product manufacturing process in commercial scale has been validated and shown to consistently produce batches of the desired quality. Process Validation and/or Evaluation.

The final assembly process of the RtU-PFS is a simple, standard, automatic assembly process. The PFS is mounted with a plunger rod and attached a label using a standard labelling and plunger rod insertion machine. The final assembly process of the auto-injector is also a simple, standard assembly process, using qualified auto-injector assembly equipment. The final assembly processes of both combination product presentations have been validated and shown to consistently produce dasiglucagon RtU-PFS and AI batches of the desired quality and performance.

The pre-filled pen is available in glass (type I) pre-filled syringe with staked stainless steel needle, rigid needle shield (polypropylene and natural rubber), and rubber plunger (bromobutyl). The pre-filled syringe is assembled into a disposable pre-filled pen with a grey cap.

The pre-filled syringe is available in glass (type I) pre-filled syringe with staked stainless steel needle, rigid needle shield/grey needle cover, rubber plunger (bromobutyl) and red plunger rod (polypropylene).

During evaluation the CHMP requested the Notified body opinion on the medical devices to be provided, which should confirm full compliance with the relevant General Safety and Performance Requirements (GSPRs) in Annex I of Regulation (EU) 2017/745. As a response the applicant provided the Notified Body opinions on the medical devices (ready-to-use pre-filled syringe and auto-injector) confirming full compliance. The response was considered satisfactory.

Leachables and extractables study was conducted during development to evaluate potential for leachables and extractables from primary packaging into finished product solution (contact solution).

Extractables studies and leachables study performed during development supports of the chosen primary packaging components for the finished product.

The choice of the container closure system has been validated by stability data and is adequate for the intended use of the product.

Manufacture of the product and process controls

The finished product is manufactured by one manufacturing site.

The manufacturing process consists of 4 main steps: compounding the finished product, sterilization by filtration, aseptic filling of the bulk solution into pre-sterilized, pyrogen-free syringes and subsequent sealing with rubber plungers and finally assembling the filled, stoppered syringes into either an auto-injector (AI) or a ready-to-use prefilled syringe (RtU-PFS) to form the finished combination products.

The process is considered to be a non-standard manufacturing process since an aseptic processing is involved.

The critical process parameters (CPPs) were identified by a risk-based approach using a process risk assessment. The following steps in the drug product process were identified as critical: Compounding, sterile filtration, filling and visual inspection. The in-process controls are adequate for this type of manufacturing process. An overview of the control strategy for each of the critical process steps is provided in the dossier; this is acceptable.

Appropriate validation of the manufacturing process has been performed using three consecutive commercial scale batches. The results from manufacture of the dasiglucagon injection 1 mg/mL PFS PPQ batches demonstrate low intra- and inter-batch variability confirming that the commercial manufacturing process in the commercial scale is reproducible. The compounding, sterile filtration, filling and visual inspection process of the finished product can be considered suitable for the intended use.

Also the final assembly process of both the auto-injector and pre-filled syringe is validated and suitable for the intended use.

Product specification

The finished product release and shelf life specifications, include appropriate tests for these kind of dosage forms: appearance of solution (visual), visible particles (visual), identity (HPLC), assay (HPLC), impurities (HPLC), pH (Ph. Eur.), osmolality (Ph. Eur.), uniformity of dosage units (Ph. Eur.), extractable volume (Ph. Eur) (only pre-filled syringes), sub visible particles (Ph. Eur.), sterility (Ph. Eur.), container closure integrity (USP) (only for auto-injector), bacterial endotoxins (Ph. Eur.), dose accuracy (ISO 11608-1) (only for auto-injector) and activation force (ISO 11608-5) (only for auto-injector), deliverable volume (Ph. Eur.) (only for RtU-PFS), and break loose and glide force (ISO 11040-8) (only for RtU-PFS).

Dasiglucagon is a synthetic peptide and is therefore excluded from the scope of ICH Q3B(R2). However, the identification threshold for impurities have been set based on the principles in ICH Q3B(R2).

All impurities found above the identification threshold, in the commercial formulation at release and/or at end of shelf life have been identified. All impurities found above the qualification threshold have been qualified.

The suitability of sterility and bacterial endotoxins test procedures for use in the finished products has been demonstrated by verification testing according to compendial requirements. The combination product functional tests are performed based on relevant International Standards Organization (ISO) standards and/or Ph. Eur.

Potential presence of elemental impurities in the finished product has been assessed following a risk-based approach in line with the ICH Q3D Guideline for Elemental Impurities. Batch analysis data on using a validated method was provided, demonstrating that each relevant elemental impurity was not detected above 30% of the respective PDE. Based on the risk assessment it can be concluded that it is not necessary to include any elemental impurity controls.

During evaluation, regarding the possible presence of nitrosamines no discussion was provided regarding possible formation of nitrosamines in the active substance. The ASMF holder was requested, as MO, to provide a rationale for the risk assessment of nitrosamines presence and carry-over, also taking into consideration starting materials, process reagents and process solvents (low molecular amine precursors are observed during synthesis). Nitrite and nitrate levels of the water are not presented, the applicant indicated that a risk evaluation was conducted and that no risk of presence of

nitrosamines was identified. The applicant submitted an additional risk assessment which takes into consideration the active substance manufacturing process, herewith including the evaluation of the impact of reagents and solvents. In this risk assessment the potential presence of nitrites has been identified and analytical tests have been carried out in order to determine the nitrites content and eventual carry-on into dasiglucagon. The risk is considered negligible, but as additional measure, the manufacturer if the finished product will test for nitrites all incoming batches in the future. The response was deemed acceptable. The contribution of excipients, manufacturing equipment, production auxiliaries, WFI, and packaging materials to the nitrosamine formation has been duly evaluated and has been considered negligible.

The analytical methods used have been adequately described and appropriately validated in accordance with the ICH guidelines. Satisfactory information regarding the reference standards used for testing has been presented.

Batch analysis results are provided for commercial scale batches of PFS, AI and RtU-PFS confirming the consistency of the manufacturing process and its ability to manufacture to the intended product specification.

Stability of the product

Stability studies have been conducted using dasiglucagon injection 1 mg/mL pre-filled syringe (PFS), ready-to-use pre-filled syringe (RtU-PFS) and auto-injector (AI) batches to evaluate the stability profile, and to assign the shelf life and storage conditions for the commercial presentations dasiglucagon injection 1 mg/mL RtU-PFS and dasiglucagon injection 1 mg/mL AI.

Stability data from 4 commercial scale batches (PFS), 2 commercial scale batches (RtU-PFS), and 2 commercial scale batches (AI) stored under long term conditions (5 °C±3 °C/ambient RH (36 months), 5 °C±3 °C/ambient RH followed by storage at 25 °C±2°C/60% RH (samples are moved to 25°C storage after 18 months (PFS only), (24 months and 30 months for total storage for up to 36 months), 25°C±2°C/60% RH (12 months)) and for up to 3 months under accelerated conditions (40°C±2°C/75% RH) according to the ICH guidelines were provided. The batches are identical to those proposed for marketing and were packed in the primary packaging proposed for marketing.

Samples were tested for appearance of solution, visible particles, content of dasiglucagon, impurities, pH, osmolality, extractable volume, sub-visible particles, sterility, bacterial endotoxins, and container closure integrity. The analytical procedures used are stability indicating.

The long-term and accelerated stability data presented show that the only stability indicating attributes that change during storage of the finished product are: dasiglucagon content, total impurities, specified impurities, largest unspecified impurity. The changes in these attributes are predictable and consistent between batches and across presentations (PFS, RtU-PFS and AI), thereby also confirming that final assembly into RtU-PFS and AI does not impact release or stability data of the finished product. Furthermore, the data confirms that the degradation at 25 °C is independent of prior storage at 5 °C.

In addition, one batch of PFS, RtU-PFS and AI exposed to light as defined in the ICH Guideline on Photostability Testing of New Drug Substances and Products. The photostability data show that dasiglucagon injection is sensitive to light. The study also confirms that the protective cases (secondary packaging) and provides sufficient protection to the product from light exposure

Based on available stability data, the proposed shelf-life of 3 years when stored in a refrigerator (2 °C – 8 °C) as stated in the SmPC (sections 6.3 and 6.4) are acceptable. Store in the original protective case in order to protect from light.

During the shelf life, the medicinal product may be kept at a temperature under 25 °C for a single period no longer than 1 year, and not exceeding the original expiry date (EXP). Once the product has been stored outside the refrigerator, the product must not be returned to the refrigerator. Upon removing the medicinal product from the refrigerator, the new expiry date must be written on the protective case label and the medicinal product should be used or discarded by the new expiry date. The original expiry date should be crossed out.

Adventitious agents

No excipients derived from animal or human origin have been used.

2.3.4. Discussion on chemical, pharmaceutical and biological aspects

Information on development, manufacture and control of the active substance and finished product has been presented in a satisfactory manner.

During evaluation, two major objections were raised by the CHMP in relation compliance with Medical Device regulation and risk assessment of nitrosamines. The responses from the applicant to the MOs were considered satisfactory and all the issues were considered to be resolved.

The results of tests carried out indicate consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use.

2.3.5. Conclusions on the chemical, pharmaceutical and biological aspects

The quality of this product is considered to be acceptable when used in accordance with the conditions defined in the SmPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way.

2.4. Non-clinical aspects

2.4.1. Introduction

Dasiglucagon is a synthetic glucagon analogue peptide comprised of 29 amino acids, with 7 amino acid substitutions compared to glucagon, which have been introduced to improve physical and chemical stability in aqueous media.

Glucagon is secreted from the α-cells of pancreatic islets and is central in the regulation of glucose homeostasis, opposing the action of insulin in its effects on blood glucose levels. Glucagon stimulates hepatic glycogenolysis and gluconeogenesis in hypoglycemic states to restore glucose homeostasis and has been shown to exert effects on lipid metabolism, energy balance and body adipose tissue mass. In addition, glucagon administration produces positive inotropic and chronotropic effects on the heart, exerts spasmolytic effects on gastrointestinal smooth muscle, and stimulates growth hormone-release. Glucagon also, increases glomerular filtration rate (GFR) and decrease food intake. Glucagon stimulates insulin release in-directly via the increase in blood glucose (and presumably directly via glucagon receptors on beta-cells in the pancreas) whereas insulin inhibits glucagon release.

Dasiglucagon is also called ZP4207.

The non-clinical safety program for dasiglucagon included the following main studies:

- Repeat dose toxicity studies in mice (13 weeks), rats (26 weeks), and dogs (39 weeks).

- Toxicity study of degradation products of dasiglucagon in rats (28 days).
- Genotoxicity studies in Salmonella typhimurium strain TA100 (Ames test), human lymphocytes, and rats (in vivo micronucleus study).
- Carcinogenicity study in transgenic mice.
- Reproductive toxicity studies in rats (fertility, embryo-fetal development, and pre- and post-natal development).
- A core battery of safety pharmacology studies evaluating effects on the CNS, respiratory and cardiovascular organ systems were completed according to ICH S7A guidance.

All of the studies were conducted in accordance with Good Laboratory Practice (GLP).

No in vivo studies have been performed to evaluate pharmacokinetic drug interactions.

Scientific Advice on the adequacy of ADME and Toxicology programme, which was deemed adequate for a MAA, was given from BfArM in 2018.

2.4.2. Pharmacology

2.4.2.1. Primary pharmacodynamic studies

In vitro

The GCGRs receptor activities of dasiglucagon were compared with those of glucagon in 5 mammalian species (human, mouse, rat, rabbit and dog). The potency of dasiglucagon (EC50) on the human receptor was ranging between 9.9 and 18 pM (Study 13-219, Study 15-159). The potency of dasiglucagon was demonstrated to be comparable with that of glucagon on the human, rabbit and dog receptors, but 2- and 4-fold less potent on rat and mouse receptors, respectively.

The agonistic effect of dasiglucagon and eight impurities in the formulation on human GCGR was assessed. Dasiglucagon has an EC50 of 7.4 pM, while all the impurities proved to be weak human glucagon receptor agonists with 10- to 65,000-fold lower potency than dasiglucagon.

In vivo

The effect of dasiglucagon compared to native glucacon in terms of blood glucose profile, was evaluated.

Rats

Injection of the glucagon analog ZP4207 at 2, 6 and 20 nmol/kg induced a more potent dose-dependent blood glucose release effect in normoglycemic rats, than native glucagon (ZP2197) (study 13-047). The glucose-releasing effects, measured as AUCs (and at for glucagon) of both compounds were statistically significant ($p<0.001$, except for glucagon at 2 nmol/kg; $p<0.05$) when compared to vehicle-treated control animals. Like glucagon, ZP4207 has a short duration of action.

Injection of ZP4207 (3, 6, 20 nmol/kg) induced a dose dependent increase in blood glucose levels calculated as area under the glucose curves (AUCs, t = 45 min to t = 180 min, y = 3.7 mM) in insulin-induced hypoglycemic rats (study 13-144). These were indeed significantly larger for ZP4207 versus glucagon at 6 and 20 nmol/kg.

When comparing ZP4207 aged and non-aged formulations, no difference in insulin response was seen between the dose levels within each sex, although insulin response was more pronounced in males than in females (study 16-115). No clear difference in pharmacodynamic effect between was noted.

Rabbit

Single dosing of ZP4207 at 0.1, 0.3 and 1 mg/kg to female non-pregnant rabbits at 3-4 days interval, caused a comparable increase in blood glucose concentration up to 60 minutes post dose at all dose levels (study 16-087). No effect on body weight or food consumption was seen.

A trend of lower Cmax values for insulin and higher Cmax values for glucose was observed after IV infusion as compared to SC administration (for both dasiglucagon and glucagon), suggesting differences in glucose/insulin dynamics which depend on administration route only (study 13-164).

In conclusion, the PK and PD parameters following SC and IV administration of glucagon and ZP4207 were comparable with the exception of a higher bioavailability of ZP4207.

2.4.2.2. Secondary pharmacodynamic studies

Study 13-183 tested dasiglucagon and glucagon on two panels of cellular receptors to test selectivity for the GCGR. Profile of ZP4000 and ZP4207 in comparison to native glucagon ZP2197 on two panels of cellular receptors from DiscoveRx in order to test their selectivity for the glucagon receptor.

The compounds were assayed for both agonistic and antagonistic activities.

All three peptides were tested at 1 µM concentration and determined to be agonist on the GCG-R as expected. ZP2197 (native human glucagon) was also an agonist of the GLP1-R also as expected at this concentration.

The activity of dasiglucagon and glucagon on 239 G-protein coupled receptors was screened. Results showed that both molecules were agonists on the GCGR. Moreover, glucagon was an agonist of the glucagon-like peptide-1 receptor. The molecules had no effect on any of the other tested receptors. In In light of such data, dasiglucagon is deemed to be a selective GCGR agonist.

2.4.2.3. Safety pharmacology programme

In a cell-based assay, dasiglucagon 10 µM did not inhibit the 8 tested human cardiac ion channels (including hERG) by greater than 20%, suggesting a low potential for QT interval prolongation in vivo (study 13-166, see also Toxicology Section).

In vivo, a single subcutaneous administration of ZP4207 at the dose level of 2, 10 or 24 mg/kg to male Wistar rats elicited no effects on a battery on behavioural and physiological parameters (Irwin test), covering both central and peripheral CNS functions (study 14-021).

Subcutaneous administration of dasiglucagon to dogs was associated with gastrointestinal findings (liquid/pasty feces, observed within hours after treatment at each dose in all animals, and vomiting, in one dog after treatment at the highest dose – study 14-022 - see also Toxicology Section).

CV effects were shortening of Van de Water-corrected QTc interval at 0.4 mg/kg (a maximum of -7.5%), and increased incidence of premature ventricular beats in 2 out of 4 animals treated at 0.12 and 0.4 mg/kg, concomitant with dose-related tachycardia. The above-mentioned findings are known to be effects of glucagon in dogs; the QTc shortening and the increased incidence of premature ventricular beats were considered related to dasiglucagon treatment (both) and/or tachycardia (the latter). TK assessment was made in the 4-week toxicity study in dogs (see Toxicology Section).

GI disturbances, including expulsion of mucoid material and soft/pasty feces, were seen in a second *in vivo* study in dogs within hours after dosing (Study 16-031, See also Toxicology Section). As mentioned above, these are known to be effects of glucagon in dogs, as declared by the Applicant and endorsed by the Assessor.

CV effects were limited to non-dose-related decrease in arterial blood pressure, dose-related tachycardia, shortening of the RR, PR, and QT intervals, and increasing incidence of premature ventricular beats in animals treated at 0.02, 0.1, and 0.4 mg/kg (1/4, 2/4, and 3/4 of the animals, respectively), concomitant with tachycardia. Again, the hemodynamic effects noted in this study were considered directly or indirectly related to the pharmacological effect of dasiglucagon (glucagon receptor agonism), which is known to induce a chronotropic and inotropic effect on the heart as well as a decrease in blood pressure in dogs (Berndt TB et al. 1973)

There was non-dose-related tachypnea at 0.1 and 0.4 mg/kg not attributed to a test-item related effect, as it was noted in the vehicle control group as well.

2.4.2.4. Pharmacodynamic drug interactions

No pharmacological interactions between dasiglucagon and other drugs have been established.

2.4.3. Pharmacokinetics

Methods of analysis

Validated methods for the determination of dasiglucagon in mouse, rat, rabbit and dog plasma are based on protein precipitation followed by on-line solid-phase extraction and analysis by LC-MS/MS with an adequate LLOQ (0.500 nmol/L). These methods were used for all pivotal toxicity studies showing acceptable analytical performance. Immunogenicity assays for dasiglucagon in non-clinical species have been developed and validated for mouse, rat and dog. All these assays were based on a direct enzyme-linked immunosorbent assay methodology, using dasiglucagon-coated microtiter plates to capture ADAs and HRP labelled protein to detect captured ADAs. The validation process showed adequate sensitivity in the presence of excess drug at drug concentrations up to 4000 ng/mL (1200 nM) in mouse and rat serum and 500 ng/mL (150 nM) in dog serum. The lack of evidence on the binding affinity of protein AG/HRP with rat and dog IgM was a limitation of the immunoassay methods. However, considering that the overall binding affinity of Protein AG/HRP for rat and dog immunoglobulin is moderate and that the rat and dog studies were long-term studies, the risk of underestimation of ADA in rats and dogs is considered to be sufficiently low.

Absorption

In mice, rats and dogs, dasiglucagon had a similar PK profile to glucagon following both IV and SC administration. This was characterized by a short elimination half-life (t½), high clearance (Cl), and low volume of distribution at steady state (Vss) (see M 2.6.5.3). After SC administration, the bioavailability of dasiglucagon was higher than that of glucagon (mouse: 47.5% versus 23.9%; rat: 31.6% versus 9.72%; dog: 87% versus 42%), and both compounds exhibited absorption-rate limited kinetics.

Distribution

The determined distribution volume following IV administration corresponds to approximately 30% of the body weight, indicating that dasiglucagon is distributed in the extracellular space. Dasiglucagon and glucagon were not distributed extensively beyond body water in mice, rats and dogs. Further in vitro or in vivo distribution studies have not been performed.

Metabolism

In vitro data in hepatocytes showed formation of metabolites in all tested species (human, rat, mouse, dog, and rabbit). In vivo, the metabolism of dasiglucagon was studied in rats and dogs dosed for 8 days. A total of 16 human metabolites of dasiglucagon were detected and identified in rat plasma samples following repeated subcutaneous dosing. All putative metabolites had a similar intensity profile to ZP4207 in that they had a maximum intensity in either the 10- or 30-minute samples, then decreased or were not detected in the 1.5- or 2.5-hour time points. All metabolites appeared to decline with a rate similar to the parent molecule and did not indicate any accumulation of metabolites over time. The data indicate that dasiglucagon is cleared mainly in the blood, liver and kidneys by normal proteolytic degradation pathways, in the same way as glucagon.

Excretion

No excretion data were generated. The available data support that dasiglucagon is degraded by proteolytical cleavage of the peptide backbone.

PK drug interactions

In vitro incubation of dasiglucagon in human liver microsomes up to 25 uM was tested for CYP1A, CYP2C9, CYP2C19, CYP2D6, or CYP3A4. Based on the in vitro data, clinically relevant drug-drug interactions related to CYP450 enzymes are considered unlikely at pharmacologically relevant dasiglucagon plasma concentrations. Dasiglucagon had no clinically relevant effect on the efflux/uptake transporters: P-gp, BCRP, OATP1B1, OATP1B3, OAT1, OAT3, OCT2, MATE1, MATE2-K and OCT1.

2.4.4. Toxicology

2.4.4.1. Single dose toxicity

Toxicity of dasiglucagon was evaluated in beagle dogs following single IV administration to support IV administration in a cardiovascular/respiratory study.

Following a single intravenous bolus injection of ZP4207 at doses of 0.1 and 0.4 mg/kg, no local or systemic adverse effects were seen. The dose levels were therefore used for the subsequent main telemetry study.

2.4.4.2. Repeat dose toxicity

In mice, rats, and dogs, repeat-dose toxicity studies of 13-, 26-, and 39-weeks duration, respectively, were completed.

The repeat dose toxicity of dasiglucagon was evaluated in mice, rats and dogs and potential reproductive toxicity was assessed in rats and rabbits. Dasiglucagon was shown to have in vitro and/or in vivo pharmacological activity on the GCGR from mice, rats, dogs and rabbits; all species for which extensive historical background data are available. In the toxicity studies, dasiglucagon exposure substantially exceeded the relevant human exposure enabling the toxicological characterization.

Mice

Toxicity and TK of ZP4207 was compared to those of native glucagon, administered daily, by s.c. injection, to CByB6F1-Tg(HRAS)2Jic (wild type) mice for four weeks (study 17-143). The study aimed at dose selection for a 26-week carcinogenicity study in transgenic rashH2 mice.

Increased incidence and severity of hepatocellular rarefaction was seen in all animals treated with ZP4207 at 5 or 20 mg/kg/day and in all animals treated with native glucagon at 5 mg/kg/day. These findings in the liver are considered related to the expected pharmacology (and likely the cause of the increased liver weights in treated animals) and were considered of no toxicological significance, as declared by the Applicant.

Overall, daily s.c. administration to CByB6F1-Tg(HRAS)2Jic (wild type) mice gave no toxicologically significant findings. Therefore, doses up to 20 mg/kg and 5 mg/kg were used for the 26-week carcinogenicity study in transgenic CByB6F1-Tg(HRAS)2Jic mice.

Daily subcutaneous administration of ZP4207 at 0, 2, 10 or 24 mg/kg/day to the mouse for 13 weeks (study 15-138) gave increased hepatocellular glycogen vacuolation, with subsequent triglyceride levels and total bilirubin increase in all dose groups, with a corresponding macroscopic observation of large liver and increase in liver weights. The findings have been attributed to the pharmacological reponse to ZP4207.

In light of the results, the NOAEL was considered to be 24 mg/kg/day (AUC(0-t) of 1820 h.nmol/L in males and 1450 h.nmol/L in females (Week 13)).

Rat

Wistar rats were treated with ZP4207 for at least 28-days by daily subcutaneous administration at dose levels of 0, 2, 10 and 24 mg/kg followed by a 2-week recovery period (Study report 14-019). Higher liver weights were noted in all ZP4207-treated animals. This related with a morphologic alteration of hepatocellular cytoplasmic rarefaction due to glycogen accumulation, which is a known effect of glucagon following repeat dosing, as reported in the literature (Root, 1954). Therefore, like the glycogen accumulation is deemed to be an exaggerated pharmacological response to ZP4207, and not toxicologically relevant.

Furthermore, other findings like changes in organ weights at end of treatment (heart, kidney, spleen, prostate gland and seminal vesicle), were without any histomorphological correlate and were then considered of low toxicological relevance.

Slight salivation was noted on several occasions in animals treated at 10 and 24 mg/kg; it was not noted during recovery.

Changes in body weight (gain), terminal body weights after overnight fasting, food consumption and urinary pH were attributed to the pharmacology of ZP4207, therefore were considered not adverse.

The NOAEL for ZP4207 is 24 mg/kg/day, AUC_{last} 2160 and 1970 h*nmol/L C_{max} 2730 and 1940 nmol/L in male and female rats, respectively, on Day 28.

In a 28-day toxicity study, both aged and non-aged formulations of ZP4207 were administered s.c. at 2 ad 8mg/kg, in order to characterize the potential toxicity of degradation products following long term storage of ZP4207 (study 16-040). No differences were noted in terms of clinical signs (transient episodic freezing, decrease in bodyweight gain increase in food consumption slight increases in AST and triglycerides and decreases in cholesterol and UREA, Hepatic glycogen vacuolation secondary to the increased liver weights – the last one due to the pharmacologic response to ZP4207 administration), hyaline droplets (declared to be a common background finding in the kidney of rats, Hard, 1993).

In light of the results shown above, comparable findings were seen following administration of both aged and non-aged formulations of ZP4207. No gender differences were moreover seen in terms of TK parameters of both formulations. NOAEL was set to 8 mg/kg.

Daily s.c. administration of ZP4207 at 2, 10 or 24 mg/kg/day for 13 weeks resulted in behavioural changes at all dose levels ("transient episodic freezing absences", mouth rubbing, salivation, and reduced activity) and decreases in bodyweight gain and increases in severity and incidence of post dose observations at 10 and 24 mg/kg/day (study 15-084). Post dose observations noted included paddling, head shaking, increased activity, impaired mobility and aggression. Moreover, liver glycogen increase was observed, effect seen also in the other studies. In the liver, AST and ALT higher levels, increased together with ASP ones, effects correlating with pathological changes. Total protein and albumin concentration increased as well, while globulin concentration decreased. Consequently, this resulted in increases in the albumin to globulin ratios at all dose levels. Based on these findings, the NOAEL was considered to be 24 mg/kg/day (AUC (0-∞) of 7460 h.nmol/L in males and 2970 h.nmol/L in females).

Alike to the above commented studies, daily s.c. administration of ZP4207 to the rat at 0.5, 2 or 8 mg/kg/day for 26 weeks (study 15-129) resulted in behavioural effects ("transient episodic freezing absences", mouth rubbing, paddling, and salivation) together with effects considered related to the pharmacodynamic effects of ZP4207: glycogen vacuolation in the livers of most animals from all dose groups, which completely reversed during the recovery period; the absence of hyaline droplets in the kidneys of almost all males from all dose groups, which also reversed during the recovery period; and an increase in the incidence of chronic progressive nephropathy in the kidneys of some males and females in all dose groups, which partially reversed during the recovery period. However, there was one death at 8 mg/kg/day caused by a liver lesion attributed to dosing with ZP4207. Although pharmacological, this was considered to be adverse.

Based on the findings of this study the NOAEL is considered to be 2 mg/kg/day with a plasma exposure (AUC(0-t)) of 104 h.nmol/L in males and 107 h.nmol/L in females (Week 26).

Dog

Systemic toxicity and local tolerance of dasiglucagon was evaluated in female and male Beagle dogs following repeated daily s.c. or i.m. administrations for 28 days (study 14-020). The study assessed the

reversibility of the effects in the s.c. and i.m. high dose groups after a post-dosing recovery period of 2 weeks.

Increased glycogen content in the liver, related to a tendency for higher liver weights higher cholesterol (recovered), triglyceride (not fully recovered), and phospholipid levels, and lower inorganic phosphate levels were seen after both s.c. and i.m. administrations, although in the latter these were less pronounced.

Following 2 week-recovery period, cholesterol and phospholipid levels were comparable to control levels; triglyceride levels were not fully recovered.

Liver weights of the s.c. and i.m. treated dogs returned to baseline weights or were higher than baseline weights. Increased hepatic glycogen was noted in females treated at 0.4 mg/kg i.m. following the recovery period. However, in males treated at 0.4 mg/kg i.m. and in males and females treated at 0.4 mg/kg s.c., the increased hepatic glycogen was no longer present.

Diarrhea and feces-containing mucus, not observed during the recovery phase, were not considered adverse, as the effect was related to the pharmacology of dasiglucagon.

Higher heart rates, redness of ears, eyes, and mucous membranes (eyes and mouth) longer activated partial thromboplastin time, were not considered adverse due to the pharmacology of glucagon, or because these were minor changes ad in absence of corroborative findings.

Overall, when comparing the effects observed following the i.m. and s.c. routes of administration, the response to treatment at the 0.4 mg/kg dose level varied. Increased incidence of diarrhea, altered clinical biochemistry parameters, and higher heart rates were noted more often in animals treated subcutaneously, while redness of ears, eyes, and mucous membranes, and higher liver weights, as well as increased glycogen content of the liver (at the end of recovery), were noted more often in animals treated intramuscularly. This finding might be related to the fact that Cmax was higher in animals with i.m. administration compared to s.c. administration, while AUCs were similar to that of animals with s.c. administration, as declared by the Applicant.

Based on the findings in this study, the NOAEL for ZP4207 is 0.4 mg/kg/day, AUClast of 81.1 / 68.8 and 93.8 / 57.0 h*nmol/L for and Cmax 55.7 /104 and 74.1 / 110 nmol/L for Cmax in male and female Beagle dogs (s.c. / i.m.), respectively on Day 28.

ZP4207 given s.c. at dose levels of 0.02, 0.1 and 0.3 mg/kg for 39 weeks (study 15-085) was tolerated but did lead to a dose related change in feces consistency in terms of lose/liquid/discoloured feces. In addition, lower body weight gain was found at 0.1 and 0.3 mg/kg.

Slight increases in heart rate (due to the pharmacology of ZP4207) and corresponding decreases in RR, PR and QT interval were evident primarily at 0.3 mg/kg but also at 0.1 mg/kg. The increase noted in liver glycogen, which corresponded to the increased liver weights and liver weight ratios in all dose groups, was thought to represent a physiologic response to administration of ZP4207. The increased liver weights correlated with changes in clinical chemistry parameters, such as increases in alkaline phosphatase, cholesterol and triglycerides.

Levels of blood glucose and insulin were comparable; both were at their maximum (with the exception of females given 0.3 mg/kg/day) at the 15 min blood sampling occasion.

Levels in the blood decreased over time until returning to a background level.

Taken together, apart from the transient episodic freezing absences noted in rats and loose feces noted in dogs, all the other findings were comparable across all species and could be ascribed to the effects of GCGR agonism. See Figure below:

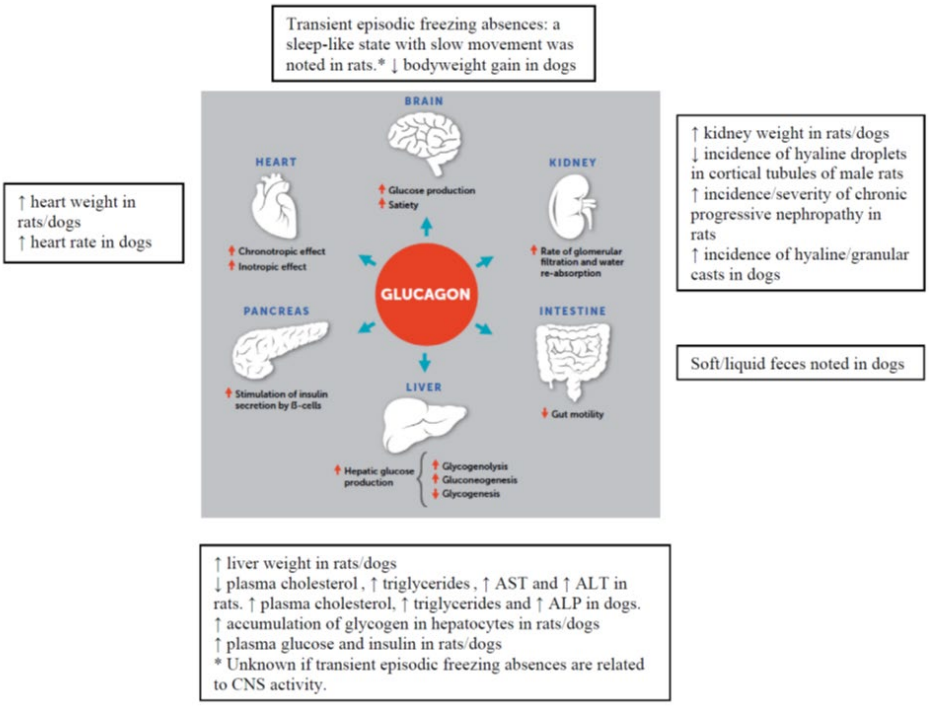


Figure 3 **Physiological effects of GCGR agonism and associated findings in the dasiglucagon repeat dose toxicity studies.**

2.4.4.3. Genotoxicity

The genotoxic effect of dasiglucagon was evaluated in accordance with the ICH S2(R1) guideline. An Ames test (Study 14-023), a chromosome aberration test in human lymphocytes (Study 14-024), and an in vivo micronucleus study in rats (Study 14-219) were conducted. These tests revealed no genotoxic potential of dasiglucagon.

ZP4207 at concentrations up to 5000 µg/plate is mutagenic in tester strain TA100 of the Salmonella typhimurium reverse mutation assay. ZP4207 is not mutagenic in the other Salmonella typhimurium tester strains (TA1537, TA100 or TA98) or Escherichia coli strain reverse mutation assay using strain WP2uvrA. The mutagenicity was confined only to incubations with metabolic activation, which was then deemed to be an artifact and not representative of a biological effect of dasiglucagon. ZP4207 does not disturb mitotic processes and cell cycle progression and does not induce numerical chromosome aberrations.

ZP4207, moreover, is not clastogenic or aneugenic in the bone marrow micronucleus test, when sampled at 24 and 48 hours post dosing of male rats up to a dose of 24 mg/kg.

2.4.4.4. Carcinogenicity

A 26-week carcinogenicity study in transgenic CByB6F1 Tg(HRAS)2Jic mice was completed where native glucagon was included as a comparator. Groups of 25 male and 25 female mice received dasiglucagon at 0 (vehicle), 1, 5 or 20 mg/kg/day or native glucagon at 0 (vehicle) or 5 mg/kg/day for 26 weeks.

The study met the requirements of a carcinogenicity study since a minimum of 22 of the 25 animals in each group survived to the end of the treatment period. In addition, the presence of neoplastic findings in the stomach of animals in the positive control group after a single intraperitoneal dose of N-methyl-n-nitrosourea (MNU) clearly demonstrated the functionality and the suitability of transgenic animals to assess the carcinogenic potential of ZP4207.

There was no evidence at the histopathological examination that ZP4207 given s.c. up to 20 mg/kg or Native glucagon up to 5 mg/kg, had any effect on tumour profile, demonstrating that ZP4207 and Native Glucagon were not carcinogenic to the transgenic CByB6F1-Tg(HRAS)2Jic mouse.

2.4.4.5. Reproductive and developmental toxicity

Reproductive and developmental toxicity for dasiglucagon were conducted in accordance with the ICH M3 (R2) guideline. Studies were conducted in rats and rabbits, the pivotal non-clinical safety studies were conducted according to Good Laboratory Practice (GLP) and reproductive toxicity studies, consisting of a fertility study in rats, embryofetal development studies in rats and rabbits and a pre- and post- natal development study in rats, were completed.

A fertility study (Study 17-132) was performed in male and female rats which were administered with daily SC doses of 0.5, 2, or 8 mg/kg/day for two weeks prior to pairing for mating.

An Embryo-fetal development study (15-131) to establish the MDT and dose-range finding studies were performed in rats (14-219) and rabbits (15-132, and 15-133) following SC injection in non-pregnant animals. These studies were performed in order to define dose levels for subsequent embryotoxicity studies in pregnant animals (15-002 in rats and 15-134 in rabbits). Animals received respectively SC doses of up to 24 mg/kg/day (Study 15-002) and SC doses up to 6 mg/kg/day (Study 15-134).

Study 15-002 in rats

In rats, treatment-related embryo-fetal effects included slightly lower mean fetal weight in the 10 and 24 mg/kg/day groups compared to control and historical range, with an associated delay in ossification of a few bones (principally the sternebrae and caudal vertebrae), secondary to the reduced maternal body weight gain in these groups during the latter phase of gestation (GD 15 to GD 20). Other minor fetal skeletal findings noted at a greater frequency in the treated groups (2 and 24 mg/kg/day) statistically significative, compared with the concurrent control and historical control data, included thick and/or wavy ribs and incomplete ossification of the interparietal and parietal bones.

The high dose of 24 mg/kg/day was selected as the NOAEL for embryo-fetal toxicity.

At this dose level on G17, exposure in terms of Cmax and AUC0-last was 1900 nmol/L and 1850 nmol*h/L, respectively.

Study 15-134 in rabbits

Healthy rabbits were treated daily with 0,1 - 0,3 – 1 mg/Kg/die with SC dasiglucagon for 14 days. Findings, including skeletal and visceral malformations were observed.

In particular, animals that received dose 0.3 mg/kg/day (20 times the human dose based on AUC), exhibited fetal skeletal and visceral malformations at no maternal toxicity.

On the other hand, animals treated with 1 mg/kg/day (100 times the human dose based on AUC), exhibited lower fetal body weight and delayed bone ossification, at a dose that also induced maternal toxicity in terms of decreased body weight gain.

2.4.4.6. Toxicokinetic data

Toxicokinetic assessment was included in the toxicity studies with dasiglucagon (4-week toxicity study in dogs - study 14-020), in line with the ICH S3A guideline.

Parameters		Group 2 0.02 mg/kg, sc		Group 3 0.12 mg/kg, sc		Group 4 0.4 mg/kg, sc		Group 5 0.4 mg/kg, im	
		Male	Female	Male	Female	Male	Female	Male	Female
Day 1									
t_{last}	(h)	3	3-4.5[$]	3-4.5[$]	3-4.5[$]	4.5-7[$]	4.5-7[$]	1.5-7[$]	1.5-3[$]
t_{max}	(h)	0.667	0.667-0.25[$]	0.667-0.25[$]	0.667-0.25[$]	0.667	0.667-0.25[$]	0.25	0.25
C_{max}	(nmol/L)	2.04	4.70	20.8	15.3	35.7	48.0	91.9	94.6
# C_{max}	(kg*nmol/L/mg)	102	235	173	128	89.4	120	230	237
AUC_{last}	(h*nmol/L)	3.01	4.37	22.4	19.5	65.4	74.4	57.3	55.1
# AUC_{last}	(h*kg*nmol/L/mg)	150	219	187	162	164	186	143	138
AUC_{∞}	(h*nmol/L)	n/a	4.48	22.6	19.7	66.1	74.6	57.6	53.6
# AUC_{∞}	(h*kg*nmol/L/mg)	n/a	224	188	164	165	187	144	134
$t_{½}$	(h)	n/a	0.680	0.527	0.614	0.612	0.542	0.404	0.289
Day 28									
t_{last}	(h)	3	3	3-4.5[$]	3-4.5[$]	3-7[$]	4.5	1.5-7[$]	1.5-24[$]
t_{max}	(h)	0.667-0.25[$]	0.667	0.667	0.25	0.667	0.667	0.25	0.25
C_{max}	(nmol/L)	1.69	5.95	29.3	17.4	55.7	74.1	104	110
# C_{max}	(kg*nmol/L/mg)	84.3	298	244	145	139	185	259	274
AUC_{last}	(h*nmol/L)	2.55	4.95	31.0	18.8	81.1	93.8	68.8	57.0
# AUC_{last}	(h*kg*nmol/L/mg)	127	247	258	157	203	234	172	143
$t_{½}$	(h)	n/a	n/a	0.511	0.459	0.590	0.435	0.566	n/a

[$] : range
\# : dose-normalized to 1 mg/kg
n/c : could not be calculated

According to data from the study report, the plasma concentrations of ZP4207 increased rapidly after subcutaneous administration. The peak plasma concentration, Cmax, was reached 15 to 40 minutes after dosing. After intramuscular administration (Group 5) also a rapid increase was noted in the plasma concentration with a Cmax 15 minutes after administration, the first blood collection time point. In general, the Cmax is faster and higher after intramuscular administration compared with subcutaneous administration, while no difference in exposure (AUC) is noted.
Following subcutaneous administration apparent individual half-lives varied from 0.372 to 0.820 hours in males and from 0.328 to 0.864 hours in females, where accurate determination was possible. After intramuscular administration the apparent individual half-lives varied from 0.270 to 1.42 hours in males and from 0.251 to 0.332 hours in females.
The variability per group in the TK parameters, evaluated by %CV was high and ranged between 1 to 101%.

Dose proportionality was evaluated by comparing the exposure parameters, Cmax and AUC, at doses of 0.02 to 0.4 mg/kg after subcutaneous administration. Furthermore, a comparison has been made between subcutaneous and intramuscular administration at a dose level of 0.4 mg/kg. Values were compared to the preceding dose. The Cmax values increased with increasing dose in a more or less dose-proportional manner from 0.02 to 0.4 mg/kg on Day 1 and 28. The exposure to ZP4207, in terms of AUClast, increased with increasing dose in males and females. A more or less dose proportional increase was noted over the given dose range in males and females. A relative low exposure is noted in the males of the low dose group (0.02 mg/kg) on Day 1 and 28. In order to compensate for the observed tlast values, 3 hours at 0.02 mg/kg s.c. and 1.5 hours after 0.4 mg/kg i.m. administration, partial areas up to 1.5 and 3 hours were calculated for all groups. Average results are presented in the table below:

Parameters		Group 2 0.02 mg/kg, sc		Group 3 0.12 mg/kg, sc		Group 4 0.4 mg/kg, sc		Group 5 0.4 mg/kg, im	
		Male	Female	Male	Male	Male	Female	Male	Female
Day 1									
$AUC_{0-1.5}$	(h*nmol/L)	2.19	3.72	16.7	13.6	40.1	47.2	53.7	52.3
#$AUC_{0-1.5}$	(h*kg*nmol/L/mg)	110	186	139	113	100	118	134	131
AUC_{0-3}	(h*nmol/L)	3.01	4.32	21.7	18.5	59.9	70.7	58.2[1]	53.6[2]
#AUC_{0-3}	(h*kg*nmol/L/mg)	151	216	181	154	150	177	146[1]	134[2]
Day 28									
$AUC_{0-1.5}$	(h*nmol/L)	1.84	4.43	25.9	15.0	54.1	70.6	63.1	55.9
#$AUC_{0-1.5}$	(h*kg*nmol/L/mg)	92.0	222	216	125	135	177	158	140
AUC_{0-3}	(h*nmol/L)	2.55	4.95	30.5	18.6	74.8	90.9	74.4[1]	63.7[3]
#AUC_{0-3}	(h*kg*nmol/L/mg)	128	248	254	155	187	227	186[1]	159[3]

: dose-normalized to 1 mg/kg
[1]: n=4, [2]: n=3, [3]: n=2

Based on the partial areas a dose proportional increase is noted over 0.02 to 0.4 mg/kg s.c. in both genders. No difference in exposure was noted between 0.4 mg/kg ZP4207 when given subcutaneous or intramuscular.

Time-dependent change in exposure was evaluated by comparing AUC last values after repeated administration to AUC∞ or AUClast on Day 1, and by comparing Cmax values in time. At the low dose and mid dose of 0.02 and 0.12 mg/kg s.c., Cmax and AUC values were similar on Day 1 and Day 28 in both male and female animals. At the high dose group at 0.4 mg/kg subcutaneous slightly higher Cmax values were noted after repeated administration while no difference in exposure was noted. After intramuscular administration at 0.4 mg/kg ZP4207 Cmax and AUC values were comparable after repeated administration compared with Day 1 in both males and females, indicating no accumulation or induction at this dose level.
After 4 weeks of subcutaneous administration at 0.02, 0.12 and 0.4 mg/kg and intramuscular at 0.4 mg/kg, no apparent gender differences were noted for Cmax and AUC.

2.4.4.7. Local Tolerance

Local tolerance was investigated in the context of the toxicity studies.

2.4.4.8. Other toxicity studies

Antigenicity

Anti-drug antibodies were detected in mice, rats, and dogs and were more frequent in animals' groups that received the highest dose. About 40-50% of the ADA-positive rats showed cross-binding to glucagon.

In rats, following 13 and 26 weeks of treatment in dose groups with higher ADA frequency (≥8 mg/kg/day), the average exposure (AUC) was increased. The ADA frequency and titers of consistently ADA-positive rats were reduced from 13 to 26 weeks of treatment, indicating a transient response in most animals.

In dogs, ADAs were detected and demonstrated only in the high dose groups, and the frequency of cross-binding to glucagon was lower in comparison to the data from rats.

Studies conducted to evaluate any pharmacological or toxicological effects of the degradation products of the dasiglucagon drug product concluded that the toxicological and pharmacological profile of both degraded and non-degraded drug product are comparable, with no increased risk of ADA formation.

Mechanistic studies

Mechanistic studies were completed to explore the finding described as "transient episodic freezing absences in rats", whose clinical signs also occurred in repeat toxicity studies in rats. Such finding only occurred in rats. The aetiology of such clinical conditions is currently unknown, however it occurred in rats, also with glucagon administration. The finding is transient, animals can have a rapid full recovery and the overall well-being is maintained.

2.4.5. Ecotoxicity/environmental risk assessment

No excretion data are available for dasiglucagon but it is expected to behave the same as other peptides with a similar molecular weight. Hence, after glomerular filtration, proteases present in the proximal tubuli will degrade dasiglucagon into peptide fragments which are then reabsorbed. Hence, no unchanged dasiglucagon is likely to be excreted and enter the environment.

In vitro studies with human hepatocytes produced a total of 15 proteolytic metabolites of dasiglucagon (Study 15-076). The many proteolytic cleavage products detected indicate that dasiglucagon, when dosed in vivo, would be cleared as native glucagon mainly in blood and liver by normal proteolytic degradation pathways. The amino acid Aib (2-Aminoisobutyric acid) incorporated in the sequence of dasiglucagon is a naturally occurring non-protein amino acid that is not metabolized or catabolized. Data from literature show that Aib is excreted in urine and feces.

2.4.6. Discussion on non-clinical aspects

The glucagon receptors (GCGR) play an important role in maintenance of glucose homeostasis and, as such, they are considered to be a valuable target for the treatment of hypoglycemia. Glucagon and their analogues represent potential therapeutic agents for acute hypoglycemia in diabetic patients. Dasiglucagon is synthetic peptide comprised of 29 a.a. This glucagon analogue has 7 amino acid substitutions and is free of amino group at the N-terminus and a carboxylic acid at the C-terminus. The non-clinical studies were performed with dasiglucagon (drug substance code: ZP4207) in comparison to native glucagon (drug substance code: ZP2197). The hydrochloride salt of dasiglucagon was used for toxicity testing and is also the salt of dasiglucagon used in the drug product. In study 16-040 degraded and nondegraded dasiglucagon was formulated with the same excipients used for the drug product, but at 4 mg/mL, pH 7. In all other non-clinical safety studies, dasiglucagon was formulated in a vehicle comprised of sodium phosphate salts (Na2HPO4, NaH2PO4) and propylene glycol, in water for injection. The non-clinical data package is rather extensive for the intended therapeutic indication.

Pharmacology

The pharmacodynamic profile of dasiglucagon was characterized in in vitro and in vivo studies and compared to glucagon. A pharmacodynamic response comparable to glucagon was confirmed in both rats and dogs, the primary species used for toxicity testing of dasiglucagon. Any off-target effects were assessed in an in vitro screen of agonistic or antagonistic effects of 239 G-protein coupled receptors. A core battery of safety pharmacology studies was conducted including an in vitro assay for effects on cardiac ion channels, an Irwin test in rats for assessment of effects on the CNS, and telemetry studies in dogs following both IV and SC administration to assess effects on the respiratory and cardiovascular systems.

In vitro, the potency of dasiglucagon was demonstrated to be comparable with that of glucagon on the human, rabbit and dog receptors, but 2- and 4-fold less potent on rat and mouse receptors, respectively. Moreover, combinations of dasiglucagon with 20% of each of the eight impurities did not affect the EC50, suggesting lack of impurities competitive inhibition (also supported by Study 16-040 - see Toxicology Section).

The in vivo pharmacodynamic data suggest that dasiglucagon is a potent and efficacious compound showing the capacity to increase the blood glucose levels in all investigated species (rats, rabbits, dogs). In vivo, results from study in normoglycemic rats demonstrated that administration of dasiglucagon induced a dose-dependent blood glucose-releasing effect similar to glucagon; however, the effect was

prolonged following dasiglucagon administration, as evidenced by an increased blood glucose AUC. In addition, the results support that dasiglucagon has a short duration of action (i.e. maximum effect within 1 hour and a return to baseline levels within <2.5 hours), similar to glucagon. In hypoglicemic rats, an animal model that was representative of a hypoglycemic episode in humans, the insulin response was similar between the dose levels within each sex, but the insulin response in males was more pronounced than in females.
Overall, degraded and non-degraded formulations of 2 or 8 mg/kg dasiglucagon administered to male and female rats did not affect blood glucose concentration, except at 30 minutes post dose for females, but caused an increase in insulin. The effect on insulin was greater in males compared with females. No clear difference in pharmacodynamic effect between degraded and non-degraded formulations were noted. The reason for not seeing the expected increase in blood glucose is unknown.

Dasiglucagon was evaluated for potential CNS effects in the rat, CVS and respiratory effects in telemeterized conscious dogs, and in an in vitro hERG channel assay. All the in vivo safety pharmacology studies conducted were GLP compliant and in accordance with the ICH S7A guidance.

Dasiglucagon was evaluated for its effects on cardiac ion channels, and the results from in vitro testing revealed that at a concentration of 10 µM, dasiglucagon did not significantly affect any of the eight tested human cardiac ion channels, including hERG. This suggests that dasiglucagon is unlikely to cause significant QT interval prolongation in vivo. In a GLP-compliant study with male Wistar rats (Study 14-021), dasiglucagon was assessed using the Irwin test, which examines behavioural and physiological parameters. After a single subcutaneous dose of dasiglucagon (2, 10, or 24 mg/kg), no drug-related effects on animal behaviour, physiology, or nervous system function were observed. Gastrointestinal disturbances were noted, consistent with known glucagon effects in dogs, but they did not adversely impact overall health.

In a study involving telemetered male Beagle dogs, the effects of intravenous dasiglucagon on cardiovascular and respiratory functions were investigated. The results indicated dose-related tachycardia and other cardiovascular effects, attributed to dasiglucagon's pharmacological action as a glucagon receptor agonist. Tachypnea was also observed, likely related to the administration procedure.

Furthermore, a study in male Beagle dogs following subcutaneous dasiglucagon administration assessed cardiovascular and respiratory parameters, ECGs, and body temperature. Dasiglucagon induced dose-related tachycardia and QTc interval shortening, along with an increased incidence of premature ventricular beats. Gastrointestinal disturbances were observed, consistent with known glucagon effects in dogs.

In summary, these studies suggest that dasiglucagon may induce some cardiovascular effects, such as tachycardia and QTc interval shortening, primarily due to its pharmacological properties. However, these effects were generally considered consistent with the expected effects of glucagon in dogs and did not have a significant adverse impact on overall health or vital functions. Toxicokinetic evaluation was not included in these studies.

Pharmacokinetics

The pharmacokinetics of dasiglucagon in comparison to glucagon was evaluated in mice, rats, dogs after single dose administration by i.v. and s.c. administration, and in mice, rats, dogs and rabbits after repeated s.c. dosing as part of the toxicological evaluation. The PK parameters calculations were based on plasma concentrations of dasiglucagon obtained from the analyses with a liquid chromatography and mass spectrometry (LC-MS) detection assays that were validated according to the Guideline on bioanalytical method validation. The employed analytical methods are considered suitable for the analysis of dasiglucagon/glucagon in species-specific K2-EDTA plasma samples over the validated concentration range and storage conditions.

The PK of dasiglucagon (ZP4207) and glucagon (ZP2197) following i.v. and s.c. administration in mice, rats and dogs was characterised by short elimination half-life, high clearance and low volume of distribution at steady state. The absolute s.c. bioavailability of dasiglucagon differed between the non-clinical species, ranging from 31.6% in rats to almost complete (97%) in dogs. In general, dasiglucagon showed higher bioavailability compared to glucagon.

Neither plasma protein binding nor specific distribution studies were performed due to assay performance issues. Considering the intended clinical use of the product as a single s.c. administration in emergency situations of hypoglycemia and based on the data from the pharmacokinetic studies, additional studies are not deemed necessary.

Apart from in vitro data in human and animal species serum/plasma and hepatocytes, no in silico data could be found on dasiglucagon metabolites. The applicant was asked to discuss whether human metabolites are expected to exert any activity of clinical concern. The applicant referenced to literature data assessing structure-activity relation of human glucagon and its proteolytic metabolites on the glucagon receptor. The data suggest that proteolytic metabolites of glucagon are not active on the glucagon receptor with high activity. In absence of actual data of the activity of dasiglucagon metabolites that are formed in vivo in humans, no firm conclusion on the PD activity of clinical relevance for dasiglucagon metabolites can be given. However, based on the available 28-days toxicology data in rats with 16 identified metabolites which appeared to decline with a rate similar to the parent compound and showed no accumulation over time, plus considering dasiglucagon is a peptide analogue of human glucagon cleared by normal proteolytic degradation pathways, it is not expected that metabolites would exert any activity of clinical concern. The issue is considered resolved.

No excretion data for dasiglucagon were generated. The available data support that dasiglucagon is degraded by proteolytical cleavage of the peptide backbone. There is no information of excretion of dasiglucagon to milk in the PK sections, however, based on data from pre- and post-natal development study in Rats (Study 19-179), generation offspring were exposed to test item via milk but there were no signs attributed to dasiglucagon during lactation. In addition, considering the intended clinical use of the product as a single s.c. administration in emergency situations of hypoglycemia and based on the data from the pharmacokinetic studies, additional studies are not deemed necessary. Finally, Dasiglucagon is cleared from the bloodstream very quickly and thus the amount excreted in the milk of nursing mothers following treatment of severe hypoglycaemic reactions is expected to be extremely small. As dasiglucagon is degraded in the digestive tract and cannot be absorbed in its intact form, it will not exert any metabolic effect in the child. Data on milk excretion are reflected in section 4.6 of the SmPC.

No clinically relevant PK related interactions of dasiglucagon have been identified. Therefore, no dasiglucagon dose adjustment is required when co-administered with other drugs.

Validated methods for the determination of dasiglucagon in mouse, rat, rabbit and dog plasma are based on protein precipitation followed by on-line solid-phase extraction and analysis by LC-MS/MS with an adequate LLOQ (0.500 nmol/L). These methods were used for all pivotal toxicity studies showing acceptable analytical performance. Immunogenicity assays for dasiglucagon in non-clinical species have been developed and validated for mouse, rat and dog. All these assays were based on a direct enzyme-linked immunosorbent assay methodology, using dasiglucagon-coated microtiter plates to capture ADAs and HRP labelled protein to detect captured ADAs. The validation process showed adequate sensitivity in the presence of excess drug at drug concentrations up to 4000 ng/mL (1200 nM) in mouse and rat serum and 500 ng/mL (150 nM) in dog serum. The lack of evidence on the binding affinity of protein AG/HRP with rat and dog IgM was a limitation of the immunoassay methods. However, as stated by the applicant, considering that the overall binding affinity of Protein AG/HRP for rat and dog immunoglobulin is moderate and that the rat and dog studies were long-term studies, the risk of underestimation of ADA in rats and dogs is considered to be sufficiently low.

In both rats and dogs, dasiglucagon had similar PK to glucagon, characterized by a short t½, high Cl, and low Vss. After SC administration, the bioavailability of dasiglucagon was higher than that of glucagon, and both compounds exhibited absorption-rate limited elimination kinetics. Dasiglucagon did not cause clinically relevant inhibition of any of the human CYP enzymes or drug transporters tested. Sixteen proteolytic metabolites were identified following incubation in human matrices (plasma, serum, and hepatocytes), and none of the identified metabolites were specific to humans. The many proteolytic cleavage products detected from these studies indicated that dasiglucagon, when dosed in vivo, is cleared as native glucagon mainly in blood and liver by normal proteolytic degradation pathways.

Toxicology

The toxicity of dasiglucagon was evaluated in mice, rats, dogs and rabbits, and dasiglucagon was shown to have in vitro and/or in vivo pharmacological activity on the GCGR in all of these species. Dasiglucagon

exposure achieved in toxicity studies substantially exceeded the relevant human exposure and was sufficient for the toxicological characterisation of the product.

The main effects observed in general toxicity studies could be directly related to dasiglucagon pharmacology, with GI tract, liver and kidney as the main target organs.

Clinical signs after dosing included mouth rubbing, paddling, salivation and reduced activity in rats of both sexes at dose levels ≥ 2 mg/kg/day. Clinical observations in dog seen across all dose groups consisted of thin build appearance, sluggish behaviour, increased salivation and loose/liquid/discoloured mucoid feces. A dose dependent increase in observations of loose/liquid brown feces was noted. Similar GI effects, such as nausea and diarrhoea, have also been noted in the clinical use of dasiglucagon.

Clinical signs described as "transient episodic freezing" was noted at all dose levels in rat. Although this effect has not been described before, the same effects could be seen in rats receiving native glucagon (study 16-135). A daily dose of 1 mg/kg/day administration of dasiglucagon to male rats in s.c. bolus caused freezing, but not in animals dosed via constant infusion (Study 18-053). The exposure in terms of AUC was comparable in the two groups whereas exposure in terms of Cmax was approximately 40 times higher in the bolus dosed group compared to the group dosed as a constant s.c. infusion. Therefore, the effects can be attributed to dasiglucagon exposure in terms of Cmax. The etiology behind these signs is unknown but considering that the animals could easily come out of the freezing state, and that full recovery was seen by the next morning, they were not considered adverse. This effect has not been observed in any other species; therefore, the relevance of this finding is considered low.

Repeated dose study durations exceeded the observation duration needed for a single dose use. Nevertheless, effects on the liver such as increased weights secondary to glycogen vacuolation seen in mice, rats and dogs (and additionally, increased total protein, albumin, albumin to globulin ratio, and triglycerides, ALT, AST, decreased cholesterol, and total globulin seen in rats, and increased levels of alkaline phosphatase, cholesterol, and triglycerides and decreased levels of urea and creatinine seen in dogs), can be linked to repeated hyperglycaemia caused by chronic dasiglucagon administration to normoglycemic animals. Exposures at the NOAELs for these effects were significantly higher than clinical exposures. The effects have therefore demonstrated that dasiglucagon is pharmacologically active in these species and that the appropriate animal models have been used. However, the observed chronic toxicity effects are of little relevance for the proposed posology of a single dose.

Effects on the rat kidneys were noted as increased kidney weights in both sexes and the reduction in hyaline droplets in males, which suggested an alteration in the production and/or metabolism of α2μ-globulin in male rats. In dogs, the increase in kidney weights was noted in both sexes. Since these findings were mild and without an effect on the kidney function, the significance of these findings for human single dose use is probably low.

Additionally, in the 39-week study, there was an increase in prostate-to-brain weight ratios in male dogs, and in ovary- and uterus-to-brain weight ratios in female dogs given dasiglucagon. Prostate- and uterus-to-brain weight ratios were still increased in animals previously given 0.3 mg/kg/day, suggesting there was no reversal of these findings during the recovery period. The applicant argued that organ weight evaluated in isolation is an uncertain indicator of treatment related effects. It was agreed, that without histopathological changes to the organs, weight differences between groups can be dismissed as incidental.

Based on the results of the chronic toxicity studies, the NOAELs for rats and dogs were established at 2 mg/kg/day and 0.1 mg/kg/day, respectively.

Although dasiglucagon was positive in the strain TA100 in the Ames test, it was negative in all other strains and mammalian in vitro and in vivo genotoxicity testing. Similar false positive results have been observed in other glucagon products. In an internal report it has been shown that the sequence of dasiglucagon is identical to native glucagon at the end where the histidine is located. It is possible that S9 induces cleavage primarily between AA 2 and 3 and then secondary between AA 1 and 2, and that histidine is released in the presence of S9, enabling bacteria to grow without converting to wildtype. Hence, dasiglucagon can be considered non-genotoxic.

Dasiglucagon was not carcinogenic in a 26-week carcinogenicity study in transgenic CByB6F1-Tg(HRAS)2Jic mice. It is unknown if glucagon or dasiglucagon have the potential to stimulate transformed cells and act as tumour promoting agents. However, it has been shown that glucagon directly enhances colon cancer cell growth through regulation of proliferative signalling including the AMPK and MAPK pathways. In the context of this MA application, the risk after a single injection is considered minimal.

The applicant was asked to discuss the reasons why exposures in Study 16-040 were markedly lower than at comparable doses in the Study 15-129, especially considering that the formulation in these two studies was different. Also, in order to qualify the degradation products, the applicant was asked to calculate the margins of exposure in the Study 16-040 in comparison to clinical exposure.

The exposures of dasiglucagon of non-degraded formulations in rat study 16-040 were markedly lower than at equal doses in the rat study 15-129. The applicant explained the higher exposures at week 26 in the study 15-129 as an effect of the ADA, however this effect would also be present in the study 16-040 on Day 28. In the Study Report 15-129, it is stated that: *of the remaining 21 (35%) animals confirmed positive after 26 weeks, 17 had decreased titres and 4 animals had equivalent titre level (increased by no more than 189) after 26 weeks. Most titres of samples from antibody positive animals were reduced from week 13 to week 26.* Therefore, in the case of dasiglucagon, ADAs in rat decrease, and not increase, the exposure to dasiglucagon. Besides, the difference in exposure can also be observed on the Day 1 (see the table below).

As the differences in exposure seem consistent throughout groups, sex and time, it can be assumed that they are not due to normal variation.

DAY 1			16-040	15-129	16-040	15-129
	mg/kg/day	gender	Cmax	Cmax	AUC	AUC
Non-aged	2	M	226	259	95.2	88.7
	2	F	162	238	51.0	94.3
	8	M	592	975	262	467
	8	F	973	1440	411	701
aged	2	M	126		47.7	
	2	F	108		41.6	
	8	M	482		236	
	8	F	533		211	
DAY 28/WEEK 26			16-040	15-129	16-040	15-129
	mg/kg/day	gender	Cmax	Cmax	AUC	AUC
Non-aged	2	M	191	170	87.2	104
	2	F	136	330	61.9	107
	8	M	870	1450	457	950
	8	F	485	1700	247	1630
aged	2	M	113		44.6	
	2	F	77.7		33.8	
	8	M	350		174	
	8	F	343		164	

In study 16-040 degraded and non-degraded dasiglucagon was formulated with the same excipients used for the drug product (Tromethamine, Sodium chloride, Water for injections, Hydrochloric acid, Sodium hydroxide), but at 4 mg/mL, pH 7, instead of 1 mg/mL, pH 6.5. All other toxicity studies, on the other hand, were performed with dasiglucagon formulated in a vehicle comprised of sodium phosphate salts (Na2HPO4, NaH2PO4) and propylene glycol, in water for injection.

As expressed in the question 44, this was a concern because with the formulations used in studies 15-129 and 16-040 not being the same, the differences in exposure might be indicating an inherent difference in bioavailability of the two formulations.

Similarly, but more precisely than HED, the exposures in animals tell us if the doses used in animal studies are in excess of maximal doses in humans. The exposures to dasiglucagon with the formulation used in all toxicity studies except the 16-040, were satisfactory (see Non-clinical Overview, Table 4.11). The only remaining issue is whether the exposures to dasiglucagon (and its degradation products) achieved in study 16-040 were also high enough for toxicological assessment. The applicant has not provided the exposure margins achieved in this study, but from the exposure data, it can be concluded

that dasiglucagon AUC exposures in the study 16-040 were roughly between 19x to 156x higher, and the Cmax was 103x to 619x higher than those achieved in humans with a dose of 0.6 mg. With that, it can be concluded that degradation products levels in the batch 15H05 is as qualified.

In view of lower exposures with the aged formulation, the applicant was asked to discuss the finding that liver size increase and the severity of glycogen vacuolation was higher in animals given 8 mg/kg/day of aged product in comparison to 8 mg/kg/day of non-aged product. The applicant has argued that the doses used in the study 16-040 are well above the Emax, hence the differences between the findings in the degraded and non-degraded dasiglucagon test groups must be considered as normal biological variation. It is still unusual that the formulation with purity causes higher severity of glycogen vacuolation than the non-degraded formulation. The effect could point to a pharmacologic activity of degradation products. However, since this medicinal product is used as a single dose in hypoglycaemic situations, this finding from a repeat dose study is not considered relevant in the context of this MA application.

Concerning reproductive and developmental toxicity, dasiglucagon had no effect on mating, fertility, or early embryonic development in rats administered with dose levels up to 8 mg/kg/day. Embryo toxicity studies were conducted in rats (Study 15-002) and rabbits (study 15-134).

With regards to rat embryo-toxicity study, findings occurred were statistically significant, however, the applicant retains unlikely any association with treatment because of the absence of dose-related increase in the incidence of these findings. The applicant was requested to argument such assumption, also considered that occurrence was out of historical control. Argumentation was provided on the delayed ossification and thick/wavy ribs seen in EFD studies, which are supposed to be secondary effects to lower weight gain. It is to be noted that even if in a non-direct way, the effects are indeed treatment-related, as mentioned in the study report conclusions. Nevertheless, such minor foetal findings are considered to be of no toxicological significance. Section 5.3 of the SmPC informs properly on the results of non-clinical studies.

With regards to rabbit's embryo-toxicity study (15-134), the malformations noted in the offspring of rabbits receiving dasiglucagon 1 mg/kg/day, were considered related to the pharmacological response to dasiglucagon in terms of increases in blood glucose. Such findings were comparable to those in offspring from human and animal diabetic mothers and those observed in the embryo-fetal development study with GlucaGen®.

Given that the pharmacological effect and the types of malformations noted for GlucaGen and dasiglucagon are similar and for both it exists a causal relationship to teratogenic effects, the adverse effects on embryofetal development noted for GlucaGen and dasiglucagon are considered similar.

Considering dasiglucagon is intended as a single dose rescue treatment in association with acute severe hypoglycemia, the treatment would have a short duration of the blood glucose increase and therefore the risk is considered minimal. In addition, considering the severity of hypoglycemia conditions, where restoration of normal blood glucose levels is vital, the hypothetical positive risk/benefit balance should be considered.

However, the reproductive toxicity observed in rabbits occurs also at a dose of 0.3 mg/kg/day (20 times the human dose based on AUC), with no maternal toxicity as indicated in the study report. This is not reassuring concerning the threshold exposure according to ICH S5 guideline.

In the light of the clinical safety concern on use of dasiglucagon during pregnancy and considering that the relevance of findings in the rabbits for human pregnancy cannot be excluded, Sections 4.6 and 5.3 of the SmPC have been updated accordingly.

No dasiglucagon-related effect on pre- and post- natal development in rats occurred when tested at dose levels up to 2 mg/kg/day.

Dasiglucagon is cleared from the bloodstream very quickly and thus the amount excreted in the milk of nursing mothers following treatment of severe hypoglycaemic reactions is expected to be extremely small. As dasiglucagon is degraded in the digestive tract and cannot be absorbed in its intact form, it will not exert any metabolic effect in the child. Results from repeat-dose toxicity study indicate that dasiglucagon is not a local toxicant.

ADAs were detected in mice rats and dogs, with apparently no changes in the safety or toxicity profiles compared to ADA-negative animals. Inconsistency was present in data percentages of animals positive for ADAs, for studies 15-138 and 14-019, reported in the dossier with respect to data reported in the study report: the discrepancy was clarified by the applicant and it was considered acceptable.

The studies performed to evaluate any pharmacological or toxicological effects of the degradation products of the dasiglucagon drug product concluded that the toxicological and pharmacological profile of both degraded and non-degraded drug product are comparable, with no increased risk of ADA formation.

Clinical signs corresponding to "Transient episodic freezing absences" were noted in the 4-, 13-, and 26-week toxicity studies in rats. Study 16-135 has compared clinical signs in rats receiving dasiglucagon and glucagon. In the study, freezing absences (described in the report as unnatural resting position) were noted in animals receiving both molecules, which suggests that the effect is a result of GCGR agonism and not specific to dasiglucagon. Study 18-053 investigated the relationship between these signs and exposure in terms of Cmax and AUC. By administration of the same dose (1 mg/kg/day) to groups of male rats either as a SC bolus or as a constant SC infusion, a similar AUC but different Cmax values were obtained. Such results seem to suggest these clinical signs are related to dasiglucagon exposure in terms of Cmax. The "transient episodic freezing absences" aetiology in rats, is currently unknown. Clinical signs of such condition are transient, animals could easily come out of the freezing state, and fully recovery from the condition. The overall well-being of the animals seems to be unmodified, therefore, such findings were not considered adverse. In addition, clinical signs corresponding to the freezing absences in rats have not been observed in non-clinical safety studies in mice, rabbits, or dogs and were only noted in rats following repeated dosing, supporting that it is a finding specific to rats.

According to Applicant "The ADA frequency and titers of consistently ADA-positive rats decreased between 13 and 26 weeks of treatment, indicating a transient response in most animals". However, the results from rat studies do not indicate a transient but rather a persistent ADA response. An American Association of Pharmaceutical Scientists expert group have defined a persistent ADA response as one that is detectable for at least 16 weeks or wherein the last ADA sampling timepoint is positive irrespective of titer. According to study-report 15-129 on rats (8 mg/kg/day) 62% where positive for ADA at week 13 while 37% where positive at week 26. The possible clinical implications of ADA response seen in repeat dose toxicity in 15-129 rat study were already explained by the applicant in the Summary of Immunogenicity. Although the formation of such antibodies could prolong dasiglucagon adverse events, data obtained during the phase 3 clinical development support the assessment of low risk.

With regards to the persisting ADA response seen in study 15-129, it is to consider that the detection of antibodies was not associated with a change in toxicity profile when compared to ADA-negative animals or change in exposure for dose levels <8 mg/kg/day (the dose groups with a lower ADA incidence compared to the ≥8 mg/kg/day ones).

The cross-reactivity towards glucagon does not seem insignificant. According to study report 16-167 on dogs, the cross-reactivity towards glucagon appeared independent of dose and time. Of concern, in one group, 48% of all ADA-positive dogs were positive for anti-glucagon at week 26. Study report 16-052 on rats also reports similar pattern in cross-reactivity where 63% of tested rats were positive for anti-glucagon at 'end of recovery'. Therefore, cross-reactivities up to 63% and 48% were rat studies. The clinical impact has already discussed in the Integrated Summary of Immunogenicity.

ERA

Dasiglucagon is a natural substance, the use of which will not alter the concentration or distribution of the substance in the environment. Therefore, dasiglucagon is not expected to pose a risk to the environment.

Assessment of paediatric data on non-clinical aspects

Juvenile toxicity studies were not considered relevant to support single doses of dasiglucagon in the paediatric population. For the claimed indication, i.e rescue treatment of severe hypoglycemia, dasiglucagon will be used acutely as a single dose. Single doses of dasiglucagon are expected to exert only short-term pharmacodynamics effects, which were considered better assessed in the clinical setting.

2.4.7. Conclusion on the non-clinical aspects

PD

In light of the in vitro and in vivo data, the non-clinical pharmacology profile of dasiglucagon is comparable to glucagon. The core battery of safety pharmacology studies evaluating effects on the CNS, respiratory and cardiovascular organ systems were completed according to ICH S7A guidance. Dasiglucagon had no significant effect on 8 key human cardiac ion channels in vitro, suggesting a low potential for QT interval prolongation in vivo. Administration of dasiglucagon to rats did not exert any effect on animal behaviour, physiology, or nervous system function as assessed by the Irwin test. In telemetered dogs, the primary physiological effects of dasiglucagon were loose feces, decreased blood pressure and tachycardia, which are known effects of GCGR agonism.

PK

The lack of evidence on the binding affinity of protein AG/HRP with rat and dog IgM was a limitation of the immunoassay methods. However, taking into account that the overall binding affinity of Protein AG/HRP for rat and dog immunoglobulin is moderate and that the rat and dog studies were long-term studies, the risk of underestimation of ADA in rats and dogs is considered to be sufficiently low.

Toxicology

The exposure in terms of AUC and Cmax in diabetic adults and children given a dose of 0.6 mg dasiglucagon is covered by NOAEL exposure in rats and dogs defined in chronic toxicity studies and NOAEL exposure defined in embryo-fetal development studies in rats and rabbits.

Reproduction toxicology

Overall, the reproduction toxicology programme is exhaustive and performed according to main EMA relevant guidelines. In rats, maternal toxicity, in terms of decreased body weight gain, lower fetal body weight, and delayed bone ossification, was observed at ≥10 mg/kg/day (≥475 times the human dose, based on AUC). In rabbits, lower fetal body weight and delayed bone ossification were observed at 1 mg/kg/day (100 times the human dose, based on AUC), a dose that also induced maternal toxicity in terms of decreased body weight gain. At ≥0.3 mg/kg/day (≥20 times the human dose, based on AUC), dasiglucagon caused fetal skeletal and visceral malformations with no maternal toxicity. No adverse fetal developmental effects were observed at 0.1 mg/kg/day, corresponding to exposure 7 times to the human dose based on AUC. Such information is included in the SmPC.

Local tolerance

According to results from repeat dose toxicity studies, the product is deemed to be not local toxicant.

Other Studies

Anti-drug antibodies were detected in mice, rats, and dogs and were most frequent in animals from the highest dose groups. ADAs presence did not appear to be associated with changes in the safety or toxicity profiles compared to ADA-negative animals.

Further studies to evaluate any pharmacological or toxicological effects of the degradation products of the dasiglucagon drug product included a 4-week toxicity study in rats, a single dose PD study, and an in vitro study to assess possible agonistic effects on the GCGR. These studies concluded that the toxicological and pharmacological profile of both degraded and non-degraded drug product are comparable, with no increased risk of ADA formation.

Clinical signs of "transient episodic freezing absences" condition were investigated in non-clinical studies. Such signs are transient, animals could easily come out of the freezing state, and fully recovery from the condition. The overall well-being of the animals seems to be unmodified, therefore, such findings were not considered adverse.

The MA for dasiglucagon is approvable from non-clinical point of view.

2.5. Clinical aspects

2.5.1. Introduction

GCP aspects

The Clinical trials were performed in accordance with GCP as claimed by the applicant.

2.5.2. Clinical pharmacology

2.5.2.1. Pharmacokinetics

Analytical methods

Methods for the determination of dasiglucagon in human plasma were based on offline solid phase extraction (SPE) followed by online SPE and highly selective liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) with multiple reaction monitoring. A stable labelled internal standard (ZP5517) was used in validation study. All assays were validated in compliance with the European Medicines Agency Guideline on Bioanalytical Method Validation.

The characteristics of the validated assays are presented in Table 1.

Table 1 - Summary of assay characheristics for dasiglucagon in human plasma

Method	LLOQ (nmol/L)	ULOQ (nmol/L)	Inter-assay CV (%)	Inter-assay bias (%)	Validation study
LC-MS/MS	0.010	1.0	2.9 – 12.4	-5.5 – 11.0	14-170
LC-MS/MS	0.010	1.0	2.76 – 4.7	-13.7 – 3.0	15-170
LC-MS/MS	0.020	1.0	3.5 – 16.9	-2.3 – -0.3	17-063

Interference from glucagon was investigated in trial 14013 where samples taken at 30 minutes from subjects dosed with GlucaGen were assayed for dasiglucagon without showing any detectable signal. Testing of dilution of samples with a concentration above the calibration range was included. Human

plasma samples were stable at -80°C for the period tested of 182 days using 8 mL BD P800 blood sampling tubes (study 14-170) and for 188 days using 5 mL BD K3EDTA (ethylenediaminetetraacetic acid) aprotinin blood sampling tubes (study 15-170). The change in sampling tubes was made due to practical issues with the P800 tubes. To support the pediatric trial 17086, a low volume (0.4 mL) blood sampling method was developed, and the samples were stable for 227 days (study 17-063).

Method 14-170 was used to analyse samples from studies 14013 and 15007, method 15-170 was used to analyse samples from studies 15126, 16051, 16098, 16136, 16137, 17084, 17144, 17145, while method 17-063 was used to analyse samples from pediatric study 17086.

The ADA assessment was performed using a multi-tiered testing approach according to guidance on immunogenicity assessment of therapeutic proteins. (EMEA/CHMP/BMWP/14327/2006 Rev 1,18 May 2017).

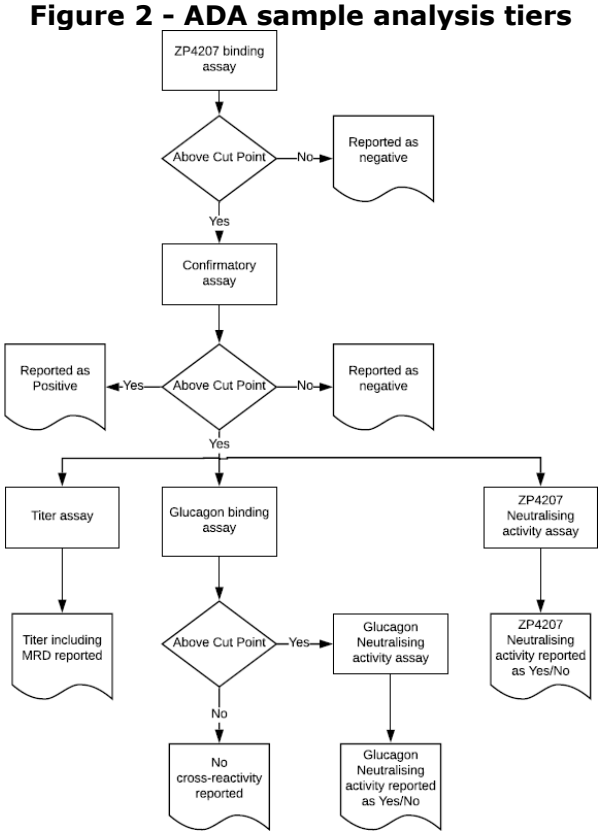
Tier 1 – screening for anti-dasiglucagon antibodies in dasiglucagon-treated patients was done in an ELISA-based assay.

Tier 2: In positive screening samples, the same assay (ELISA) with inclusion of excess dasiglucagon was used as confirmatory analysis.

Tier 3: Confirmed positive samples were analyzed for potentially neutralizing antibodies in a cell-based assay.

A similar set of assays were used to analyze for anti-glucagon antibodies in glucagon-treated patients. Furthermore, anti-dasiglucagon positive samples were analyzed in the anti-glucagon antibody analyses in order to assess for cross-reactivity to glucagon. The ADA sample analysis tiers for the dasiglucagon-treated patients are outlined in Figure 1.

Figure 2 - ADA sample analysis tiers



An overview of the validation reports is presented in Table 2.

Table 2 - Development and validation reports for screening, confirmatory and titer ADA assays used during the clinical development of dasiglucagon for rescue treatment

ADA Validation Report Title	**Purpose**	**Relevant for anti dasiglucagon/glucagon**
Study 14-077: Development of screening and confirmatory anti-ZP4207 antibody assays in human serum	Assay development	Dasiglucagon
Study 14-078: Development of screening and confirmatory anti-ZP2197 antibody assays in human serum	Assay development	Glucagon
Study 14-173: Amended Final Report, Validation of an Analytical Method for the Detection of Anti- ZP4207 antibodies in Human Serum, March 2019	Validation	Dasiglucagon
Study 14-174: Amended Final Report, Validation of an Analytical Method for the Detection of Anti- Glucagon IgM and IgG antibodies in Human Serum, March 2019	Validation	Glucagon
Study 16-047: Characterization of Rabbit anti-mouse IgG/HRP Critical Reagent when used in the Detection of Human IgG and IgM Non- Regulatory Study	Characterization of critical reagent	Both dasiglucagon andglucagon
Study 17-126: Characterization of the ADA detection reagent (rabbit a- Mouse Ig/HRP) applied to the clinical ADA assays for anti-ZP4207 (Study 14-173) and anti-glucagon (study 14-174)	Characterization of critical reagent	Both dasiglucagon and glucagon
Study 18-056: Development of Additional human IgG/IgM sensitivity controls for the clinical ZP4207 and glucagon ADA assays	Development of IgG/IgM sensitivity quality control samples	Both dasiglucagon and glucagon
Study 18-076: Partial Validation of an Analytical Method for the Detection of Anti-Glucagon IgM and IgG antibodies in Human Serum	Validation of IgG/IgM sensitivity quality control samples	glucagon
Study 18-077: Partial Validation of an Analytical Method for the Detection of Anti-ZP4207 IgM and IgG antibodies in Human Serum	Validation of IgG/IgM sensitivity quality control samples	dasiglucagon
Study 14-205: Development of an assay to detect neutralizing antibodies against ZP4207	Assay development	Both dasiglucagon and glucagon
Study 15-039: Validation of an assay to detect neutralizing antibodies toZP4207 by measuring cAMP in glucagon	Validation	dasiglucagon

ADA Validation Report Title	Purpose	Relevant for anti dasiglucagon/glucagon
receptor transfected cell line – Amendment		
Study 15-039 Addendum 1: Validation of an Assay to Detect Neutralizing Antibodies to ZP4207by Measuring Cyclic Adenosine Monophosphate (cAMP) in a Glucagon Receptor Transfected Cell Line	Partial validation tosupport change in Casein	dasiglucagon
Study 15-039 Addendum 2: Validation of an Assay to Detect Neutralizing Antibodies to ZP4207by measuring Cyclic Adenosine Monophosphate (cAMP) in a Glucagon Receptor Transfected Cell Line	Partial validation tosupport change in key reagent (cAMP) and titration of neutralizing effect	Dasiglucagon
Study 15-040: Validation of an Assay to Detect Neutralizing Antibodies to Glucagon by Measuring Cyclic Adenosine Monophosphate (cAMP) in a Glucagon Receptor Transfected Cell Line	Validation	Glucagon
Study 15-040 Addendum: Validation of an Assay to Detect Neutralizing Antibodies to Glucagon by Measuring Cyclic Adenosine Monophosphate (cAMP) in a Glucagon Receptor Transfected Cell Line	Partial validation to support change in key reagent (cAMP) and titration of ADA positive samples	Glucagon

The in vitro neutralizing antibody (NAb) assays were based on glucagon receptor transfected human embryonic kidney cells. Samples and antibody positive controls (neat serum concentrations: LPC 60ng/mL, MPC 120 ng/mL and HPC 250ng/mL) were diluted in assay buffer and pre-incubated with 80pM dasiglucagon for a minimum of 15 minutes at 37° C. After pre-incubation with dasiglucagon, cells were treated with the samples and controls in assay buffer at the minimum required serum dilution of 1:10 for approximately 15 minutes. After incubation, samples were aspirated from the cells, and the cells were lysed for approximately 15 minutes using a cell lysis buffer. The cyclic adenosine monophosphate (cAMP) in the cell lysate was measured using the MSD® Cyclic AMP competitive assay.

The assay is a competitive ELISA in which the signal increases in the presence of neutralizing anti-drug antibodies.

The following neutralizing antibody (NAB) assays have been validated for ZP4207: 14-205 and 15-039.

The following neutralizing antibody (NAB) assay has been validated for glucagon: 15-040.

The validation of the NAb assays included assessment of the assay cut point, sensitivity, specificity, selectivity, drug tolerance, antibody and control precision, assay robustness, short-term and freeze/thaw stability. Appropriate controls were run on each plate during validation to monitor the performance of the assay.

Evaluation and Qualification of Models

Four PopPK models were developed for dasiglucagon:

- PK/PD model 16-147 was developed based on phase 2 data from trial 15126 to support the dose selection of dasiglucagon
- PK/PD model 19-077, developed from model 16-147 and updated and refined with data from the five phase 3 trials of dasiglucagon conducted in adult and paediatric patients with T1DM, has the main objective to quantify the impact of specific covariates on dasiglucagon PK in order to explain between-subject variability
- PK/PD model 20-046, developed from model 16-147 and updated and refined with data from the Studies 16137, 17084, 17086 and 17145. The main objective of the model update was to quantify the impact of subject covariates on glucose response to treatment with dasiglucagon to explain between-subject variability.
- PK/PD model 17-094 was developed based on phase 2 data from trial 15126 to support the paediatric dose selection of dasiglucagon

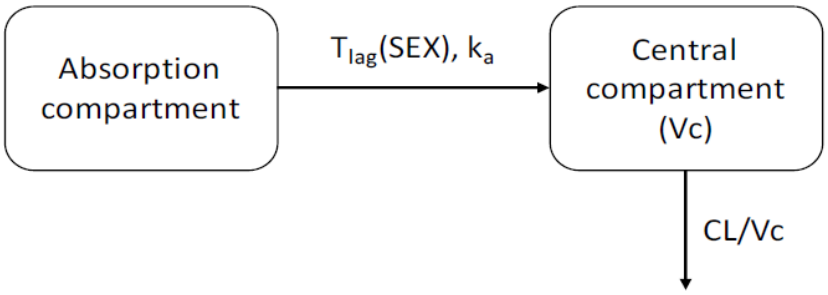
Table 3 shows a summary of continuous and categorical patient covariates included in the PK analysis datasets.

Table 3 - Summary of baseline covariates included in the PK analysis dataset (n=54).

Covariate	Categories	Patients or mean (sd)
DOSE	0.1 mg/0.3 mg/0.6 mg/1 mg	6/16/16/16
AGE (years)	Mean (sd)	36.1 (8.54)
WT (kg)	Mean (sd)	76.6 (7.58)
ALT (U/L)	Mean (sd)	19.7 (12)
AST (U/L)	Mean (sd)	20.4 (6.19)
CRCL (mL/min)	Mean (sd)	125 (19.4)
SEX	Female/Male	24/30

The linear elimination of the log-transformed dasiglucagon concentration supports the selection of a one-compartment elimination model, with linear lagged SC absorption and first order linear elimination, as shown in Figure 2.

Figure 3 - **Structure of the final dasiglucagon PK model. The GLPG0634 dose enters the absorption compartment and flows into the central compartment following a gender-dependent lag-time.**



The stepwise addition of covariates ($p<0.01$) in the dasiglucagon PK model led to the inclusion of sex on the absorption lag time (Tlag). SEX on Tlag remained as the only statistically significant ($p<0.001$) covariate in the final PK model.

Table 4 shows the estimated parameter values based on the final dasiglucagon PK model. All parameters were estimated with good precision (RSE<35%) and acceptable shrinkage (34% on ka, otherwise <10%). Based on the estimated PK parameters, the elimination rate constant was 1.8 h-1, corresponding to an elimination half-life of approximately 23 minutes.

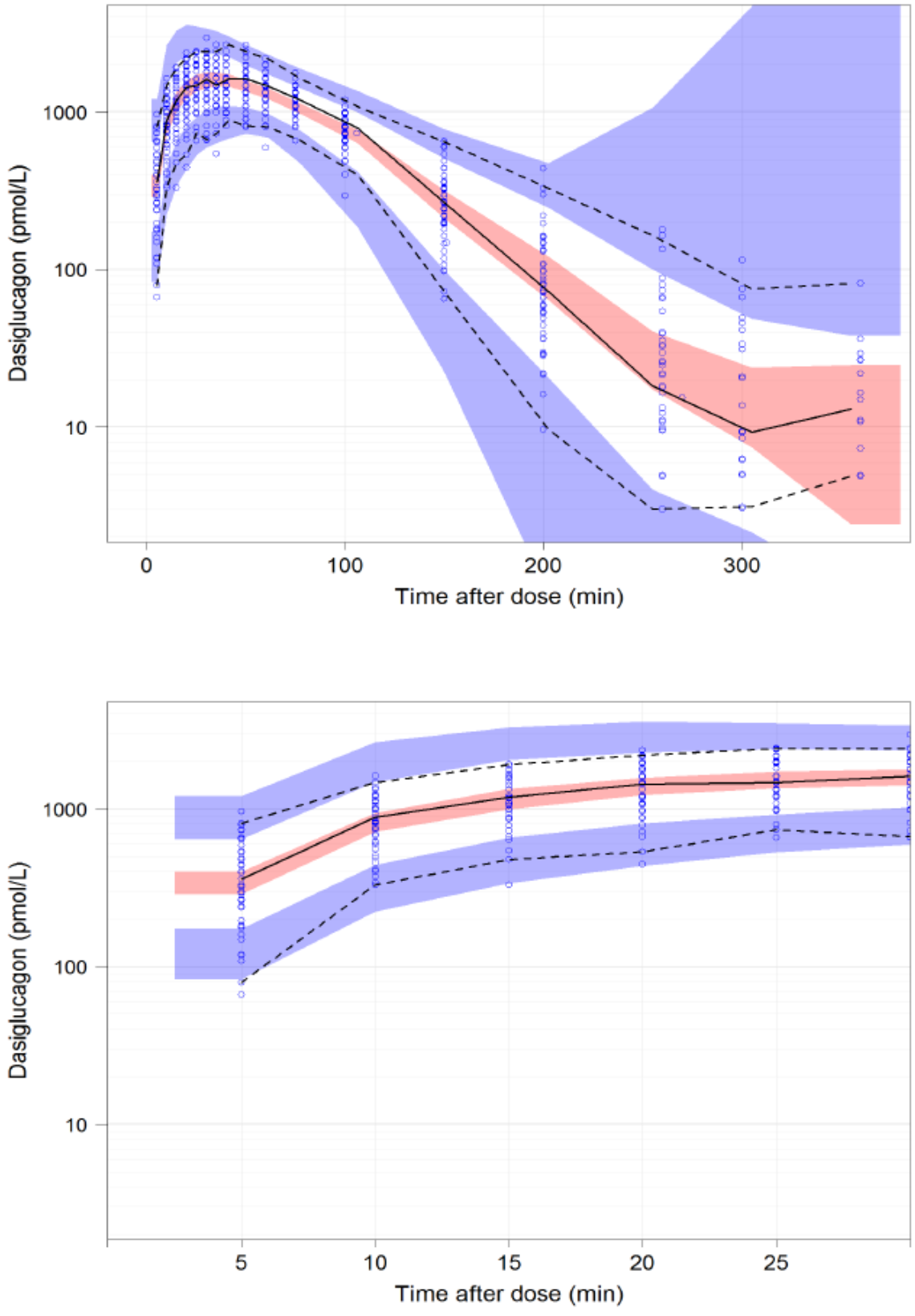
Table 4 - dasiglucagon PK model parameter estimates with relative standard errors (RSE) and bootstrap confidence intervals (317/500 converged replicates). RUV and IIV: residual- and interindividual-variability, respectively.

Description	**Value**	**RSE (%)**	**95% CI**
Objective function value	-1642		[-1806; -1508]
CL (L/h)	68.59	2.064	[65.79; 71.57]
V_c (L)	37.15	4.717	[31.46; 42.02]
k_a (h^{-1})	1.691	2.963	[1.44; 1.843]
T_{lag} in male patients (min)	1.561	12.64	[0.9179; 2.027]
T_{lag} relative increase in female patients	0.4722	34.24	[0.08232; 1.573]
Proportional RUV (CV%)	39.18	5.074	[36.26; 40.83]
Additive RUV (pmol/L)	4.879	21.37	[2.846; 13.06]
IIV CL (CV%)	14.77	20.82	[11.31; 17.6]
IIV V_c (CV%)	43.48	13.8	[32.37; 51.96]
IIV k_a (CV%)	27.48	32.67	[20.02; 32.94]

The appropriateness of the structural and random effect components of the final population PK model was further assessed VPC.

Figure 3 shows the prediction-corrected VPC including data from all dose-levels during 6 hours and during the first 30 minutes after dose.

Figure 4 - Prediction-corrected VPC showing data from all treatment arms across the entire trial (top) and during the first 30 minutes after the dose (bottom).



Figures 4, 5, and 6 show goodness-of-fit plot and residual based diagnostics of the dasiglucagon PK model. Both population and individual predictions vs observations appear to scatter randomly around the line of unity, indicating that the model is unbiased. This was confirmed by the conditional weighted residuals (CWRES) which appear unbiased when plotted against population predictions and time since first dose. Finally, the QQ plot indicates that the distribution of CWRES appears to follow the expected standard normal distribution. The majority of dasiglucagon records appear close to the line of unity with only a few samples with high and low concentrations under- and over-predicted (respectively) by the model.

Figure 5 - Goodness-of-fit plot based on the final dasiglucagon PK model showing population (left) and individual (right) model predictions vs observed dasiglucagon concentrations on linear (top) and log-transformed (bottom) scales.

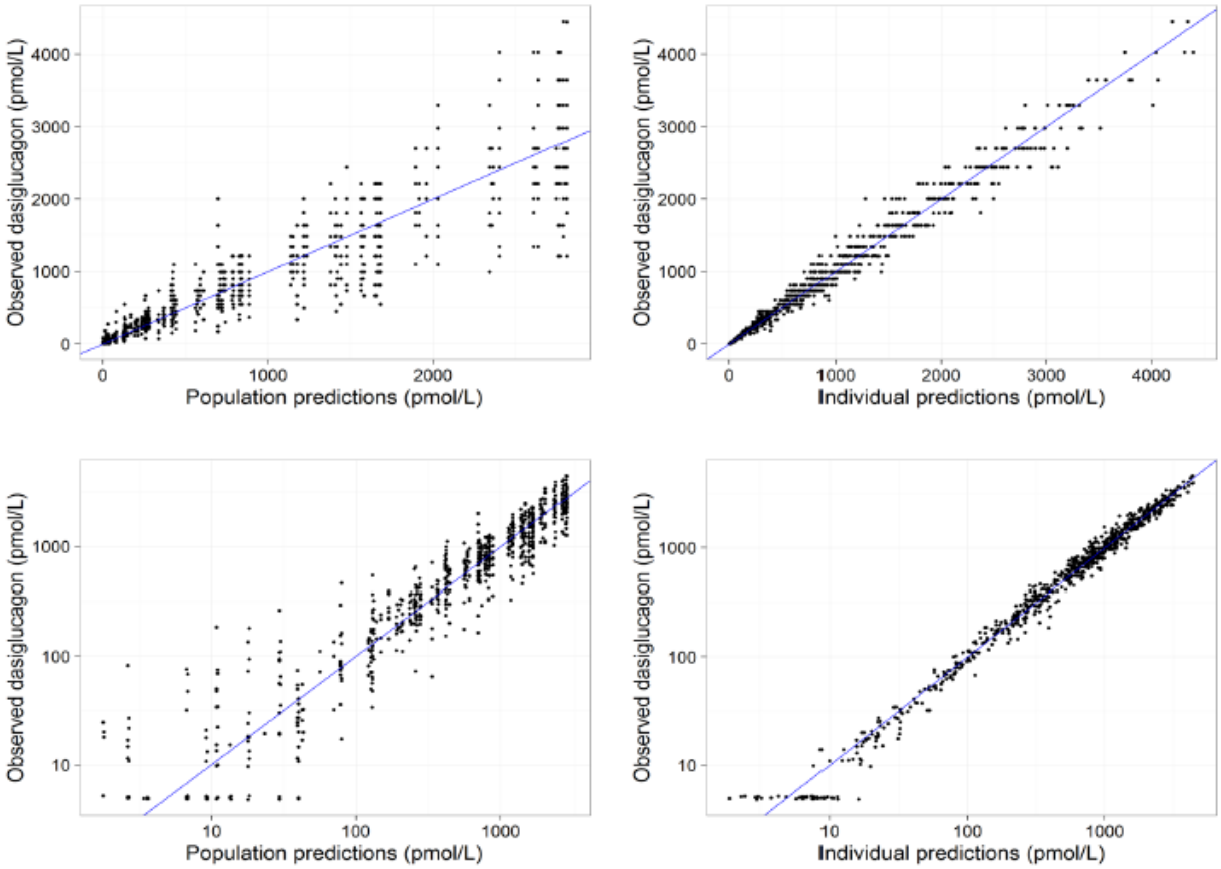


Figure 6 - Residual-based diagnostics of the final dasiglucagon PK model showing conditional weighted residuals (CWRES) vs population predictions (left) and time after dose (right)). Red line (gray area) shows a LOESS fit (95%CI)

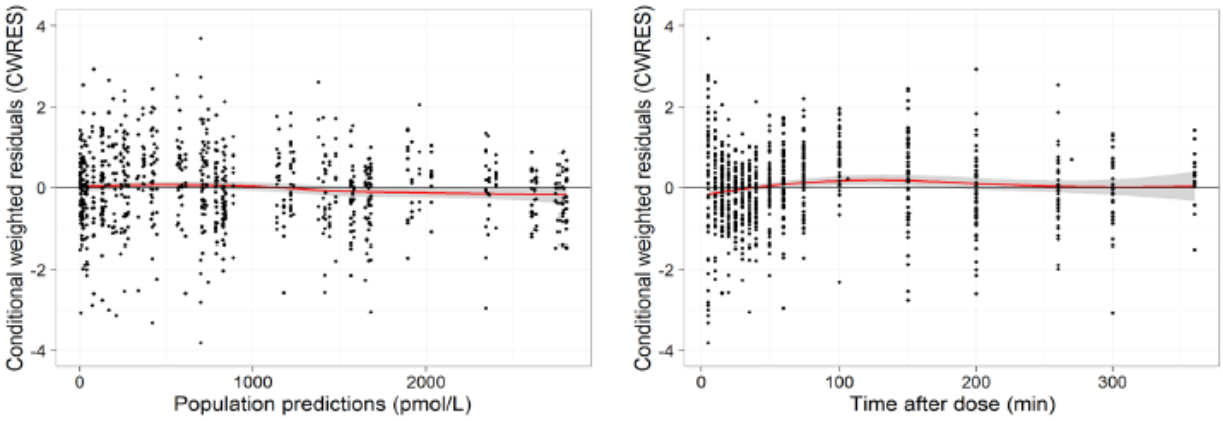
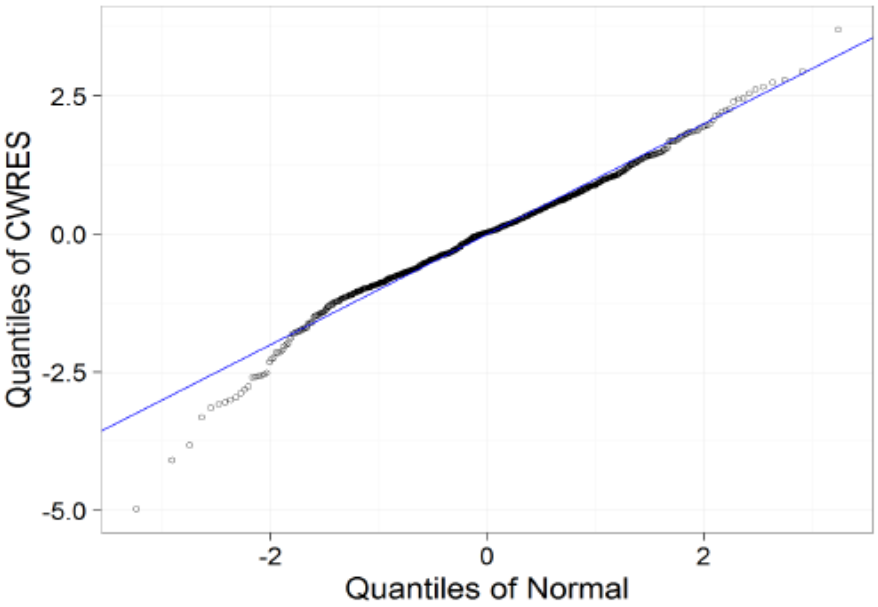


Figure 7 - QQ-plot comparing the conditional weighted residuals (CWRES) of the final dasiglucagon PK model to a standard normal distribution. Dots show CWRES, while the blue line is the line of unity.



The PK/PD analysis dataset was created merging observed glucose concentrations following treatment with dasiglucagon with dosing history, patient covariates and individual PK parameters based on the developed final PK model.

The observed glucose concentration following treatment with dasiglucagon was adequately described by an indirect response model depending on individual dasiglucagon concentration time-profiles predicted by the developed population-PK model.

The VPC indicated that observed between-subject variability was adequately described by the developed PK/PD model despite the individual estimated KOUT was higher than expected in the 0.1 mg dose group, and EMAX and PLAT showing a decreasing and increasing trend with dose, respectively.

Figure 7 shows the VPC including data from all dose levels and time points in the analysis dataset, while Figure 8 shows the first 30 minutes after dose. While most observed data fall within the confidence intervals of the simulated data, the model shows a slight trend towards over-predicting the glucose response after the first hour in the lowest dose group. This implies that the predictive performance of the model is good for doses above 0.1 mg, extrapolation to lower doses (micro doses) should be considered carefully.

Figure 8 - VPC showing glucose response stratified by treatment arm. Solid (dashed) curves show median (5th and 95th percentile) of observed data; red (blue) shaded areas show the 95% CI of the simulated median (outer percentile bands) based on 1000 replicates.

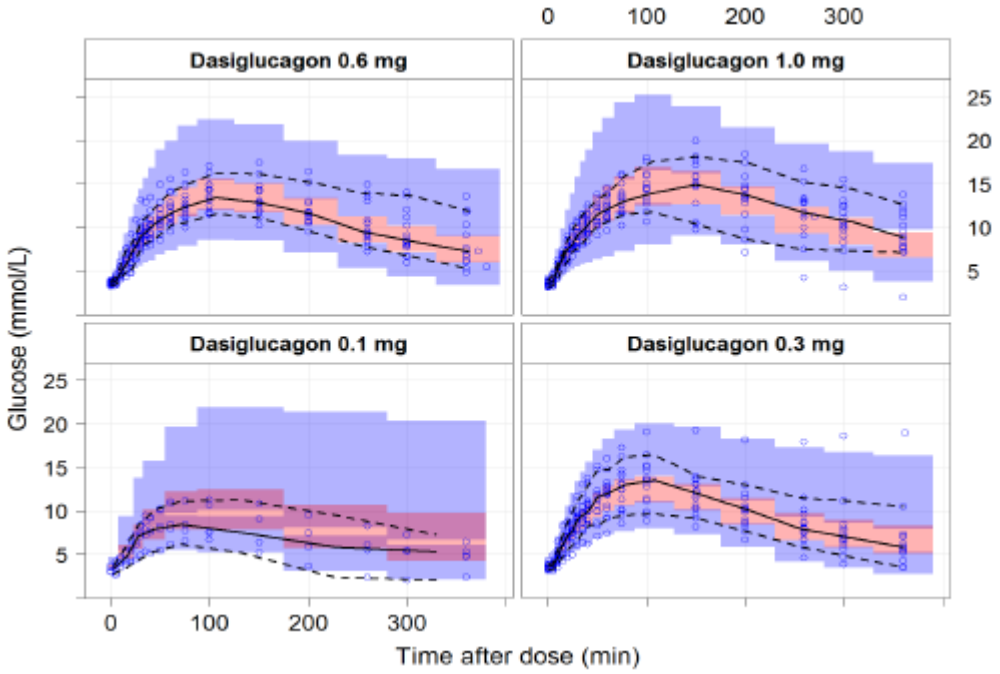
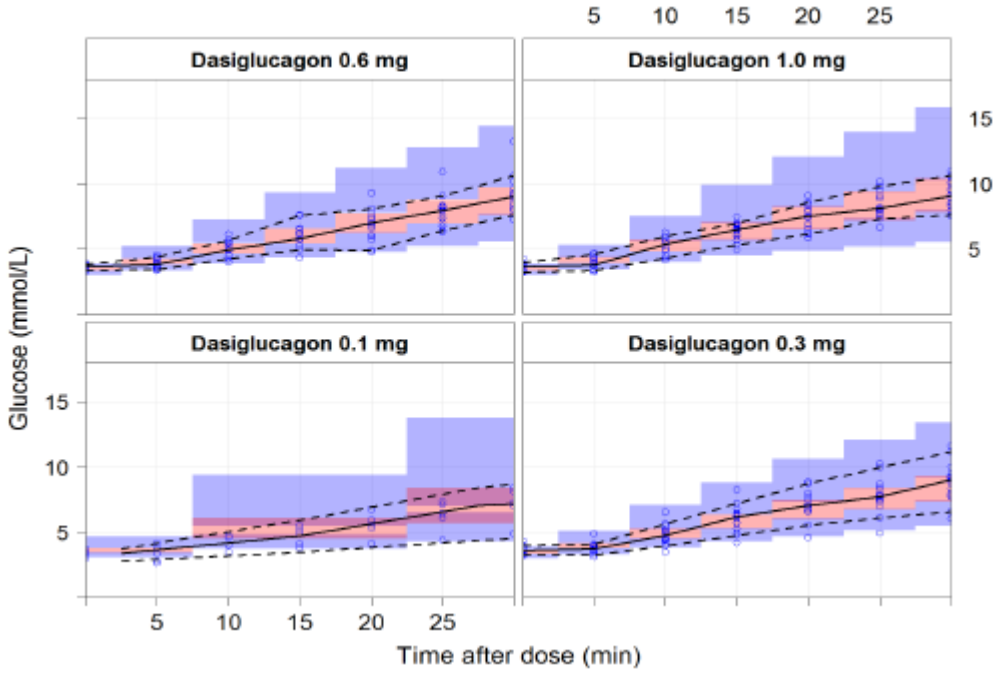


Figure 9 - VPC showing glucose response in the first 30 minutes after dose, stratified by treatment arm.



<u>Study 17-094 - Population PK Modelling and Simulation of Dasiglucagon in Pediatric Patients with Type I Diabetes Mellitus</u>

The objective of this PopPK model was to support the pediatric dose selection of dasiglucagon as a single dose for the acute treatment of severe hypoglycemic events in pediatric patients with Type 1 Diabetes Mellitus (T1DM).

Simulations of dasiglucagon and glucose concentrations following doses of 0.60 mg and 0.30 mg were performed, using previously developed population pharmacokinetic (pop-PK) and pharmacokinetic-pharmacodynamic (PKPD) models in an adult patient population (16-147). The pop-PK model was updated with allometric scaling to accommodate pediatrics.

In simulations, the updated pop-PK model was used as the driver of the glucose response in an unchanged existing PKPD model. A summary of the simulated dasiglucagon exposure and glucose concentrations for pediatrics with typical weight values of 25 kg, 35 kg, and 45 kg, as well as a reference adult population (77 kg) were performed and the pediatric pop-PK model showed dose proportional concentration and exposure. However, for a given dose, pediatric patients with lower body weights, were predicted to have higher dasiglucagon exposure (AUC) and maximum concentration (CMAX) compared to adult patients, while time to maximal dasiglucagon concentration (TMAX), elimination half-life (T1/2), and mean residence time (MRT) were predicted to be lower in pediatrics compared to adults.

The higher exposure simulated for the 0.60 mg compared to 0.30 mg did not result in substantial differences in glucose concentrations between the two doses.

Although the difference was small, the time to achieve glucose CFB >=20 mg/dL was somewhat shorter for the 0.60 mg compared to the 0.30 mg dose.

Study 19-077 - Population Pharmacokinetic Modeling of Dasiglucagon in Subjects with Type I Diabetes Mellitus

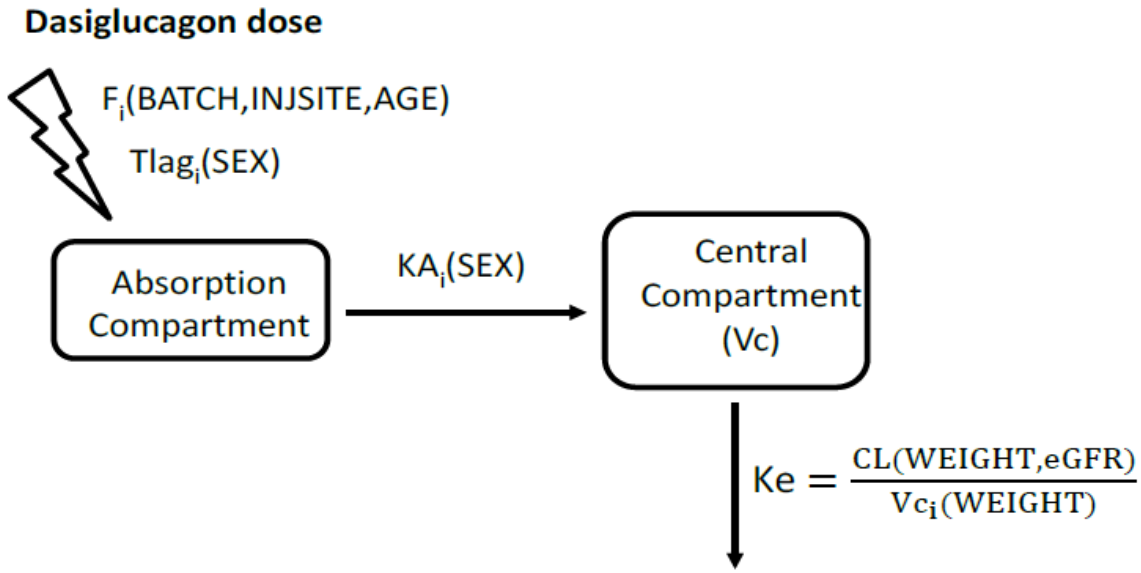
The objective of this PopPK model was to update the previously developed population PK model 16-147 (based on data from trial 15126 only) for dasiglucagon to include data from recently completed trials 16136, 16137, 17084, 17086 and 17145. The main objective of the model update was to quantify the impact of subject covariates on dasiglucagon PK in order to explain between-subject variability.

Using the base population PK model, a covariate analysis was performed. The influence of continuous and categorical covariates was tested for their significance on KA, CL, V, Tlag and F.

During the forward covariate selection step, the following covariate-parameter relationships were found to be significant: MDRD eGFR on CL, age and injection site on F, and body weight and sex on KA. After the backwards elimination step, only MDRD eGFR effect on CL, injection site effect on F and sex effect on KA remained in the model.

In the final model, the dasiglucagon concentration was described by a 1-compartment model with first-order delayed absorption and first-order linear elimination of dasiglucagon, including allometric scaling by body weight on CL and Vc, injection site effect, age effect and batch effect on F, MDRD eGFR effect on CL and sex effect on KA. A schematic representation of the final model with corresponding system of differential and mathematical equations is provided in Figure 9.

Figure 10 - Structure of the Final Dasiglucagon PK Model



The parameter estimates for the final population PK model are presented in Table 5 below.

Table 5 - Final Model (Model 029) Parameter Estimates for Dasiglucagon PK

Parameters	Symbol#	Final Model Estimates		Bootstrap estimates		
		Value	95% CI	Mean	95% CI	
CL (L/hr)		65.3	[63.5; 67.2]	65.1	[63.1; 67.1]	
Vc (L)		33.2	[30.4; 36.3]	33.1	[30; 36.4]	
KA (hr^{-1})		1.13	[1.05; 1.21]	1.12	[1.02; 1.22]	
T_{lag} (minutes)		0.47	[0.33; 0.68]	0.48	[0.30; 0.65]	
BATCH-F: Dual storage vs regular (ratio)	$1 + b_3$	0.866	[0.836; 0.89]	0.866	[0.838; 0.889]	
Weight exponent for CL		0.75 (Fixed)		0.75 (Fixed)		
Weight exponent for Vc		1 (Fixed)		1 (Fixed)		
INJSITE-F						
Thigh vs abdomen (ratio)	$1 + b4$	0.69	[0.62; 0.75]	0.68	[0.63; 0.75]	
Buttocks/deltoid vs abdomen (ratio)	$1 + b5$	0.86	[0.80; 0.91]	0.86	[0.80; 0.91]	
AGE-F : Age_{kp} (years)	b_7	24	[24.0; 24.0]*	24	[20.9; 27.2]*	
Age > kp: 1						
Age ≤ kp: $1 + \theta*(Age-Age_{kp})$	b_6	0.0275	[0.0182; 0.0369]	0.0272	[0.0165; 0.0466]	
SEX-KA: Female vs Male (ratio)	$1 + b_1$	1.29	[1.23; 1.35]	1.29	[1.20; 1.39]	
eGFR-CL: $(eGFR/95.16)^{\theta}$	b_2	0.19	[0.08; 0.31]	0.19	[0.07; 0.29]	
Random effects						**Shrinkage (%)**
IIV on Vc (CV%)		37	[30.8; 42.5]	36.7	[31.1; 42.8]	21.5
IIV on KA (CV%)		26.8	[22.8; 30.3]	26.8	[22.4; 31.0]	29.6
IIV on T_{lag} (CV%)		163	[111; 224]	159	[111; 238]	40.8
IIV on F (CV%)		20.9	[18.5; 23]	20.4	[18.3; 22.7]	4.6
Residual error						
Proportional (CV%)		42.3	[40.7; 43.9]	42.2	[40.3; 44.2]	--
Additive (pmol/L)		19	[14.1; 23.8]	19.1	[13.4; 33.1]	10

Source: parmest.docx and parmest-bootstrap.docx

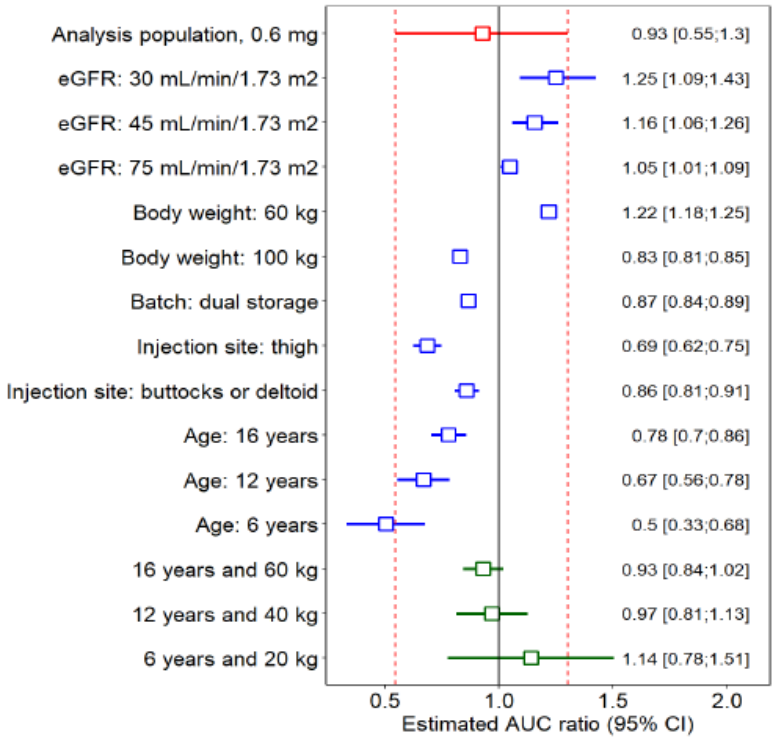
#: Symbol used in the mathematical description of final model in Figure 4. *: Estimated 95% CI based on the bootstrap analysis more realistic than based on the parametric method. Abbreviations: AGE-F=age effect on F, Age_{kp}=age knot point in the piece-wise linear relationship between F and age, BATCH-F=batch effect on F, CI=confidence interval, CL=clearance, CV=coefficient of variation, eGFR=estimated glomerular filtration rate, eGFR-CL=eGFR effect on CL, F=relative bioavailability, IIV=inter-individual variability, INJSITE-F=injection site effect on F, KA=absorption rate constant; kp=knot point, SEX-KA=sex effect on KA, T_{lag}=absorption lag time, Vc=central volume of distribution.

The η- shrinkage was 4.6% for F, 21.5% for Vc, 29.6% for KA and 40.8% for Tlag. The ε-shrinkage was 10%.

The 95% CI of all parameter estimates exclude zero and indicated that the respective parameter values were statistically significantly different from zero.

Figure 10 illustrates the magnitude of covariate effects on dasiglucagon exposure (AUC0-inf) relative to the reference subject profile relative to exposure predicted for a reference subject of at least 24 years and 78 kg body weight who received 0.6 mg dasiglucagon from the regular batch through an abdominal injection. The predictions of individual covariate effects did not account for correlations between covariates.

Figure 11 - Relative Effect of Covariates on Dasiglucagon Exposure



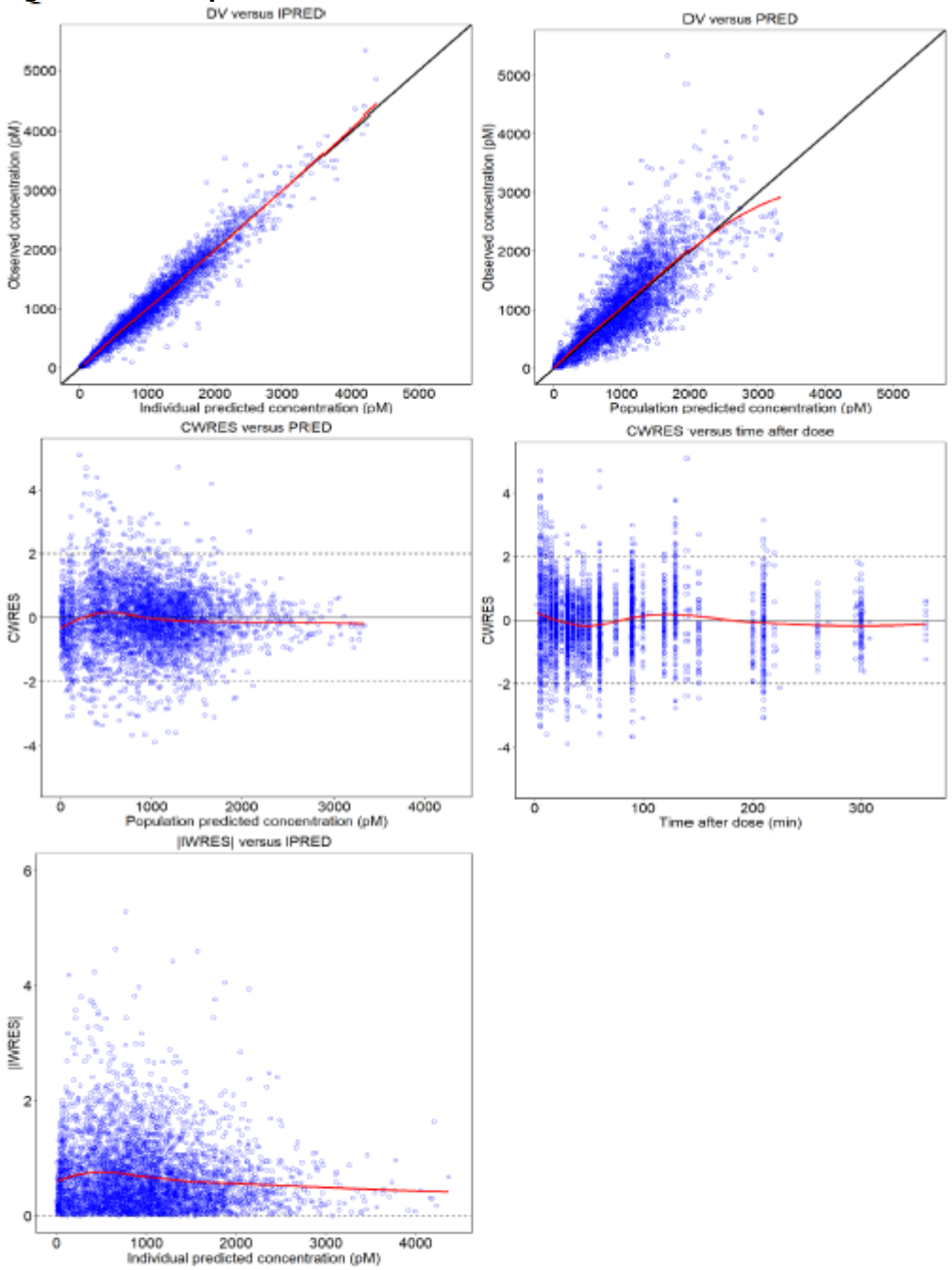
Source: parmest.docx

Notes: Exposures are relative to a reference subject of at least 24 years and 78 kg body weight who received 0.6 mg dasiglucagon from the regular batch through an abdominal injection. The red marker (error bar) shows the median (5^{th} to 95^{th} percentile interval) of model-predicted individual exposures for all subjects in the analysis dataset (n=321) following a subcutaneous dasiglucagon dose of 0.6 mg. Blue markers (error bars) show the model-predicted (95% CI) relative exposure for selected sub-populations. Green markers (error bars) show the predicted relative exposure in children when considering correlations between age (16, 12, and 6 years, respectively), body weight (60, 40, and 20 kg, respectively), and renal function (MDRD eGFR of 106, 187, and 273 mL/min/1.73m^2, respectively).

GOF plots show that the model described the observed data well and showed no trends in the residual plots (CWRES vs PRED and |IWRES| vs IPRED) (

Figure 11).

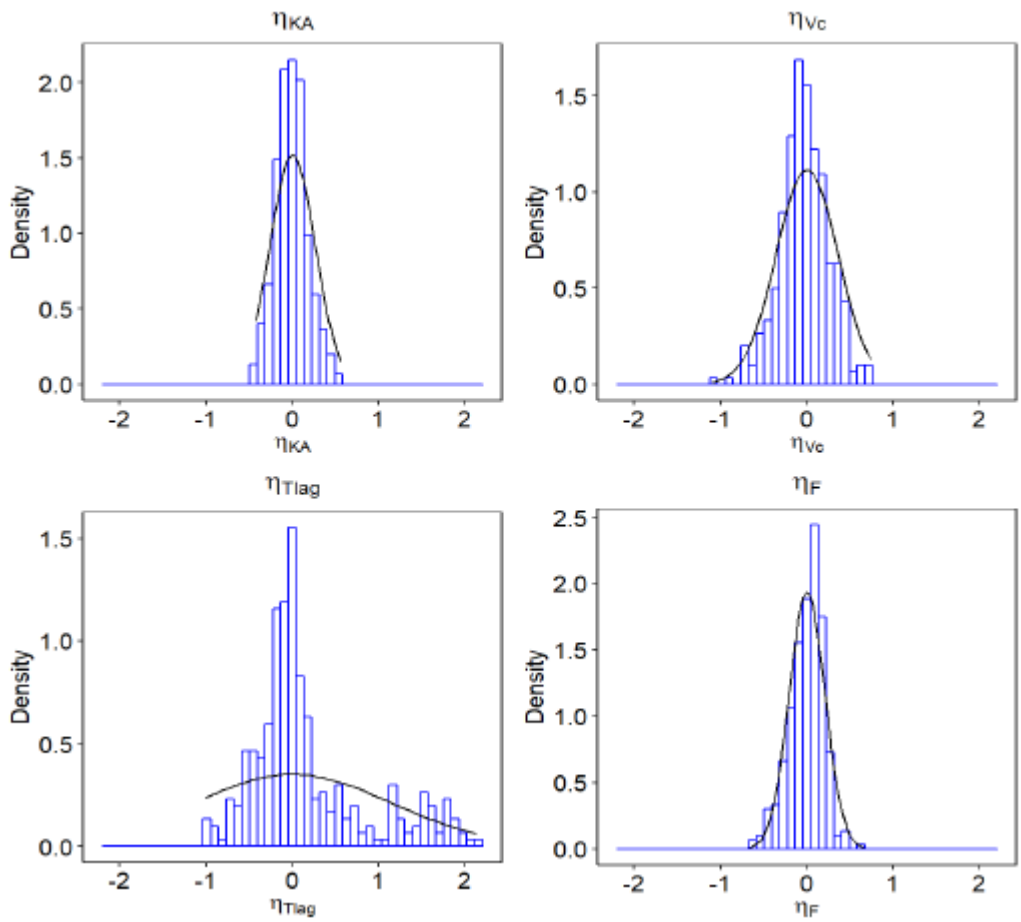
Figure 12 - GOF plots for the final model



Source: gof-plots.docx
Notes: Dots are individual data points and solid red lines are smoothed LOESS lines. The solid black lines in the 2 plots in the first row are lines of identity. The dashed lines in the 2 plots in the second row show the boundaries of the CWRES ± 2 interval.
Abbreviations: CWRES=conditional weighted residuals, DV=dependent variable (ie, dasiglucagon concentration), GOF=goodness of fit, IPRED=individual predictions, |IWRES|=absolute values of individual weighted residuals, PRED=population predictions.

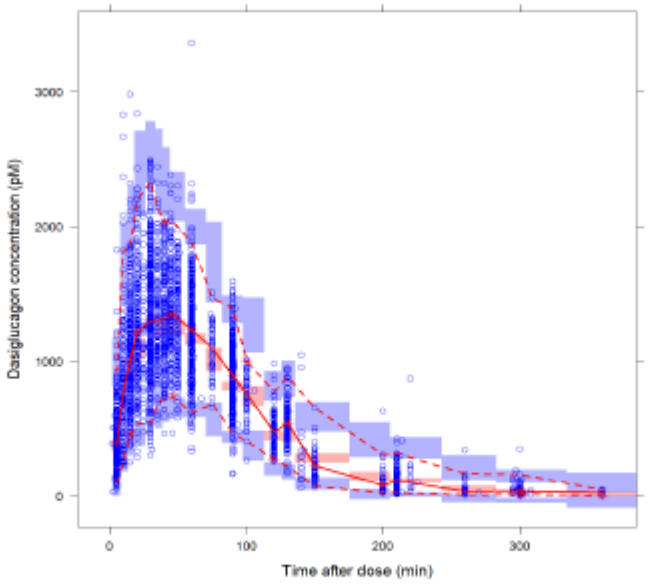
Examination of random effects and prediction-corrected VPC were performed to assess the robustness of the final population PK model. Overall, η-distributions were close to normal, reflecting the adequacy of the exponential models for IIV (Figure 12).

Figure 13 - Distribution of Random Effects on KA, Vc, Tlag and F for the Final Model



The prediction-corrected VPC for the population PK model is displayed in Figure 13.

Figure 14 - Prediction-Corrected VPC of the Final Model



Source: vpc.docx
Notes: Blue dots are observed data points; red solid line is the observed median; red dashed lines are observed p5 and p95. The pink area is the 95% prediction interval (PI) of the simulated median, and purple areas are the 95% PI of the simulated p5 and p95 based on 500 replicates.

Study 20-046 - Pharmacokinetic/Pharmacodynamic Modeling of Dasiglucagon in Subjects with Type 1 Diabetes Mellitus

The objective of this PK/PD model was to update the previously developed pharmacokinetic/pharmacodynamics (PKPD) model 16-147 (based on data from Study 15126 only) for dasiglucagon with data from Studies 16137, 17084, 17086 and 17145. The main objective of the model update was to quantify the impact of subject covariates on glucose response to treatment with dasiglucagon to explain between-subject variability.

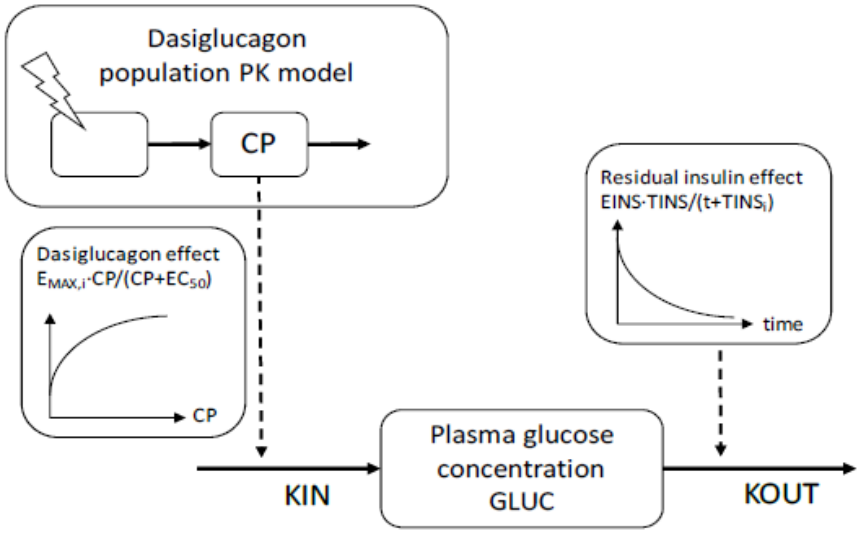
The evaluation of covariates in the PKPD model focused on the most clinically relevant covariates.

The updated population PK model consisted of a 1-compartment model with first-order delayed absorption and first-order linear elimination. Systemic clearance (CL) and central volume of distribution (Vc) were predicted to decrease with decreasing body weight, following an allometric scaling model populated with coefficients based on literature values. The covariate model implemented in the updated population PK model included statistically significant effects of injection site and age on bioavailability (F), sex on the rate of absorption (KA), and modification of diet in renal disease (MDRD) estimated glomerular filtration rate (eGFR) on systemic CL.

A schematic representation of the final PKPD model with corresponding system of differential and mathematical equations is provided in

Figure **14**.

Figure 15 - Structure of the final dasiglucagon PK model



The parameter estimates for the final PKPD model are presented in Table 6.

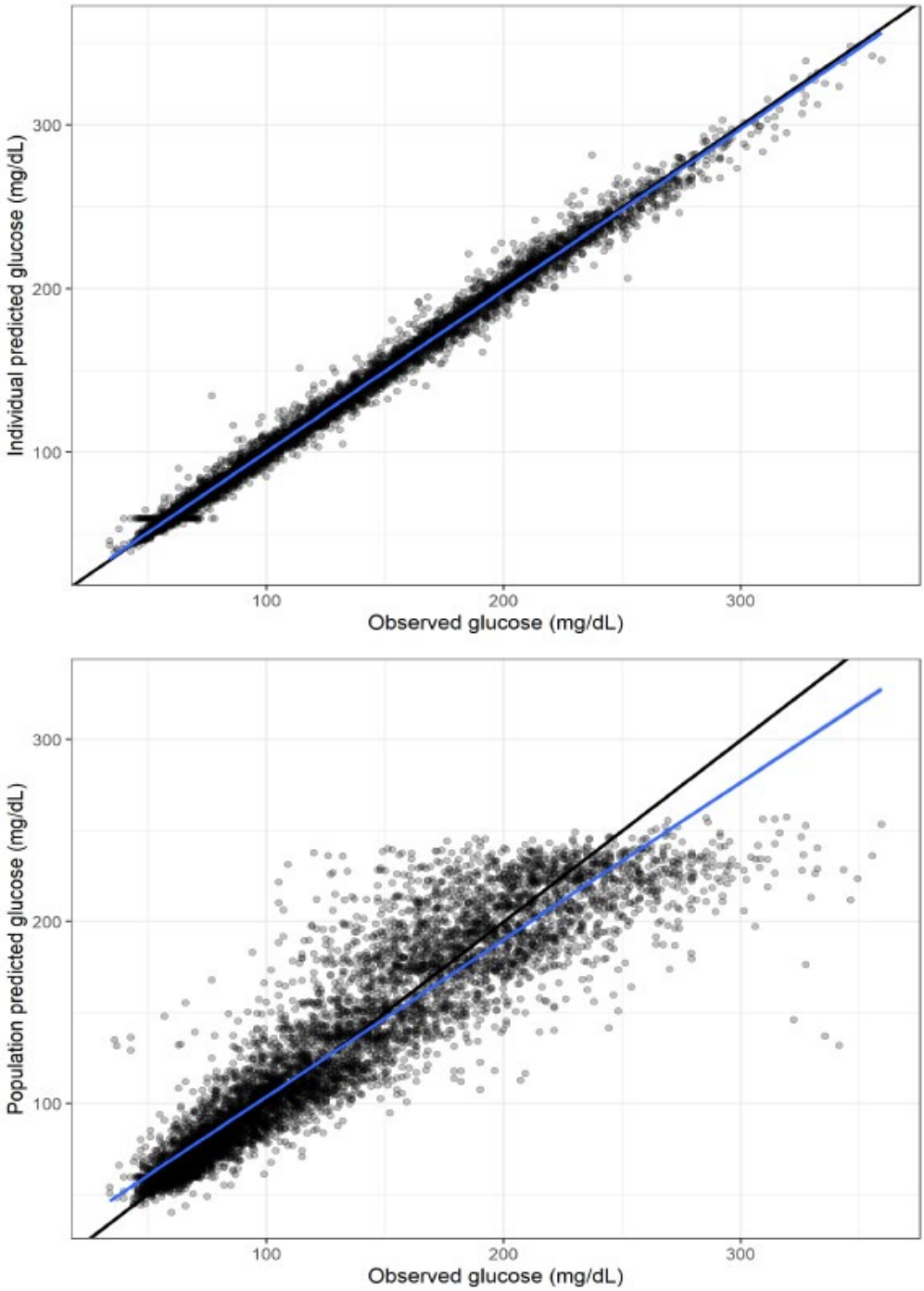
Table 6 - Parameter estimates of the final PKPD model (run134.mod)

Parameter	NONMEM	Estimate (%RSE)	Bootstrap 95%CI	Untransformed estimate
Glucose steady state (PLAT)	THETA15	4.874 (0.899%)	[4.768 ; 4.949]	130.8 mg/dL
Glucose elimination rate (K_{OUT})	THETA16	0.5141 (5.54%)	[0.4578 ; 0.5765]	1.672/hr
Maximal dasiglucagon effect (E_{MAX}) on glucose release rate (K_{IN})	THETA17	0.1461 (53.8%)	[0.007343 ; 0.328]	115.7% change
Dasiglucagon concentration at half-maximal effect on K_{IN} (EC_{50})	THETA18	-0.8169 (12.5%)	[-1.052 ; -0.6315]	130.6 pmol/L
Estimated baseline glucose (BASE)	THETA20	4.084 (0.12%)	[4.074 ; 4.093]	59.35 mg/dL
Maximal residual insulin effect on K_{OUT} (EINS)	THETA21	2.278 (4.11%)	[2.036 ; 2.445]	975.8% change
Time to half-maximal insulin effect on K_{OUT} (T_{INS})	THETA22	-5.006 (3.13%)	[-5.307 ; -4.614]	0.4019 minutes
Glucose steady state in pediatric vs adult subjects (PLAT PED)	THETA23	0.106 (41.7%)	[0.02542 ; 0.1997]	10.6% change
Estimated baseline glucose in pediatric vs adult subjects (BASE PED)	THETA25	0.2118 (8.31%)	[0.177 ; 0.2476]	21.18% change
Effect of 10% increase of observed baseline glucose on K_{OUT}	THETA27	-0.7703 (22%)	[-1.117 ; -0.4506]	-7.078% change
Effect of US vs non-US region on K_{OUT}	THETA28	-0.1426 (27.7%)	[-0.215 ; -0.05554]	-14.26% change
Effect of 10% increase of observed baseline glucose on T_{INS}	THETA29	-4.712 (9.42%)	[-5.562 ; -3.889]	-36.18% change
Proportional RUV	SIGMA.1.1.	0.002543 (2.19%)	[0.002315 ; 0.002767]	5.043%$_{CV}$
IIV on PLAT	OMEGA.1.1.	0.1813 (5.41%)	[0.1433 ; 0.222]	42.58%CV
PLAT-K_{OUT} correlation	OMEGA.2.1.	-0.06752 (11.2%)	[-0.1017 ; -0.03721]	-41.42
IIV on K_{OUT}	OMEGA.2.2.	0.1466 (6.32%)	[0.1122 ; 0.185]	38.29%CV
PLAT-E_{MAX} correlation	OMEGA.3.1.	-0.2224 (6.03%)	[-0.2742 ; -0.1693]	-79.43
K_{OUT}-E_{MAX} correlation	OMEGA.3.2.	0.001667 (571%)	[-0.03318 ; 0.04487]	0.662
IIV on E_{MAX}	OMEGA.3.3.	0.4326 (6.18%)	[0.3249 ; 0.5379]	65.78%CV
PLAT-T_{INS} correlation	OMEGA.4.1.	0.2328 (8.81%)	[0.1497 ; 0.3102]	57.04
K_{OUT}-T_{INS} correlation	OMEGA.4.2.	-0.1481 (12.4%)	[-0.221 ; -0.07145]	-40.35
E_{MAX}-T_{INS} correlation	OMEGA.4.3.	-0.1209 (23.8%)	[-0.2375 ; 0.000691]	-19.18
IIV on T_{INS}	OMEGA.4.4.	0.9192 (5.9%)	[0.7176 ; 1.155]	95.87%CV

Shrinkage was less than 30% for all random effects except for random effects on the EMAX parameter (36%).

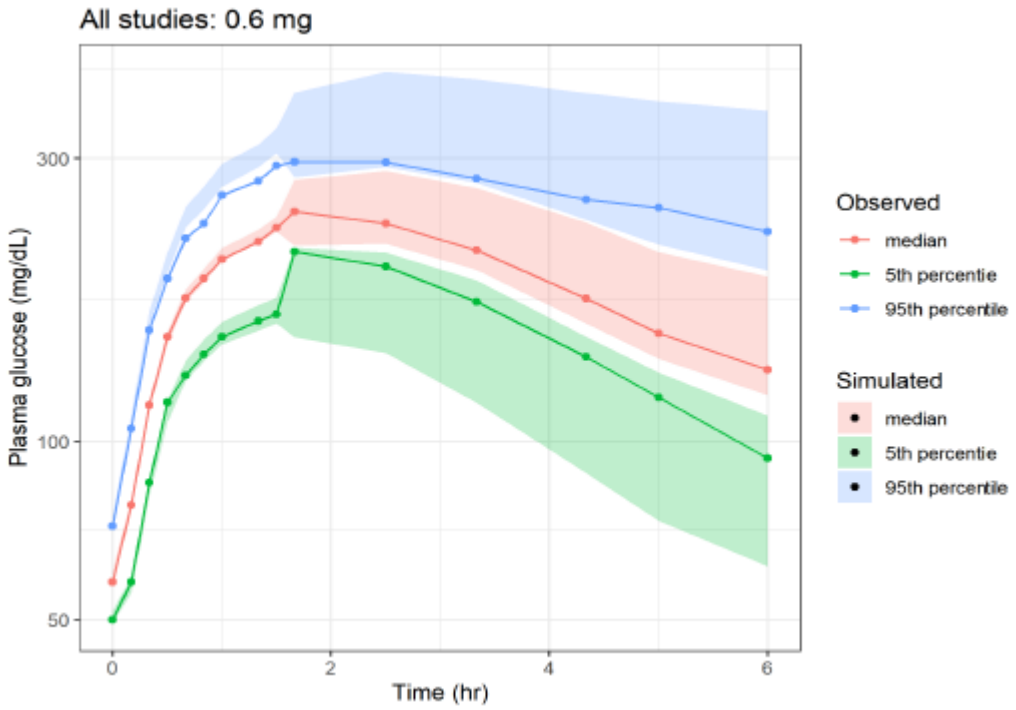
Figure 15 shows the GOF at the individual (top panel) and population level (bottom panel). The individual predictions scattered randomly and closely around the line of unity, indicating that the structural and random model components allowed the model to accurately reproduce the individual observed glucose data. The GOF plot of the population predictions also showed no substantial bias. However, the linear regression of population predicted vs observed glucose concentrations had a slope slightly less than 1. This indicated a slight under- and over-prediction of the highest and lowest glucose concentrations, respectively, reflecting regression to the mean.

Figure 16 - Goodness-of-fit of observed glucose data and individual model predictions



A VPC of the full time course of available glucose concentrations for subjects treated with 0.6 mg dasiglucagon across all 5 studies is shown in Figure 16.

Figure 17 - VPC of the final PKPD model including all 0.6 mg dose groups



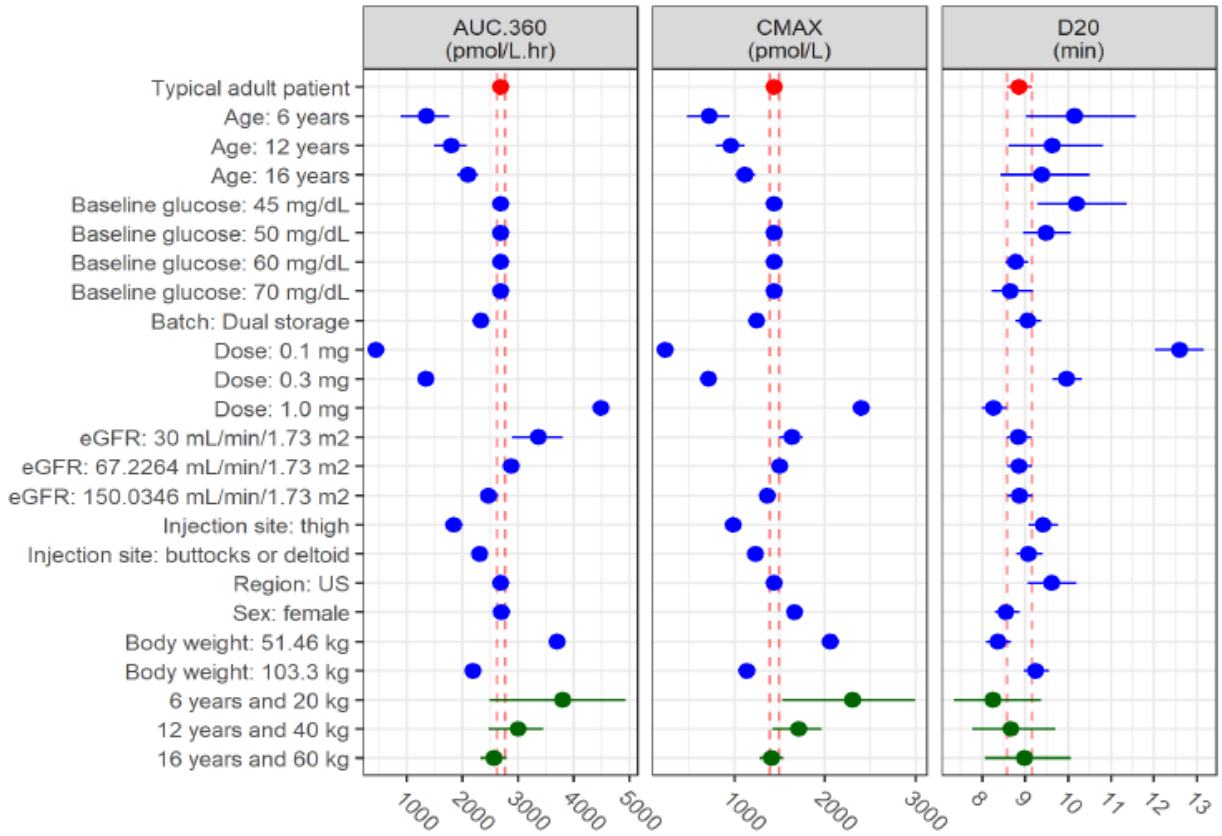
The VPC confirmed that the final PKPD model was able to accurately reproduce the median, 5th percentile, and 95th percentile of observed data in most of the considered strata of the analysis dataset. An exception was the 0.1 mg dasiglucagon dose group in Study 15126, in which the observed glucose concentrations were over-predicted by the model and the placebo data.

Overall, the model showed a tendency to over-predict variability. This tendency was most pronounced in the placebo arms.

The impact of covariates on dasiglucagon PK and glucose PD metrics was simulated. The predicted AUC0-360, CMAX, and D20 is shown in Figure 17.

The prediction for the typical subject is shown in red with vertical lines, indicating the 95%CI related to the uncertainty of parameter estimates. Dasiglucagon dose was the only predictor in the model with clinically significant impact on D20. Other covariates had limited impact on D20.

Figure 18 - Impact of covariates on dasiglucagon AUC and CMAX, and time to a 20 mg/dL glucose concentration increase from baseline



An alternative population PK model was discussed in addition to the final population PK model. The alternative model included trial (17086 vs other trials) as a predictor of relative F instead of the piece-wise linear relationship between age and F included in the final population PK model. Simulations indicated that PK and PD predictions in adult subjects were similar with the final and alternative population PK models (Figure 18), while for paediatric subjects up to 7 years of age, the predicted dasiglucagon exposures were substantially higher with the alternative PK model than with the final PK model (Figure 19). The analysis dataset used to estimate the population PK models included paediatric subjects between 7 and 17 years of age, and the predictions of dasiglucagon PK in younger subjects represents an extrapolation outside the range of observed data.

Figure 19 - Median and 5th-95th percentile of dasiglucagon AUC and CMAX, and time to a 20 mg/dL glucose concentration increase from baseline vs age in paediatric subjects at least 7 years old

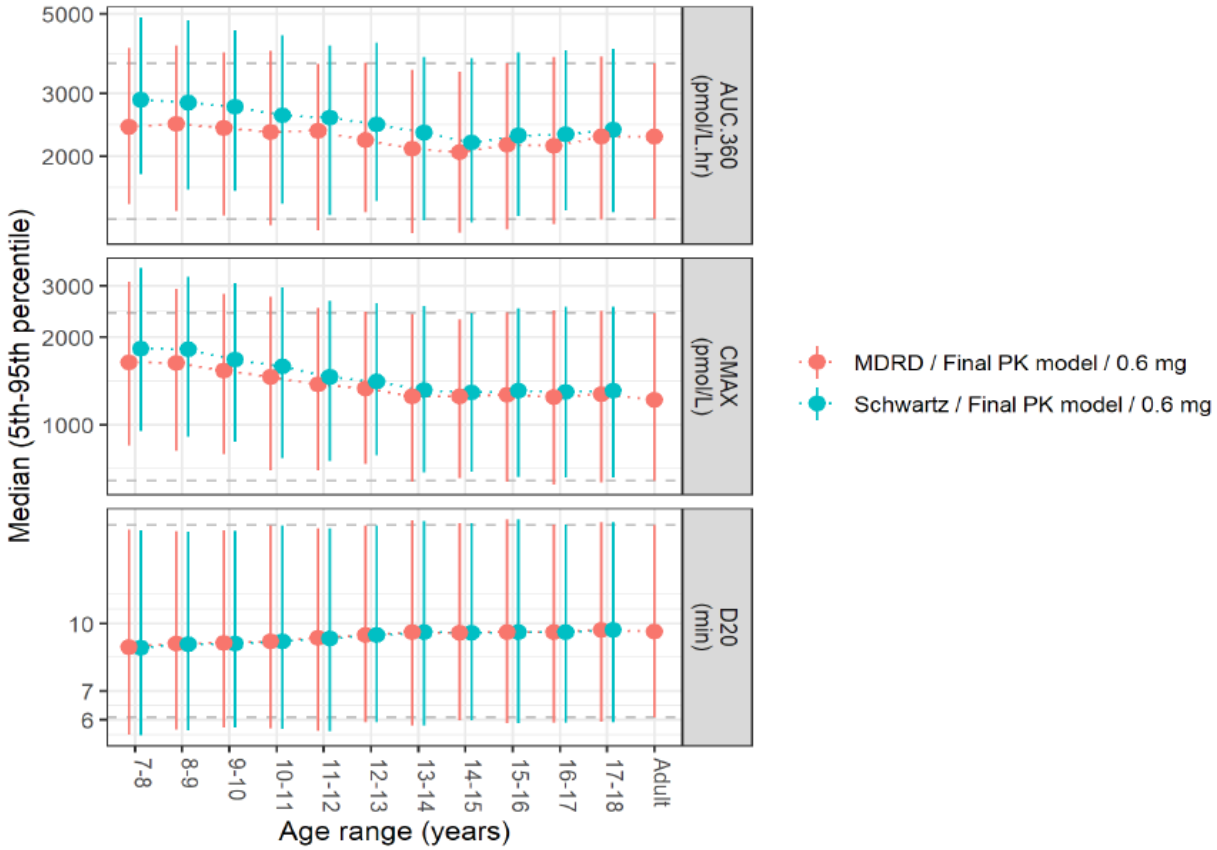
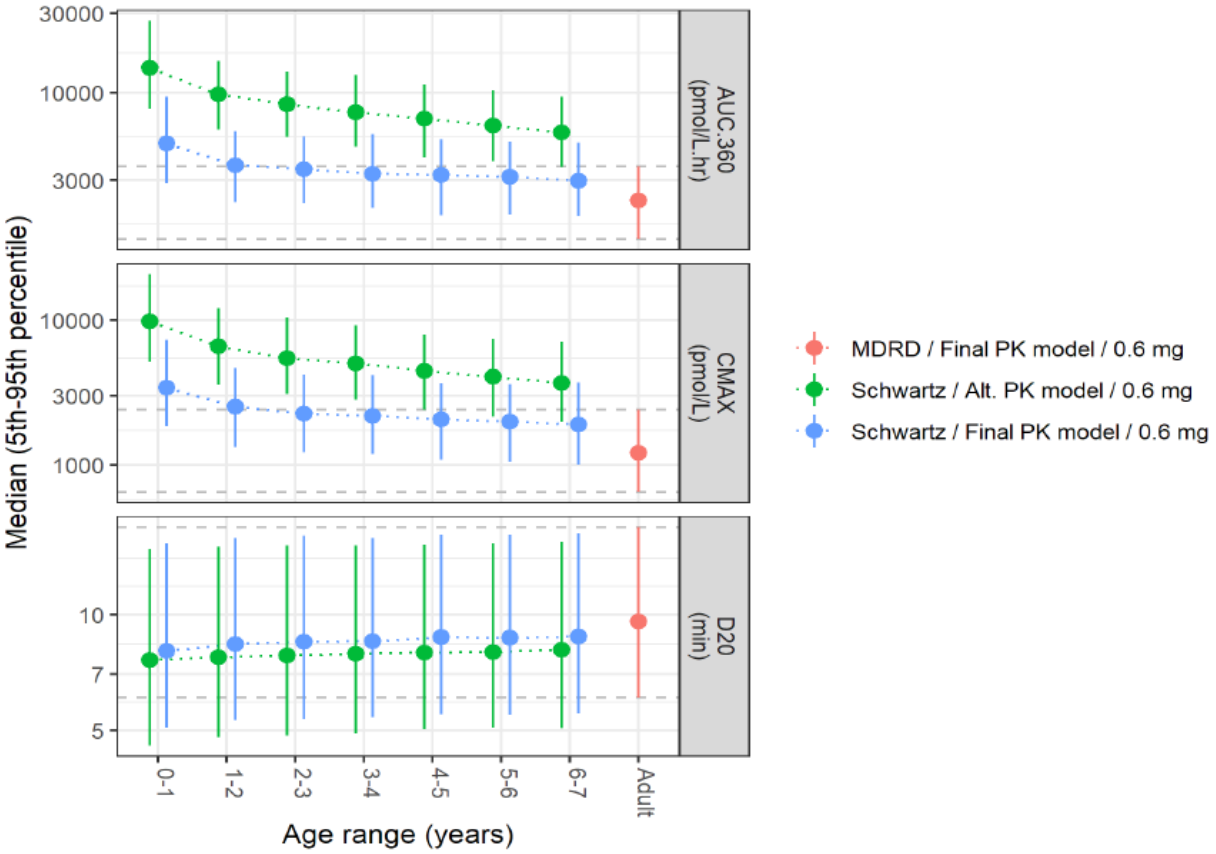


Figure 20 - Median and 5th-95th percentile of dasiglucagon AUC and CMAX, and time to a 20 mg/dL glucose concentration increase from baseline vs age in paediatric subjects of up to 7 years old



Dasiglucagon exposures and maximal concentrations were similar across most of the paediatric age range but appeared substantially higher with the alternative model for paediatric subjects below 2 years of age. Based on these considerations, the largest overlap between the distributions of exposure predicted for adult subjects following treatment with 0.6 mg dasiglucagon and paediatric subjects between 1 and 2 years of age could be obtained with a paediatric dose of 0.3 mg based on the final PK model and 0.1 mg based on the alternative PK model.

Absorption

In each of the 9 trials evaluating dasiglucagon for the treatment of severe hypoglycemia, the route of administration was SC; the IV/QTc trial 17144 also evaluated dasiglucagon exposure after IV administration. Trial 14013 was the only trial that evaluated both SC and intramuscular (IM) administration of dasiglucagon.

In line with observations for human glucagon, and consistent with the literature, the mean absorption rate of dasiglucagon was faster following IM versus SC injection (Trial 14013). In addition, the pharmacodynamic effect of a 0.7 mg IM dasiglucagon dose (percentage of subjects achieving a plasma glucose increase of ≥20 mg/dL [1.1 mmol/L] within 30 minutes of dosing) was equivalent to a 1.0 mg IM dose of GlucaGen (Trial 14013), the approved dose of GlucaGen for SC and IM injection for the treatment of severe hypoglycemia.

Consequently, based on the above findings, accidental IM administration of the to-be-marketed formulation of dasiglucagon is not expected to lead to a clinically relevant difference compared to SC administration.

Thigh injection is expected to lead to lower exposures if compared to abdominal, buttock or deltoid injection. However, dasiglucagon exposure was predicted to be slightly lower also for deltoid and buttock, compared to abdomen.

Bioavailability

The phase 1 IV/QTc trial 17144, a randomized, double-blind, placebo-controlled, dose-escalation trial, with an open-label, non-controlled SC cohort, evaluated the bioavailability of dasiglucagon following SC compared to IV administration in healthy subjects. In the IV dose cohorts, dasiglucagon was administered through an IV line in a peripheral vein and in the SC cohort, dasiglucagon was administered as a SC injection in the abdomen.

Absolute bioavailability was evaluated by comparing the total exposure (AUC0-∞) of SC dasiglucagon with IV dasiglucagon (both 0.6 mg). In total, data from 8 subjects receiving 0.6 mg IV dasiglucagon and data from 6 subjects receiving 0.6 mg SC dasiglucagon were used to assess absolute bioavailability.

The absolute bioavailability after SC administration of 0.6 mg dasiglucagon (estimated from the ratio of geometric mean AUC0-∞ [SC/IV]) was 51%; estimated treatment ratio was 50.99% [38.55; 67.44]95% CI (Table 7).

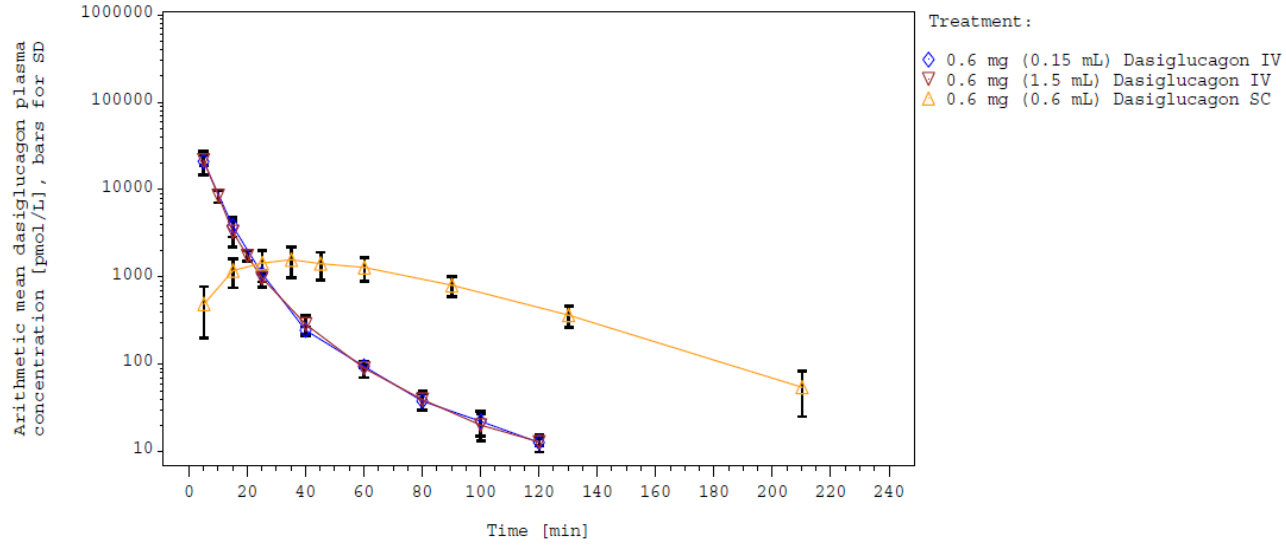
Table 7 - 95% CI for absolute SC bioavailability (%), typical PK profiles only (0.6 mg only)

Pharmacokinetic population

Model	PK Parameter	Absolute bioavailability (F) [%]	Lower limit of 95% CI [%]	Upper limit of 95% CI [%]
SC/IV-0.15 mL	Cmax	7.53	4.27	13.28
	AUC0-inf	50.77	30.94	83.31
SC/IV-1.5 mL	Cmax	7.08	4.89	10.26
	AUC0-inf	51.12	36.77	71.07
SC/IV-volume pooled	Cmax	7.25	5.27	9.97
	AUC0-inf	50.99	38.55	67.44

Figure 21 - 0.6 mg dasiglucagon IV vs SC, group concentration-time curves, typ. PK profiles only, log scale

Pharmacokinetic analysis set.



Distribution

The volume of distribution of dasiglucagon (Vz/f) after administration of a single 0.6 mg SC dose and the Vz/f of 1.0 mg GlucaGen after a single SC dose are presented in Table 8.

Table 8 - Volume of distribution of 0.6 mg dasiglucagon and 1.0 mg GlucaGen after a single subcutaneous dose

Trial number	N	V_z/f (L) Geometric mean (CV%)	
		Dasiglucagon	GlucaGen
Adult patients with T1DM			
Trial 15126	16 / 33	46.6 (39.5)	104 (38.0)
Trial 17084[a]	87 / –	49.2 (44.4)	
Trial 17084[b]	86 / –	56.6 (43.3)	
Pediatric patients with T1DM			
Trial 17086	20 / 10	86.4 (62.2)	373 (81.1)
Healthy adult subjects			
Trial 17144	6 / –	55.0 (41.2)	

Abbreviations: CV, coefficient of variation; N, number of subjects; T1DM, type 1 diabetes mellitus; V_z/f, apparent volume of distribution.
Notes: [a]Dasiglucagon batch stored under refrigerated conditions.
[b]Dasiglucagon batch reflecting storage under the intended dual storage conditions.
N are shown for dasiglucagon / GlucaGen. For the GlucaGen V_z/f endpoint in trial 15126, but not in trial 17086, the baseline-adjusted and truncated value is presented.

In adults with T1DM, the geometric mean Vz/f of 0.6 mg dasiglucagon was approximately 50 L, ranging from 46.6 to 56.6 L.

The geometric mean Vz/f of dasiglucagon was higher in paediatric patients with T1DM, at approximately 86 L, compared with adult patients.

In the phase 1 IV/QTc trial 17144, the geometric mean Vz/f of 0.6 mg dasiglucagon administered SC in healthy adults was similar to that observed in adults with T1DM.

After a single IV administration of 0.6 mg dasiglucagon in trial 17144, the geometric mean Vss was found to be approximately 5 L (Trial 17144), indicating that dasiglucagon is primarily circulating in plasma.

Elimination

The elimination of dasiglucagon in terms of the apparent total clearance from plasma (CL/f) and the elimination half-life (t½) after a single 0.6 mg dose is presented in Table 9. For reference, the elimination of 1.0 mg GlucaGen after a single SC dose is also shown.

After reaching a Cmax at approximately 35 minutes after SC administration, plasma concentrations of dasiglucagon decreased by a geometric mean t½ of approximately 30 minutes (range 29 to 35 minutes) in adults with T1DM. Geometric mean CL/f ranged from 59 to 69 L/h.

The geometric mean t½ values were comparable for both dasiglucagon batches in the bridging trial 17084.

The geometric mean t½ in pediatric patients with T1DM of was approximately 37 minutes, with no difference between children and adolescents. The geometric mean CL/f in pediatric patients was somewhat higher (~96 L/h) than in adults, driven by a higher CL/f in adolescents (109.8 [42.7] L/h) than in children (78.6 [42.0] L/h).

In healthy adult subjects, the mean t½ and CL/f values were similar to those observed in adults with T1DM after SC administration. After a single IV administration of 0.6 mg dasiglucagon in trial 17144, mean CL was found to be approximately 38 L/h (Trial 17144,

Table 10), i.e., approximately half the CL/f after SC administration. Dasiglucagon was rapidly eliminated, with a t½ following IV and SC administration of approximately 20 minutes (Trial 17144,

table 10) and 30 minutes (Table 9), respectively. This indicates that the t½ after SC administration is influenced by the absorption rate (flip-flop kinetics).

Table 9 - Elimination of 0.6 mg dasiglucagon and 1.0 mg GlucaGen after a single subcutaneous dose

Trial number	N	CL/f (L/h) Geometric mean (CV%)		$t_{½}$ (min) Geometric mean (CV%)	
		Dasiglucagon	GlucaGen	Dasiglucagon	GlucaGen
Adult patients with T1DM					
Trial 15126	16 / 33	68.0 (14.8)	180 (22.9)	28.5 (25.2)	24.1 (28.7)
Trial 17084[a]	87 / –	59.1 (22.5)		34.6 (30.8)	
Trial 17084[b]	86 / –	68.7 (19.2)		34.3 (29.7)	
Pediatric patients with T1DM					
Trial 17086	20 / 10	96.1 (45.1)	188 (70.3)	37.4 (37.4)	–[c]
Healthy adult subjects					
Trial 17144	6 / –	75.1 (30.9)		30.5 (14.5)	

Abbreviations: CL/f, apparent total clearance from plasma; CV, coefficient of variation; N, number of subjects; $t_{½}$, elimination half-life; T1DM, type 1 diabetes mellitus.

Notes: [a]Dasiglucagon batch stored under refrigerated conditions.

[b]Dasiglucagon batch reflecting storage under the intended dual storage conditions.

[c]The GlucaGen t½ value was not baseline-adjusted as in trial 15126 and the value obtained (82.6 [27.2] minutes) was therefore abnormally high.

N are shown for dasiglucagon / GlucaGen. For the GlucaGen PK endpoints in trial 15126, but not in trial 17086, the baseline-adjusted and truncated values are presented.

Table 10 - Pharmacokinetic characteristics for IV dose cohorts, typical profiles only

Variable	Statistic	0.03 mg (0.15 mL) IV	0.1 mg (0.25 mL) IV	0.3 mg (0.75 mL) IV	0.6 mg (0.15 mL) IV	0.6 mg (1.5 mL) IV	1.5 mg (1.5 mL) IV
	N	4	5	5	3	5	6
$AUC_{0\text{-}4\ h}$ [h×pmol/L]	GeoMean	251	707	2593	4653	4621	13656
	GeoSD	1.33	1.26	1.20	1.32	1.13	1.24
$AUC_{0\text{-}\infty}$ [h×pmol/L]	GeoMean	251	707	2593	4653	4621	13656
	GeoSD	1.33	1.26	1.20	1.32	1.13	1.24
C_{max} [pmol/L]	GeoMean	1111	2966	11336	20370	21666	57896
	GeoSD	1.33	1.46	1.25	1.39	1.14	1.31
C_0 [pmol/L]	GeoMean	1855	4730	19807	33818	38906	119672
	GeoSD	1.36	1.75	1.27	1.5	1.17	1.49
λz [h^{-1}]	GeoMean	6.46	4.71	3.01	2.00	2.26	2.37
	GeoSD	1.08	1.26	1.19	1.34	1.09	1.42
$t_{½\lambda z}$ [h]	GeoMean	0.107	0.147	0.23	0.346	0.307	0.293
	GeoSD	1.08	1.26	1.19	1.34	1.09	1.42
CL [L/h]	GeoMean	35.4	41.8	34.2	38.1	38.4	32.5
	GeoSD	1.33	1.26	1.20	1.32	1.13	1.24
Vss [L]	GeoMean	4.20	5.44	4.20	5.06	5.14	4.12
	GeoSD	1.35	1.69	1.28	1.57	1.17	1.44
MRT [h]	GeoMean	0.119	0.13	0.123	0.133	0.134	0.127
	GeoSD	1.04	1.35	1.07	1.19	1.09	1.17

Note: C_0 is the estimated concentration at end-of-infusion; C_{max} is the maximum observed concentration
GeoMean: geometric mean, GeoSD: geometric standard deviation, IV=intravenous

Based on the molecular weight of dasiglucagon, it is expected that dasiglucagon is excreted by filtration in the kidneys. Following glomerular filtration, peptides are degraded by the proteases present in the proximal tubuli and the peptide fragments are reabsorbed.

No in vivo human metabolite studies have been performed.

The metabolism of dasiglucagon was also studied in vivo in rats and dogs dosed for 8 days. In the conducted in vitro and in vivo metabolism studies, only proteolytic metabolites were identified. In total, 16 human proteolytic metabolites were identified and detected across species. The data indicate that dasiglucagon is cleared mainly in the blood, liver and kidneys by normal proteolytic degradation pathways, in the same way as glucagon.

Structure-activity relationship analysis indicated that the metabolites are not expected to be active on the glucagon receptor with high activity.

Dose proportionality and time dependencies

Dose proportionality of dasiglucagon PK was investigated in the dose-finding trial 15126, in which SC dasiglucagon doses of 0.1, 0.3, 0.6 and 1.0 mg were evaluated. Dose proportionality results from trial 15126 are shown in

Table 11.

Table 11 - Analysis of dose proportionality of dasiglucagon PK endpoints: Trial 15126

Parameter	Slope	95% CI
$AUC_{0\text{-}30\ min}$	0.885	(0.7403; 1.0292)
$AUC_{0\text{-}360\ min}$	1.029	(0.9667; 1.0904)
$AUC_{0\text{-}inf}$	1.030	(0.9683; 1.0916)
C_{max}	0.888	(0.7791; 0.9972)

Abbreviations: $AUC_{0\text{-}inf}$, area under the plasma concentration-time curve from time 0 to infinity; $AUC_{0\text{-}30\ min}$, area under the plasma concentration-time curve from time 0 to 30 minutes; $AUC_{0\text{-}360\ min}$, area under the plasma concentration-time curve from time 0 to 360 minutes; CI, confidence interval; C_{max}, maximum plasma concentration.

Dose proportionality was considered demonstrated if the 95% CI of the slope included "1". The upper limit of the 95% CI was marginally below 1 only for Cmax.

Dasiglucagon is not intended to be used for multiple administrations, however, trial 15007, a randomized, placebo-controlled, double-blind trial investigated and evaluated the safety, tolerability, PK and PD of multiple ascending doses of dasiglucagon administered SC to healthy subjects. Dose-proportional exposure without relevant changes in PK parameters was also indicated between first and last dosing. No accumulation of ZP4207 over the 5-days dosing period was observed.

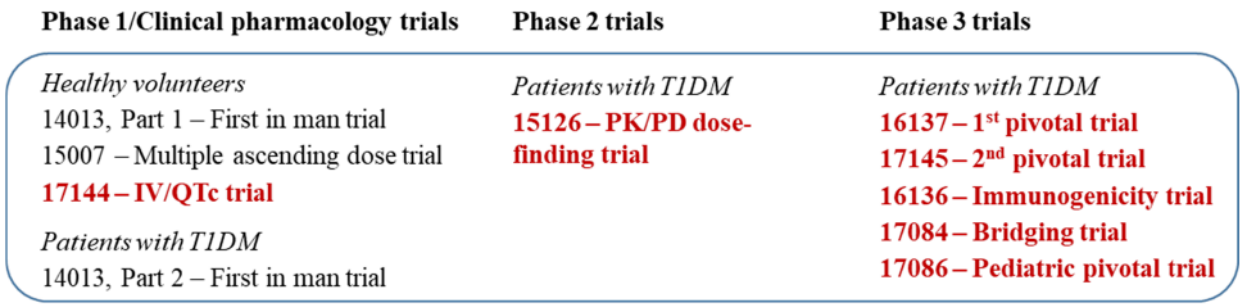
Pharmacokinetic in the target population

Dasiglucagon PK was mainly evaluated in population with T1DM. Two initial phase 1 trials (trials 14013 and 15007) tested an early formulation of dasiglucagon in healthy subjects, but the formulation was subsequently changed to improve the initial absorption rate.

As the formulations used in these two phase I trials is not representative of the to-be-marketed product, a direct comparison between PK in healthy subjects and in patients with T1DM has a limited interest.

Trials in bold red in Figure 21 were used to obtain main PK and PD data.

Figure 22 - Overview of clinical trials



Abbreviations: IV, intravenous; PD, pharmacodynamics; PK, pharmacokinetics; QTc, corrected QT interval; T1DM, type 1 diabetes mellitus.
The trials are all completed. Trials providing PK and PD data in this summary are shown in bold red text.

Table **12** shows clinical trials completed in the adult target population and Table 13 shows trial completed in the paediatric target population.

Table 12 - Trials contributing to the PK and PD characterization of dasiglucagon in the adult patients

Trial number Phase	Trial design	Number of randomized subjects	Dosing of dasiglucagon Dasiglucagon formulation[a]
Adult patients with T1DM			
Trial 15126 Phase 2 Dose-finding trial	A randomized, double-blind, crossover trial of single doses of dasiglucagon administered SC to characterize the PK and PD of dasiglucagon as compared to an active comparator, GlucaGen	58 adult patients with T1DM	0.1, 0.3, 0.6 and 1.0 mg SC (single doses) using a PFS Formulation B
Trial 16137 Phase 3 1st pivotal trial	A randomized, double-blind, parallel-group trial to confirm the clinical efficacy and safety of dasiglucagon administered SC as compared to placebo and with reference to an active comparator, GlucaGen	170 adult patients with T1DM	0.6 mg SC (single dose) using a PFS Formulation C
Trial 17145 Phase 3 2nd pivotal trial	A randomized, placebo-controlled, double-blind, parallel-group trial to confirm the clinical efficacy and safety of dasiglucagon administered SC	45 adult patients with T1DM	0.6 mg SC (single dose) using an auto-injector Formulation C
Trial 16136 Phase 3 Immunogenicity trial	A randomized, double-blind, parallel-group safety trial to evaluate the immunogenicity of dasiglucagon administered SC as compared to an active comparator, GlucaGen	112 adult patients with T1DM	0.6 mg SC (3 consecutive weekly doses) using a PFS Formulation C
Trial 17084 Phase 3 Bridging trial	A randomized, double-blind, crossover trial evaluating the efficacy and safety of two different dasiglucagon batches[b]	92 adult patients with T1DM	0.6 mg SC (single doses of 2 dasiglucagon batches) using a PFS Formulation C

Table 13 - Trials contributing to the PK and PD characterization of dasiglucagon in the paediatric patients

Trial number Phase	Trial design	Number of randomized subjects	Dosing of dasiglucagon Dasiglucagon formulation[a]
Pediatric patients with T1DM			
Trial 17086 Phase 3 Pediatric pivotal trial	A randomized, double-blind, parallel-group trial in pediatric patients aged 6 to <18 years to confirm the clinical efficacy and safety of dasiglucagon administered SC as compared to placebo and with reference to an active comparator, GlucaGen	42 pediatric patients with T1DM 16 children and 26 adolescents[c]	0.6 mg SC (single dose) using a PFS Formulation C

Pivotal trials 16137 and 17145

The PK and PD characteristics of a single SC dose of 0.6 mg dasiglucagon were evaluated as secondary endpoints in the two pivotal phase 3 trials, 16137 and 17145, in adult patients with T1DM.

The mean plasma dasiglucagon and glucagon concentrations versus time profile for study 16137 are shown in Figure 22, while the mean plasma dasiglucagon concentrations versus time profile for study 17145 are shown in Figure 23.

Figure 23 - Mean Dasiglucagon and Glucagon Profiles (Full Analysis Set)

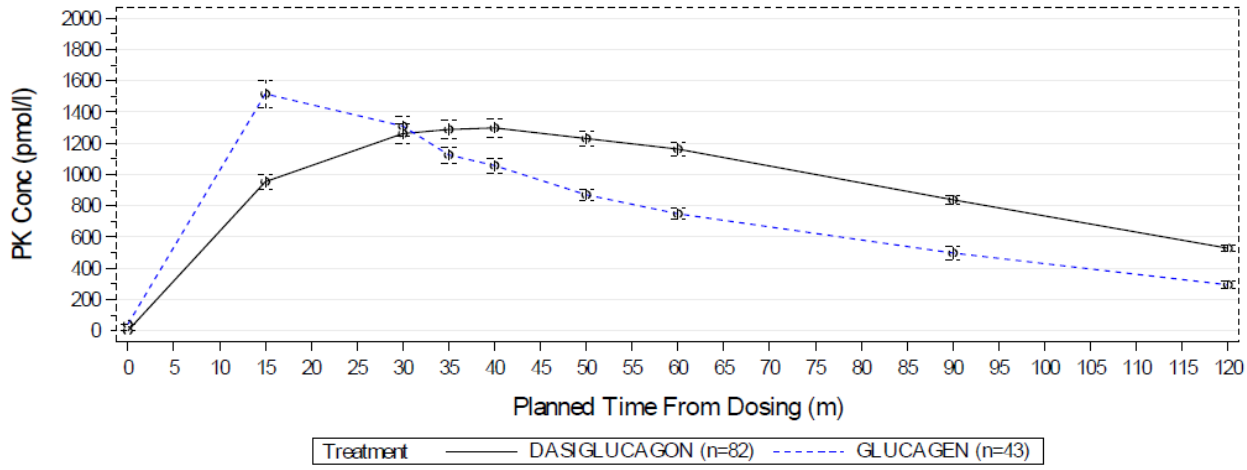
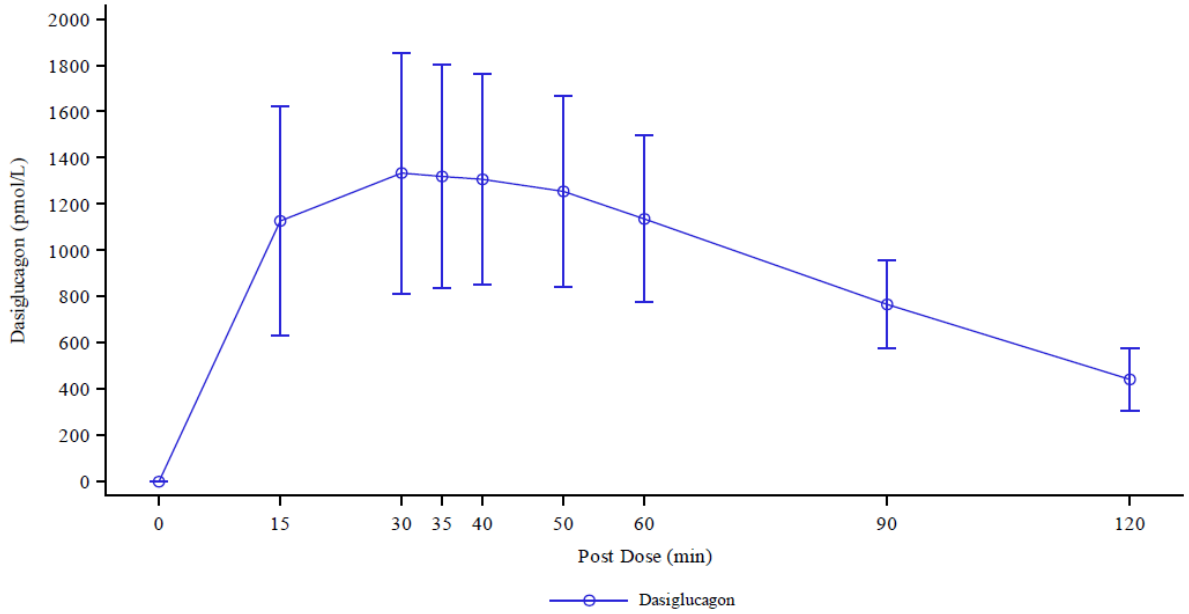


Figure 24 - Mean (± SD) Plasma Dasiglucagon Concentration: FAS Population



For study 16137, plasma AUC0-90min was similar in the dasiglucagon group and GlucaGen® group, with an LSM ratio of 0.910 (95% CI: 0.801, 1.033; p=0.144) for glucagon: dasiglucagon (Table 14).

In a post hoc analysis, plasma AUC0-120min was statistically significantly higher in the dasiglucagon group than in the GlucaGen® group, with an LSM ratio of 0.844 (95% CI: 0.749, 0.951; p= 0.006) for glucagon:dasiglucagon (Table 14). Cmax was significantly lower in the dasiglucagon group than in the GlucaGen® group, with an LSM ratio of 1.158 (95% CI: 1.010, 1.328; p=0.036) for glucagon:dasiglucagon (Table 14).

The maximum plasma concentration was reached around 40 minutes after injection in the dasiglucagon group and around 20 minutes after the injection in the GlucaGen® group (Table 14). The Tmax LSM ratio for glucagon:dasiglucagon was 0.700 (95% CI: 0.663, 0.739; <0.001).

Table 14 - Summary Statistics of Pharmacokinetic Endpoints (Full Analysis Set)

PK Parameter	Category/ Statistic	Dasiglucagon (N=82)	GlucaGen® (N=43)
$AUC_{0\text{-}90min}$ (pmol*h/L)	N	82	43
	Mean (SD)	1520 (518)	1350 (372)
	Geometric mean	1430	1300
	CV (%)	34.2	27.5
	Median	1470	1320
	Min, Max	587, 2850	475, 2080
$AUC_{0\text{-}120min}$ (pmol*h/L)	N	82	43
	Mean (SD)	1860 (580)	1550 (422)
	Geometric mean	1770	1490
	CV (%)	31.2	27.2
	Median	1780	1530
	Min, Max	771, 3300	507, 2480
C_{max} (pmol/L)	N	82	43
	Mean (SD)	1380 (519)	1570 (542)
	Geometric mean	1280	1490
	CV (%)	37.7	34.5
	Median	1330	1470
	Min, Max	530, 2990	722, 3160
T_{max} (h)	N	82	43
	Median	0.670	0.250
	Min, Max	0.250, 1.00	0.230, 0.670

For study 17145, dasiglucagon plasma concentration by time point and treatment for the FAS population is summarized below in Table 15.

All subjects randomized to dasiglucagon were dosed. The PK profile of dasiglucagon was characterized by a rapid increase after dosing, peaking at 1330 (521) pmol/L at 30 minutes, and was followed by a rapid decline to 442 (134) pmol/L at 90 minutes.

The PK parameters AUC0-90mins, AUC0-120mins, Cmax, and tmax for the FAS population are summarized by treatment group below in Table 16. Mean (SD) AUC0-90min was 1560 (506) hr*pmol/L, mean (SD) AUC0-120min was 1860 (549) hr*pmol/L, mean (SD) Cmax was 1440 (511) pmol/L and median (min, max) tmax was 35 (15, 60) minutes.

Table 15 - Mean (± SD) Dasiglucagon Plasma Concentration: FAS Population

Parameter	Time Point[1], Mean (SD)	0.6 mg Dasiglucagon (N=34)
Dasiglucagon (pmol/L)	Pre-dose at t = 0	0.0 (0)
	15 minutes post dose	1130 (496)
	30 minutes post dose	1330 (521)
	35 minutes post dose	1320 (484)
	40 minutes post dose	1310 (454)
	50 minutes post dose	1250 (413)
	60 minutes post dose	1140 (360)
	90 minutes post dose	766 (192)
	120 minutes post dose	442 (134)

Source data: Modified from Table 14.2.8

[1] Time Points of dasiglucagon concentration were: Pre-dose, and at 15, 30, 35, 40, 50, 60, 90, and 120 minutes after dosing.

- Records at Pre-dose that were below the limit of quantitation (BLQ) were set to zero.

Table 16 - PK parameters AUC0-90mins, AUC0-120mins, Cmax, and Tmax: FAS Population

Parameter	Statistics	Treatment 0.6 mg Dasiglucagon (N=34)
$AUC_{0\text{-}90mins}$ (hr*pmol/L)	n	34
	Mean (SD)	1560 (506)
	SE	86.7
	Geometric Mean (SE)	1480 (85.2)
	% CV of Geo. Mean	34.6
$AUC_{0\text{-}120mins}$ (hr*pmol/L)	n	34
	Mean (SD)	1860 (549)
	SE	94.2
	Geometric Mean (SE)	1780 (94.1)
	% CV of Geo. Mean	31.6
C_{max} (pmol/L)	n	34
	Mean (SD)	1440 (511)
	SE	87.6
	Geometric Mean (SE)	1350 (85.6)
	% CV of Geo. Mean	38.2
T_{max} (min)	n	34
	Median	35
	Min, Max	15, 60
	Geometric Mean (SE)	33.8 (2.02)
	% CV of Geo. Mean	35.9

Special populations

Impaired renal function

No dedicated renal impairment study was performed with dasiglucagon. However, the effect of renal impairment was investigated as covariate of PopPK model 19-077: the systemic CL was estimated to be lower for subjects with lower renal function.

The point estimates of the predicted exposures fell within the 5th to 95th percentile interval of individual predicted dasiglucagon exposures. Only the upper boundary of the 95% CI for exposure predicted in a subject with eGFR of 30 mL/min/1.73m2 (and body weight of 78 kg) was predicted to increase above the threshold.

Impaired hepatic function

No dedicated hepatic impairment study was performed with dasiglucagon.

Gender

Gender was included as a predictor of the absorption lag time in PopPK model 16-147, indicating that absorption lag time was longer in female (2.4 min) than in male patients (1.6 min). While the covariate effect was statistically significant ($p<0.001$), the impact on the absorption lag time was considered of limited clinical relevance.

Race

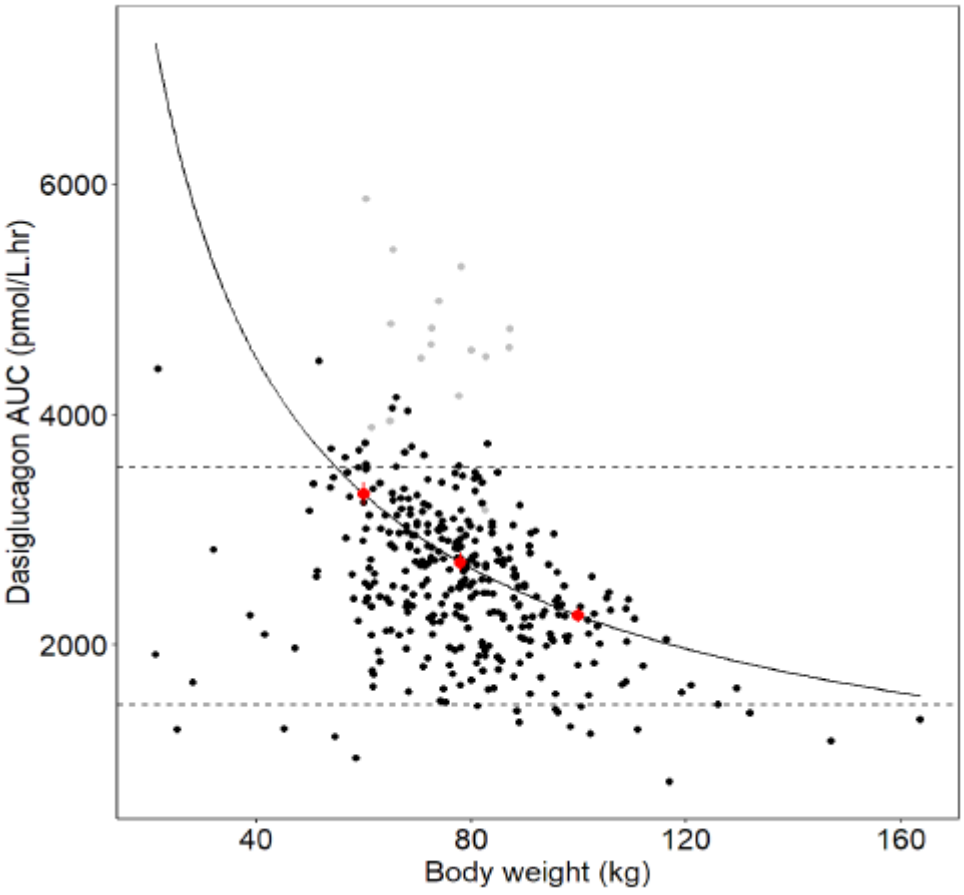
A meaningful test to estimate the effect of race and ethnicity on the population PK parameters was not possible due to the low number of Asian (n=7) and Black/African American (n=3) patients and a substantial number of patients with unknown ethnicity in the analysis (n=54).

Available data on race effect are not sufficient to draw a conclusion. It is unknown if dasiglucagon PK is influenced by race.

Weight

Figure 24 illustrates the relation between body weight, and the model-predicted dasiglucagon exposure. The effect of body weight on exposure was predicted for the range of body weights in the analysis dataset (21.2-163.7 kg). The point estimates of the predicted exposures fell within the 5th to 95th percentile interval of individual predicted dasiglucagon exposures.

Figure 25 - Impact of Body Weight on Dasiglucagon Exposure



Elderly

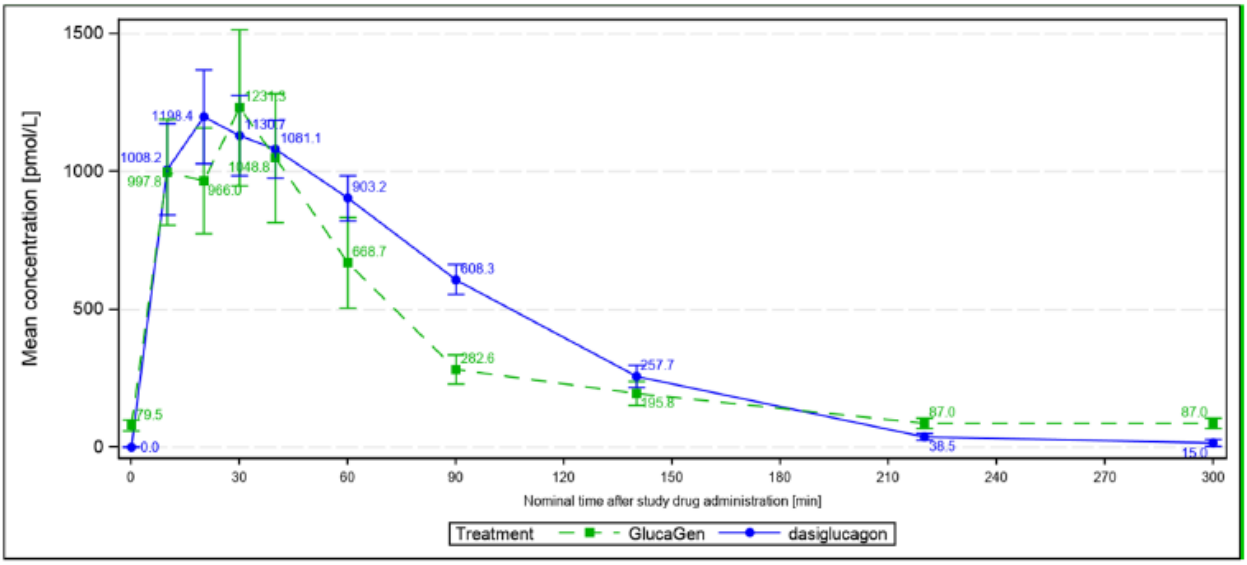
The PK of dasiglucagon in elderly subjects was not investigated. No simulations with existing PopPK model were generated for different age groups including elderly subjects.

Children

In the phase 3 trial 17086, the PK and PD effects of a single SC dose of 0.6 mg dasiglucagon were evaluated in children (aged 6 to 11 years) and adolescents (aged 12 to 17 years) with T1DM.

The mean analyte concentrations over time are illustrated in Figure 25.

Figure 26 - Mean analyte concentrations versus time - original scale (full analysis set)



Results for the PK parameters, calculated from plasma concentration data for dasiglucagon or GlucaGen, are presented in Figure 17.

Table 17 - Pharmacokinetic parameters (full analysis set)

Parameter		Dasiglucagon (N=20)	GlucaGen® (N=10)
$AUC_{0\text{-}30min}$ (h•pmol/L)	Mean (±SD)	462 (±300)	437 (±253)
	Median	413	356
	Min-max	88.7-1340	147-902
	Geom. mean	376	376
	Geom. mean CV %	78.1	63.3
$AUC_{0\text{-}300min}$ (h•pmol/L)	Mean (±SD)	1970 (±843)	1650 (±1050)
	Median	1900	1180
	Min-max	709-4440	599-3250
	Geom. mean	1810	1370
	Geom. mean CV %	44.8	72.7
C_{max} (pmol/L)	Mean (±SD)	1330 (±724)	1380 (±890)
	Median	1160	979
	Min-max	359-3410	376-2660
	Geom. mean	1160	1120
	Geom. mean CV %	61.2	80
T_{max} (h)	Mean (±SD)	0.552 (±0.338)	0.35 (±0.123)
	Median	0.35	0.333
	Min-max	0.167-1.5	0.167-0.5
	Geom. mean	0.476	0.328
	Geom. mean CV %	58.9	41.8
$AUC_{0\text{-}inf}$ (h•pmol/L)	Mean (±SD)	2010 (±854)	1830 (±1160)
	Median	1920	1250
	Min-max	719-4450	715-3590
	Geom. mean	1850	1530
	Geom. mean CV %	45.1	70.3
MRT (h)	Mean (±SD)	1.32 (±0.39)	1.9 (±0.386)
	Median	1.21	1.85
	Min-max	0.777-2.31	1.36-2.44
	Geom. mean	1.27	1.86
	Geom. mean CV %	29.5	21.1
λ_z (/h)	Mean (±SD)	1.18 (±0.422)	0.519 (±0.123)
	Median	1.07	0.544
	Min-max	0.515-2.1	0.296-0.67
	Geom. mean	1.11	0.504
	Geom. mean CV %	37.4	27.2
$t_{½}$ (h)	Mean (±SD)	0.664 (±0.248)	1.42 (±0.426)
	Median	0.647	1.27
	Min-max	0.329-1.35	1.03-2.34
	Geom. mean	0.623	1.38
	Geom. mean CV %	37.4	27.2
CL/f (L/h)	Mean (±SD)	105 (±48.9)	221 (±121)
	Median	92.7	229
	Min-max	39.9-247	80.1-401
	Geom. mean	96.1	188
	Geom. mean CV %	45.1	70.3
V_z/f (L)	Mean (±SD)	101 (±62.5)	462 (±303)
	Median	77.4	348
	Min-max	36.1-245	143-943
	Geom. mean	86.4	373
	Geom. mean CV %	62.2	81.1

The geometric mean AUC0-inf and Cmax values in the paediatric population were slightly lower than the corresponding values observed in adults and the median tmax was earlier at 21 minutes.

The geometric mean AUC and Cmax values were all higher in children than in adolescents, and the median tmax was reached slightly earlier.

Also the updated pop-PK model 19-077 simulated dasiglucagon exposure and glucose concentrations for paediatrics with typical weight values of 25 kg, 35 kg, and 45 kg, as well as a reference adult population (77 kg) and the paediatric pop-PK model showed dose proportional concentration and exposure. However, for a given dose, paediatric patients with lower body weights, were predicted to have higher dasiglucagon exposure (AUC) and maximum concentration (CMAX) compared to adult patients, while time to maximal dasiglucagon concentration (TMAX), elimination half-life (T1/2), and mean residence time (MRT) were predicted to be lower in paediatrics compared to adults.

Pharmacokinetic interaction studies

In study 13-149, the potential of dasiglucagon to inhibit cytochrome P450 (CYP) enzymes (CYP1A, 2C9, 2C19, 2D6, and 3A4) was assessed in order to identify the likelihood of dasiglucagon causing drug-drug interactions. The study showed that dasiglucagon does not cause significant inhibition of any of the CYP enzymes tested (CYP1A, CYP2C9, CYP2D6, CYP3A4), with all IC50 (half maximal inhibitory concentration) values reported as greater than the top concentration tested in the assay (25 µM).

In study 19-168, the potential of dasiglucagon to be an inhibitor of the human transporters P-gp, BCRP, OATP1B1, OATP1B3, OAT1, OAT3, OCT2, MATE1, MATE2-K and OCT1 in various in vitro cell test systems was assessed. Under the current assay conditions with the in vitro test systems utilised in this study, ZP4207 has been determined to be an inhibitor of probe substrate transport mediated via OATP1B1, OATP1B3 and OAT3 but not via P-gp, BCRP, OAT1, OCT2, MATE1, MATE2-K, and OCT1 (Table 18).

Table 18 - Summary table of observed inhibition of transporters by ZP4207

Transporter	IC_{50} (µM)
P-gp	No inhibition
BCRP	No inhibition
OATP1B1	59.2
OATP1B3	74.3
OAT1	No inhibition
OAT3	>100 (45.9 % inhibition at 100 µM)
OCT2	No inhibition
MATE1	No inhibition
MATE2-K	No inhibition
OCT1	No inhibition

Pharmacological interactions between dasiglucagon and other drugs are not known.

No in vivo drug-drug interaction studies were performed with dasiglucagon.

Pharmacokinetics using human biomaterials

As part of the clinical pharmacology program, dasiglucagon was evaluated in five in vitro human biomaterial studies conducted with human cells, plasma, or serum samples. An overview of these studies is shown in Table 19.

Table 19 - In vitro studies with human biomaterials

Study number	Type of study
Study 14-001	Metabolite identification following in vitro incubation of dasiglucagon in serum and plasma
Study 15-076	Metabolite identification following in vitro incubation of dasiglucagon in hepatocytes
Study 15-075	Metabolite clearance following in vitro incubation of dasiglucagon in hepatocytes
Study 13-149	Inhibition of CYP450 enzymes in liver microsomes
Study 19-168	Inhibition of the human transporters in MDCK-MDR1, Caco-2 and HEK293 cells

Abbreviations: CYP, cytochrome P450 enzymes which function to metabolize potentially toxic compounds.

Dasiglucagon has similar potency to glucagon on the human glucagon receptor (based on average half maximal effective concentration [EC50] values), with a rapid onset of anti-hypoglycemic effect and a short duration of action.

2.5.2.2. Pharmacodynamics

Mechanism of action

Dasiglucagon is a specific GCGR agonist with comparable potency to glucagon. The speed and magnitude of plasma glucose increase are the clinically relevant factors in the context of treating severe hypoglycaemia, i.e., the proposed indication.

Primary and Secondary pharmacology

The key plasma glucose endpoints of the trials characterising the PD were the following:

- AUE0-30 min, area under the plasma glucose effect curve above baseline from time 0 to 30 minutes
- AUE0-t, area under the plasma glucose effect curve above baseline from time 0 to 't' minutes
- AUE0-last, area under the plasma glucose effect curve above baseline from time 0 to the last available measurement
- CE30 min, plasma glucose concentration at 30 minutes after dosing and above baseline (trial 15126)
- CEmax, maximum plasma glucose concentration measurements from time 0 to 't' minutes above baseline
- TEmax, time to maximum plasma glucose concentration measurements

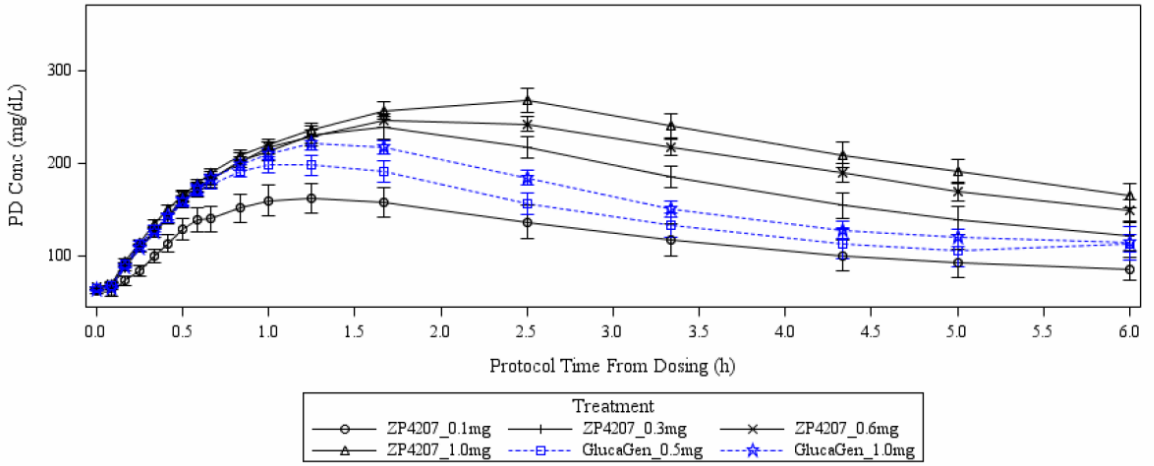
PD endpoints were derived from individual plasma glucose profiles above baseline.

Study 15126

Study 15126 was a phase 2 PK/PD dose-finding trial. It was a randomized, double-blind, crossover trial of single doses of dasiglucagon administered SC to hypoglycemic patients with T1DM to describe the PK and PD of dasiglucagon as compared to an active comparator, GlucaGen (lyophilized glucagon).

The mean glucose profiles (baseline-adjusted) following administration of dasiglucagon (0.1, 0.3, 0.6 or 1.0 mg) or GlucaGen (0.5 or 1.0 mg) are shown in the following figure.

Figure 14.2.4.3.2
Mean Glucose Profiles With Standard Error Bars (SEM) Overlaid by Treatment
Full Analysis Set



Summary statistics of the PD endpoints are displayed in the Table 20.

Table 20 - Study 15126. Summary statistics of PD endpoints

IMP	ZP4207 (dasiglucagon)				GlucaGen	
Dose	0.1 mg	0.3 mg	0.6 mg	1.0 mg	0.5 mg	1.0 mg
$AUE_{0\text{-}30min}$ [mg*h/dL]						
N	5	16	17	16	17	33
Mean	12.9	20.9	21.1	24.1	22.1	21.9
SD	5.21	6.13	6.10	5.18	5.48	5.74
CV (%)	40.5	29.3	28.9	21.5	24.7	26.2
GeoMean	11.5	19.9	20.4	23.5	21.5	21.2
Min	4.03	7.11	13.5	15.3	15.2	11.0
Median	14.0	20.5	20.6	24.8	19.9	21.3
Max	16.8	33.2	38.8	32.1	31.9	36.5
$AUE_{0\text{-}60min}$ [mg*h/dL]						
N	5	16	17	16	17	33
Mean	54.6	83.4	83.9	90.0	81.2	85.1
SD	20.1	21.2	17.8	15.3	17.5	18.5
CV (%)	36.9	25.4	21.2	17.0	21.5	21.7
GeoMean	50.5	80.6	82.3	88.8	79.5	83.2
Min	21.9	36.4	56.4	63.8	55.4	52.4
Median	59.9	82.7	83.7	88.9	81.4	82.5
Max	71.2	127	135	115	113	130
AUE [mg*h/dL]						
N	5	16	17	16	17	33
Mean	344	666	788	895	462	566
SD	149	247	165	213	273	232
CV (%)	43.4	37.1	20.9	23.8	58.9	40.9
GeoMean	320	631	772	869	405	527
Min	202	365	537	489	185	279
Median	283	649	729	918	384	529
Max	566	1400	1080	1340	1260	1270
CE_{30min} [mg/dL]						
N	5	16	17	16	17	33
Mean	66.1	93.4	98.2	100	93.5	96.5
SD	23.8	23.7	25.0	20.3	21.4	21.9
CV (%)	35.9	25.3	25.5	20.3	22.9	22.7
GeoMean	61.1	90.4	95.7	98.0	91.1	94.1
Min	25.9	47.0	66.1	70.1	64.1	63.1
Median	78.0	94.2	97.1	104	95.3	92.1
Max	84.1	141	175	140	128	137

IMP	ZP4207 (dasiglucagon)				GlucaGen	
Dose	**0.1 mg**	**0.3 mg**	**0.6 mg**	**1.0 mg**	**0.5 mg**	**1.0 mg**
CE [mg/dL]						
N	5	16	17	16	17	33
Mean	102	174	190	209	142	166
SD	33.7	44.6	32.2	40.2	42.6	42.5
CV (%)	33.1	25.6	17.0	19.2	29.9	25.5
GeoMean	97.0	169	187	206	137	161
Min	59.1	98.2	138	131	85.0	94.1
Median	99.1	178	186	202	136	159
Max	136	268	246	295	257	267
t_{max} [h]						
N	5	16	17	16	17	33
Min	0.833	1.00	1.67	1.67	0.667	0.833
Median	1.25	1.67	1.67	2.50	1.00	1.25
Max	1.67	2.50	4.33	2.50	5.00	6.12
$T_{PG \geq 70\ mg/dL}$ [min]						
N	5	16	17	16	17	33
Min	2.00	0.000	0.000	0.000	0.000	0.000
Median	10.0	6.00	6.00	6.00	6.00	7.00
Max	17.0	13.0	9.00	9.00	9.00	10.0
% of patients achieving a plasma glucose concentration ≥70 mg/dL within 30 min after treatment						
N	5	16	17	16	17	33
%	100	100	100	100	100	100
$T_{PG\ increase \geq 20 mg/dL}$ [min]						
N	5	16	17	16	17	33
Min	11.0	7.00	6.00	7.00	6.00	5.00
Median	14.0	10.0	9.00	9.00	10.0	10.0
Max	27.0	20.0	16.0	15.0	13.0	15.0
% of patients achieving a plasma glucose increase of ≥20 mg/dL within 30 min after treatment						
N	5	16	17	16	17	33
%	100	100	100	100	100	100

Cross-reference: Tables 14.2.3.1.1, 14.2.3.1.2, 14.2.3.1.3, 14.2.3.1.4, 14.2.3.1.5, 14.2.3.1.6, 14.2.3.1.7, 14.2.3.1.8, 14.2.3.1.9, and 14.2.3.1.10

The parameters AUE0-30min, AUE0-60min, AUE, CE30min and CE, markedly increased between the 0.1 and 0.3 mg dose. Higher doses only determined a slight further increase or no increase at all. Median time to achieve a plasma glucose level of at least 70 mg/dL (TPG ≥70 mg/dL) decreased between 0.1 and 0.3 mg and was the same at all higher dose levels (6 min).

At all dose levels, all patients achieved a plasma glucose level of at least 70 mg/dL within 30 minutes post-dose. Median time to achieve a plasma glucose increase by at least 20 mg/dL decreased moderately between 0.1 and 0.3 mg (from 14 to 10 min) and decreased slightly further between 0.3 and 0.6 mg (to 9 min). No further decrease was observed when increasing the dose to 1.0 mg. The time range for TPG increase ≥20 mg/dL decreased from 11-27 min at 0.1 mg to 7-20 min at 0.3 mg, to 6-16 min at 0.6 mg, and to 7-15 min at 1.0 mg. At all dose levels, all patients showed an increase in plasma glucose by at least 20 mg/dL within 30 minutes post-dose.

The simple Emax model was applied on the PD endpoints AUE, CE, AUE0-30min and CE0-30min of dasiglucagon versus dose in order to determine the maximum effect, the dose causing 50% of the maximum effect, as well as the baseline effect at point zero. The results of these calculations are displayed in the following table.

Parameter	E_0	E_{max}	ED_{50}
AUE [mg*h/dL] vs. Dose [mg]	5.72	1050	0.189
CE [mg/dL] vs. Dose [mg]	1.29	229	0.112
$AUE_{0\text{-}30min}$ [mg*h/dL] vs. Dose [mg]	0.508	25.0	0.087
CE_{30min} [mg/dL] vs. Dose [mg]	6.70	101	0.058

E_0 = baseline effect; E_{max} = maximum effect; ED_{50} = drug dose required to produce 50% of the drug-induced maximum effect
Cross-reference: Table 14.2.3.2.8

Study 16137

Study 16137 was a phase 3, randomized, double-blind, parallel trial efficacy and safety trial of dasiglucagon in the rescue treatment of hypoglycemia in patients with T1DM compared to placebo and with reference to GlucaGen®. The PK and PD characteristics of a single SC dose of 0.6 mg dasiglucagon were also evaluated as secondary endpoints. The study methodology was similar to that of Study 15126.

The median time to plasma glucose concentration ≥70 mg/dL without administration of rescue glucose was 8 minutes in both dasiglucagon and GlucaGen® groups, significantly shorter than in the placebo group (25 minutes; p<0.001).

The mean (SD) plasma glucose AUE0-30min was similar in the dasiglucagon and GlucaGen® groups and lower in the placebo group.

Table 11-5: Plasma Glucose Response as $AUE_{0\text{-}30min}$ (Full Analysis Set)

Pharmacodynamic Parameter	Category/ Statistic	Dasiglucagon (N=82)	Placebo (N=43)	GlucaGen® (N=43)
$AUE_{0\text{-}30min}$ (mg*h/dL)	Mean (SD)	21.0 (5.26)	3.57 (2.86)	20.4 (5.49)
	Median	20.5	3.21	20.6
	Min, max	6.47, 31.8	0.0, 12.5	9.75, 32.9

Source: Post-text Table 14.2.10

Study 17145

Study 17145 was a randomized, double-blind, parallel-group trial to confirm the clinical efficacy and safety of dasiglucagon in the rescue treatment of hypoglycemia in subjects with T1DM. The methodology and PD endpoints of the study were the same as those of study 16137.

The median time to plasma glucose concentration ≥70 mg/dL without administration of rescue glucose was 9 minutes.

Table 14.2.5
Secondary Efficacy Analysis: Time to first Plasma Glucose Concentration ≥ 70 mg/dL (3.9 mmol/L) from Baseline
Full Analysis Set (N=44)

Statistics	Treatment: 0.6 mg Dasiglucagon (N=34)	Treatment: Placebo (N=10)
Status, n (%)		
Subjects with event	33 (97.1)	8 (80.0)
Subjects censored	1 (2.9)	2 (20.0)
Time to Event (Minutes)		
25th percentile	7.0	20.0
Median (95% CI)	9.0 (8.0, 10.0)	27.5 (12.0, 40.0)
75th percentile	10.0	40.0
P-value (Log-Rank Test) [1]	<.0001	

[1] P-value from pairwise Log-Rank Test of treatment group difference.
Cross-reference: Listing 16.2.4

The mean (SD) plasma glucose AUE0-30min are shown in the following table.

Table 14.2.6
Secondary Efficacy Analysis: Plasma Glucose $AUE_{0\text{-}30mins}$ by Treatment
Full Analysis Set (N=44)

Parameter Statistics	Treatment: 0.6 mg Dasiglucagon (N=34)	Treatment: Placebo (N=10)
$AUE_{0\text{-}30mins}$ (hr*mg/dL)		
n	34	10
Mean (SD)	19.9 (8.41)	2.67 (3.06)
SE	1.44	0.968
Median	19.3	3.22
Min, Max	-0.01, 34.7	-1.45, 6.89
n	33	6
Geometric Mean (SE)	18.7 (1.49)	4.62 (0.658)
% CV of Geo. Mean	48.1	36

- AUE=Area Under the Effect Curve.
- Geometric mean = exp (mean (log(x))). SE of geometric mean = Geometric mean * SE of (log(x)).
- CV = Coefficient of Variation. % CV of Geometric Mean = 100% * sqrt (exp ((log (GeoSD))2)-1). GeoSD= exp (SD (log(x))).
- Negative and zero AUE are not included in calculation for geometric means.

Cross-reference: Listing 16.2.5

Study 17086

Study 17086 was a phase 3, randomized, double-blind, placebo- and active-controlled, parallel-arm trial to assess the efficacy, safety, and PK of dasiglucagon in children with T1DM, compared to placebo and GlucoGen. The mean glucose concentrations versus time are shown in the following figure.

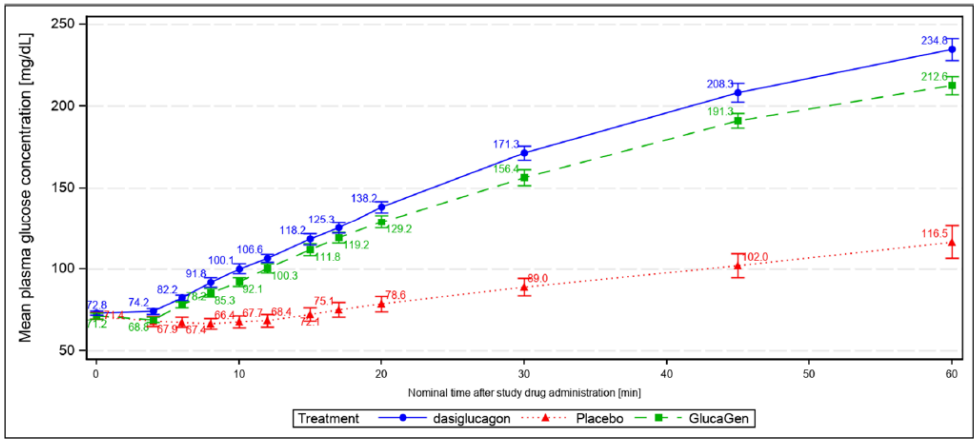
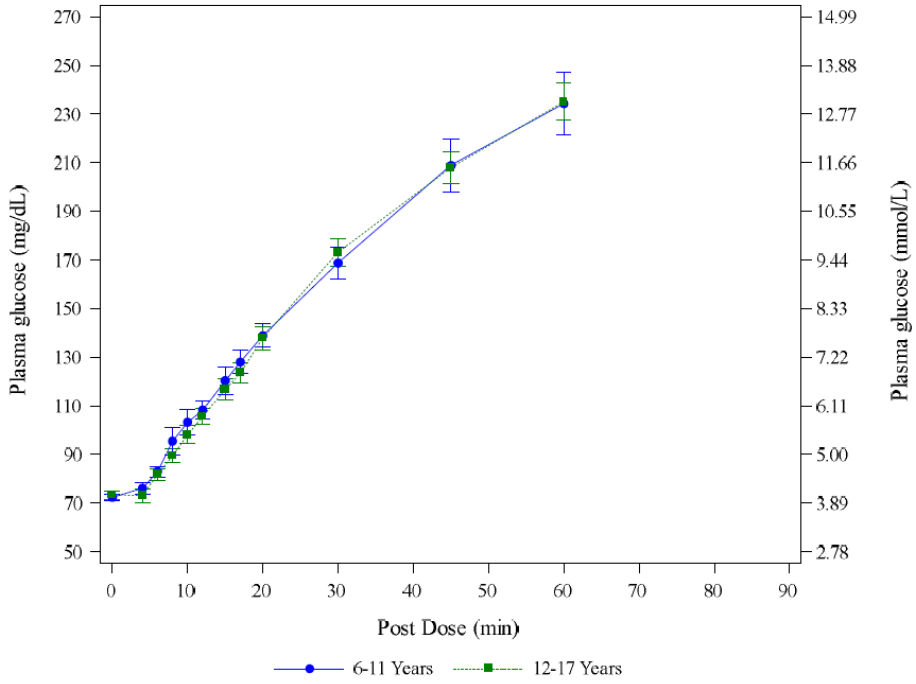


Figure 11.7: Mean glucose concentrations versus time - original scale (full analysis set)

Abbreviation: min=minutes

Source: Table 14.4.2.2

The glucose over time profile was comparable in the two age groups (6-11 and 12-17 years):



AUE0-30min values are shown in the following table.

Table 11.11: Pharmacodynamic metrics for plasma glucose (full analysis set)

Parameter		Dasiglucagon (N=20)	Placebo (N=11)	GlucaGen® (N=10)
$AUE_{0\text{-}30min}$ (mmol•h/L)	Mean (±SD)	22.83 (±6.126)	1.81 (±4.641)	19.66 (±3.410)
	Median	22.44	1.46	20.11
	Min-max	10.9-34.1	-6.3-9.1	13.8-24.1

Abbreviations: Max=maximum; Min=minimum

Source: Table 14.4.2.1

Study 17084 (Bridging study)

The aim of trial 17084 was to demonstrate non-inferiority and to evaluate safety of a single subcutaneous (SC) dose of dasiglucagon stored at the intended dual storage conditions (Batch B) relative to a dasiglucagon batch stored at refrigerated conditions (Batch A) for the treatment of hypoglycemia in patients with T1DM.

The study was performed according to a cross-over design.

PD results are shown in the following table:

Table 11.14: Pharmacodynamic metrics (full analysis set)

Parameter		Batch A (N=87)	Batch B (N=87)
CE_{max} (mg/dL)	Mean (±SD)	175.26 (±39.262)	175.99 (±36.939)
	Median	173.33	176.40
	Min-max	72.3-299.6	70.1-262.5
TE_{max} (h)	Mean (±SD)	1.47 (±0.086)	1.46 (±0.105)
	Median	1.50	1.50
	Min-max	1.0-1.5	1.0-1.5
$AUE_{0-30min}$ (h•mg/dL)	Mean (±SD)	21.38 (±6.388)	21.13 (±6.786)
	Median	21.10	21.00
	Min-max	5.1-37.3	4.6-41.2
$AUE_{0-90min}$ (h•mg/dL)	Mean (±SD)	162.56 (±36.458)	163.27 (±34.833)
	Median	158.89	162.00
	Min-max	76.0-276.0	70.2-259.0

Abbreviations: max=maximum; min=minimum
Source: Table 14.4.2.1

The results show that the two batches were similar from a PD point of view.

Immunogenicity

Study 16136 was a phase 3, randomized, double-blind, parallel-group safety trial to evaluate the immunogenicity of dasiglucagon and GlucaGen® administered subcutaneously in patients with T1DM. This trial aimed to evaluate the incidence and consequences of dasiglucagon immunogenicity as assessed by the occurrence of antidrug antibodies (ADAs) and neutralizing ADAs and of cross-reactivity with native glucagon, after 3 doses of SC administered dasiglucagon in T1DM patients. Patients with T1DM were randomly assigned in a 1:1 ratio to receive 3 SC injections of either dasiglucagon (0.6 mg) or GlucaGen® (1 mg), with 1 week between doses. Patients were followed for 15 weeks from the day of the first dose to assess the immune response. A total of 57 patients treated with dasiglucagon and 54 treated with GlucaGen completed the study and were analysed for the presence of ADA. The overall ADA incidence was zero. No patient had any confirmed treatment-induced or treatment-boosted ADA response at any measuring time after dosing.

Study 17144 - QTc interval study

Study 17144 included an evaluation of the potential effect of dasiglucagon on the QTc interval during ECG via a concentration-response analysis, to investigate whether dasiglucagon prolongs cardiac repolarization. In addition to the evaluation of cardiac repolarization, the potential effects of dasiglucagon on cardiac conduction (PR and QRS) and heart rate were investigated, as well as any treatment-emergent changes in T-wave morphology or the presence of U-waves. The study was a randomized, double-blind, placebo-controlled dose-escalation trial with an open-label, non-controlled SC cohort evaluating the safety and tolerability of a single IV administration of dasiglucagon and the bioavailability of dasiglucagon following SC compared to IV administration. The study was performed in healthy subjects.

The potential of dasiglucagon to cause QT prolongation was assessed through concentration-response analysis of data from clinical trials performed in healthy subjects, in line with ICH E14 Q&A revision R3. The placebo-treated subjects from all cohorts were pooled for the concentration-response analysis.

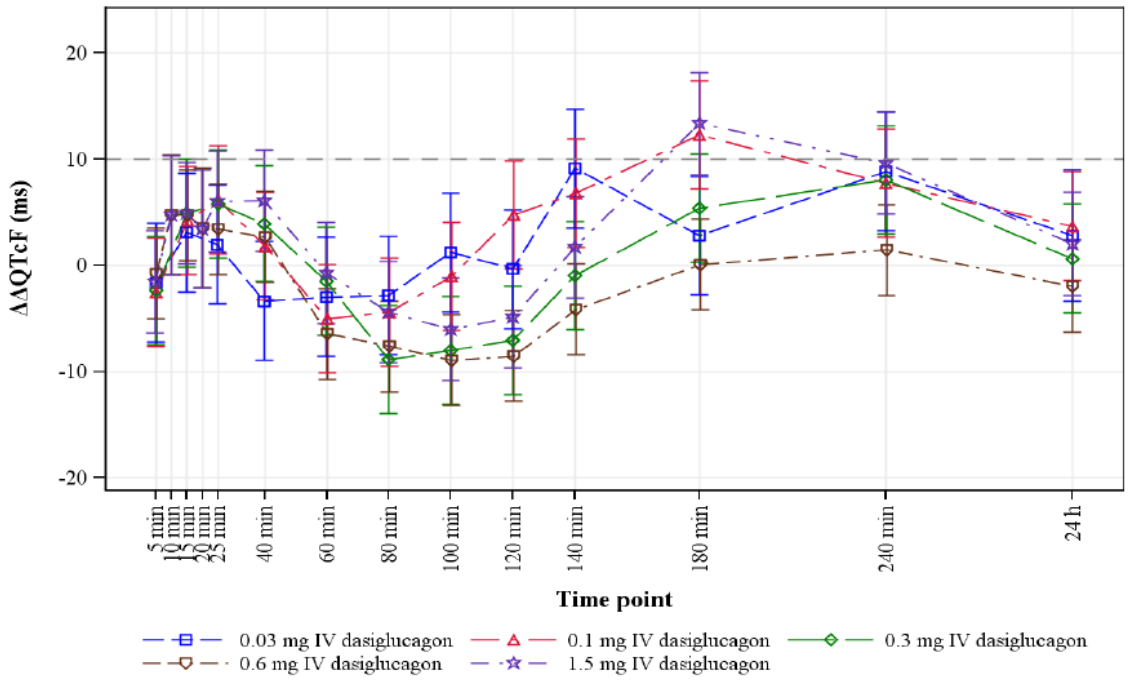
Administered doses were as follows:

- IV: 0.03, 0.1, 0.3, 0.6, and 1.5 mg
- SC: 0.6 mg.

Dasiglucagon at the studied doses did not have a clinically meaningful effect on heart rate (HR). Mean change-from-baseline HR (ΔHR) on active treatment varied between−0.2 and 10.5 bpm, and mean placebo-corrected ΔHR (ΔΔHR) was small across all dose groups, varying from −7.0 to 7.5 bpm. Due to the lack of an HR effect (mean ΔΔHR was <10 bpm), Fridericia's formula was chosen as the QT correction method (QTcF) and other correction methods such as QTcI or QTcS were not explored.

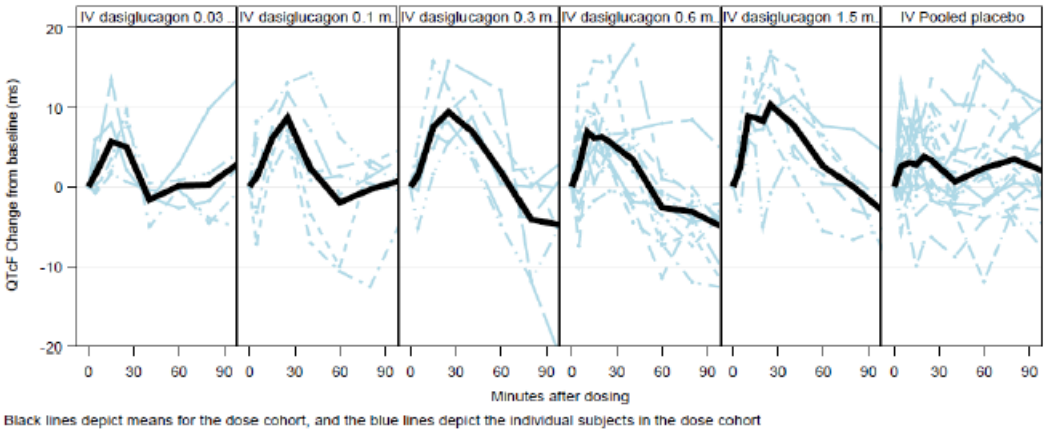
An evaluation of QTcF by-time point showed that mean change-from-baseline QTcF (ΔQTcF) on dasiglucagon was generally larger than on placebo in the first 10 to 25 minutes post-dose (mean placebo-corrected ΔQTcF (ΔΔQTcF varied between 1.9 and 6.2 ms) and again from 180 minutes to 24 hours post-dose (ΔΔQTcF varied between 0.1 and 13.4 ms, except the 0.6 mg dose group at 24 hours post-dose, where ΔΔQTcF was −1.9 ms). Within the first 40 minutes post-dose, mean ΔQTcF varied between −2.5 ms (at 40 minutes post-dose on 0.03 mg) and 9.7 ms (at 25 minutes post-dose on 0.1 mg). There was no clear relation to dose or concentration. The second increase in ΔΔQTcF was observed at 180 and 240 minutes post-dose, when mean dasiglucagon concentrations were below the lower level of quantification. At 180 minutes post-dose, the largest mean ΔΔQTcF reached 12.3 and 13.4 ms in the 0.1 and 1.5 mg dose groups.

Figure 14.2.4.S **Placebo-corrected change-from-baseline QTcF (ΔΔQTcF) across time points (QT/QTc analysis set) – subjects with typical PK profiles**



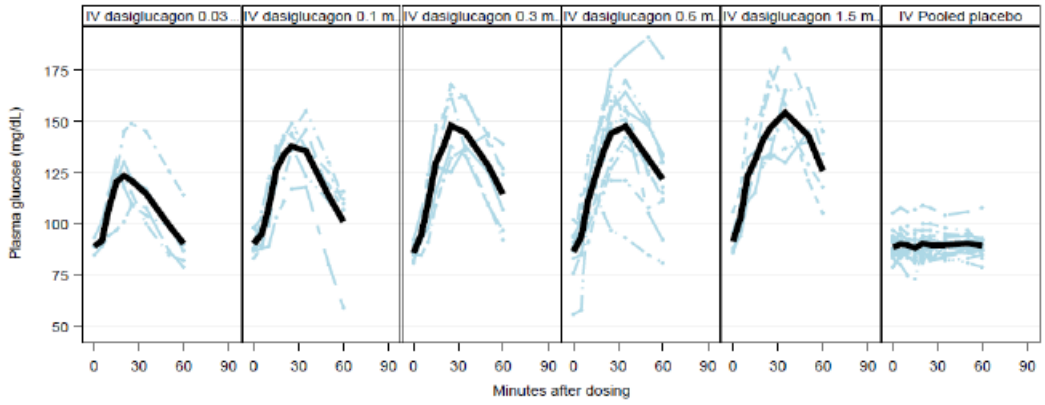
Least squares mean and 90% confidence interval based on a linear mixed-effects model:
ΔQTcF = Time+Treatment+Time*Treatment+Baseline QTcF. A compound symmetry covariance structure was used to specify the repeated measures (time within subject).

To explore the hypothesis of a relationship between the first increase in ΔQTcF and the pharmacodynamic effect, the temporal association between ΔQTcF (top panel) and plasma glucose (lower panel) was presented for subjects with typical PK profiles in the following Figure.



Black lines depict means for the dose cohort, and the blue lines depict the individual subjects in the dose cohort

Source: Figure 14.3.5.3.4



Black lines depict means for the dose cohort, and the blue lines depict the individual subjects in the dose cohort

Source: Figure 14.2.6.11

Figure 12-1: Joint plot of mean plasma glucose and mean QTcF change from baseline (ΔQTcF) for subjects with typical PK profiles

It was concluded that the first increase in ΔQTcF across all dasiglucagon dose cohorts temporally coincided with increase in plasma glucose.

The applicant concluded that, although a delayed QTcF effect for dasiglucagon was observed, the magnitude of the increase in QTcF was small and with no clear relation to dose or concentration. Furthermore, dasiglucagon at the studied doses did not have a clinically relevant effect on cardiac conduction, i.e., the PR and QRS intervals.

2.5.3. Discussion on clinical pharmacology

Analytical methods

Methods for the determination of dasiglucagon in human plasma were based on offline solid phase extraction (SPE) followed by online SPE and highly selective liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) with multiple reaction monitoring. A stable labeled internal standard (ZP5517) was used in validation study. All assays were validated in compliance with the European Medicines Agency Guideline on Bioanalytical Method Validation.

During the clinical development of dasiglucagon, glucagon treatment was included in the majority (7 out of 11) of the clinical trials as reference treatment. Therefore, two sets of ADA assays were used in parallel. One set of ADA assays was used for dasiglucagon-treated patients and another set was used

for glucagon-treated patients. All clinical ADA assays were validated in compliance with guidelines and white papers.

In case anti-ZP4207 or anti-glucagon antibodies are detected in the serum samples, assays to determine whether these serum samples contain antibodies that neutralize the activity of ZP4207 or glucagon were developed. The neutralizing antibody (NAB) assays have been validated for ZP4207 and glugagon, including assessment of the assay cut point, sensitivity, specificity, selectivity, drug tolerance, antibody and control precision, assay robustness, short-term and freeze/thaw stability. Appropriate controls were run on each plate during validation to monitor the performance of the assay.

Evaluation and qualification of models

Four PopPK / PK/PD models were developed for dasiglucagon, with the objectives of supporting the dose selection of dasiglucagon and quantifying the impact of specific covariates on dasiglucagon PK in order to explain between-subject variability. Models have been adequately developed and the performance is considered acceptable for the purpose.

ADME

The therapeutic use of dasiglucagon for the treatment of severe hypoglycemia has been investigated in a clinical development program consisting of 9 trials evaluating dasiglucagon for the treatment of severe hypoglycaemia. The route of administration was SC, except for the IV/QTc trial 17144 also evaluating dasiglucagon exposure after IV administration. Trial 14013 was the only trial that evaluated both SC and intramuscular (IM) administration of dasiglucagon. After the phase 1 trials had been conducted, an optimized dasiglucagon formulation was developed with the aim of improving the initial absorption rate. All phase 2 and 3 trials evaluated this optimized formulation. The effects on dasiglucagon exposure of specific covariates, including injection site (abdomen, buttock, deltoid, or thigh) were evaluated as part of covariate analysis of model 19-077 and 20-046. Thigh injection is expected to lead to lower exposures if compared to abdominal, buttock or deltoid injection. However, dasiglucagon exposure was predicted to be slighty lower also for deltoid and buttock, compared to abdomen.

The absolute bioavailability after SC administration of 0.6 mg dasiglucagon (estimated from the ratio of geometric mean AUC0-∞ [SC/IV]) was 51%.

In adults with T1DM, the geometric mean Vz/f of 0.6 mg dasiglucagon was approximately 50 L, ranging from 46.6 to 56.6 L, while it was higher in pediatric patients with T1DM, at approximately 86 L.

The geometric mean dasiglucagon Cmax after a single 0.6 mg SC dose (range 1265 to 1690 pmol/L across trials) was reached at approximately 35 minutes after administration in adults with T1DM and after 20 minutes in paediatric patients with T1DM. Overall, exposure after a single dose of dasiglucagon was consistent across trials.

After reaching a Cmax at approximately 35 minutes after SC administration, plasma concentrations of dasiglucagon decreased by a geometric mean t½ of approximately 30 minutes (range 29 to 35 minutes) in adults with T1DM (Geometric mean CL/f ranged from 59 to 69 L/h) and of approximately 37 minutes in pediatric patients with T1DM, with no difference between children and adolescents. The geometric mean CL/f in paediatric patients was somewhat higher (~96 L/h) than in adults, driven by a higher CL/f in adolescents (109.8 [42.7] L/h) than in children (78.6 [42.0] L/h).

No in vivo human ADME study was performed with dasiglucagon. Considering that dasiglucagon is a peptide similar to glucagon, based on its molecular weight, it is expected that dasiglucagon is excreted by filtration in the kidneys. Following glomerular filtration, peptides are degraded by the proteases present in the proximal tubuli and the peptide fragments are reabsorbed. Structure-activity relationship analysis indicated that the metabolites are not expected to be active on the glucagon receptor with high activity.

Dose proportionality and time dependency

Dose proportionality was demonstrated in the dosing range 0.1 mg - 1 mg of dasiglucagon. Dose-proportional exposure without relevant changes in PK parameters was also indicated between first and last dosing of a multiple dose study. No accumulation of ZP4207 over the 5-days dosing period was observed.

PK in target population

Dasiglucagon PK in population with T1DM was investigated both in adult and paediatric patients. The geometric mean AUC0-inf and Cmax values in the paediatric population were slightly lower than the corresponding values observed in adults and the median tmax was earlier at 21 minutes, compared to 30-45 minutes in adult studies. The geometric mean AUC and Cmax values were all higher in children than in adolescents, and the median tmax was reached slightly earlier.

However, despite the indication includes patients with T1DM and T2DM, no clinical studies were performed on patients with T2DM.

Special populations

PK in special populations has been mainly investigated through PopPK covariate analysis. A dedicated study is available for paediatric population, but no other dedicated studies have been performed to investigate PK in special populations.

Sufficient data are available for subjects with renal impairment and model simulations adequately described the expected clinical impact. No data neither model simulations are available for subjects with hepatic impairment and the lack of information should be clearly stated in SmPC.

Race, weight and gender are not expected to have a clinically significant impact on dasiglucagn PK metrics and appropriate covariate analysis was performed to identify any potential differences.

The PK of dasiglucagon in elderly subjects was not investigated, neither in clinical trials (only few patients ≥ 65 years were enrolled) or simulations.

DDI

No in vivo DDI studies were performed with dasiglucagon. In vitro studies showed that dasiglucagon does not cause significant inhibition of CYP enzymes and was not determined to be an inhibitor P-gp, BCRP, OAT1, OCT2, MATE1, MATE2-K, and OCT1. In in vitro study 19-168 dasiglucagon was determined to be an inhibitor of probe substrate transport mediated via OATP1B1, OATP1B3 and OAT3, but at not clinically relevant dasiglucagon concentrations.

Pharmacodynamic

The PD results were consistent across all studies. The studies were correctly designed and carried out. The choice of subjects, washout of current therapy with long-acting insulins and stopping insulin infusion before administering dasiglucagon were all measures that allowed the influence of insulin to be minimized and to characterize the effect of dasiglucagon alone. The results clearly show that dasiglucagon induced a rapid increase on blood glucose. The rapidity of the effect of dasiglucagon was similar to the effect of reference glucagon product, but the effect of dasiglucagon was more persistent. During the first 30-minute time interval, a dose of 0.6 mg dasiglucagon was demonstrated to be comparable to 1.0 mg GlucaGen, while a dose of 0.3 mg dasiglucagon was comparable to 0.5 mg GlucaGen in regards to the blood glucose response.

However, despite the claimed indication includes patients with T1DM and T2DM, no clinical studies were performed on patients with T2DM and the potential impact of type of diabetes on dasiglucagon PD, considering that T2DM patients can have co-morbidities along with possibly different basal levels of glucagon and glucagon receptor expression.

The bridging study was correctly designed and performed. The results show that the two batches were similar from a PD point of view. However, there is uncertainty about exact storage conditions of Batch B. Furthermore, the storage conditions reported in the SmPC should be clarified (See Efficacy section).

Dasiglucagon impact on QT interval was evaluated in a dedicated study, after single IV doses administration of 0.03, 0.1, 0.3, 0.6, and 1.5 mg of dasiglucagon. Plots of ΔQTcF and ΔΔQTcF by-time point showed two peaks, the first at 10 to 25 minutes post-dose (mean ΔΔQTcF varied between 1.9 and 6.2 ms), the second from 180 minutes to 24 hours post-dose (ΔΔQTcF varied between 0.1 and 13.4 ms). The concentration-QTc analysis allowed excluding that these changes were related to dasiglucagon concentration (or dose). However, the clinical relevance of this effect is negligible for the first peak registered at 10 to 25 minutes post-dose, but not for the second ΔΔQTcF variation occurring at 180-240 min post-dose that exceeded 10 ms. The applicant's explanation for the second peak in ΔΔQTcF is that it is partly driven by a negative placebo response in ΔQTcF at 180 and 240 min post dosing (180 min: mean ΔQTcF of -3.1 ms; 240 min: mean ΔQTcF of -4.7 ms). It is also considered that the second ΔΔQTcF peak influenced by several confounding factors including large between-subject variation, counter-regulatory hormonal responses to hyperglycemia, and the consumption of a snack offered to the subjects 90 min post-dose, which is reasonable.

The proposed SmPC, section 4.5, lists 4 PD interactions, including those with indomethacin and warfarin, but the source of data supporting these interactions is not derived from ad hoc clinical interaction studies. It is referred to clinical studies investigating the interaction between glucagon and indomethacin (Schmitt, 1987; Ref. 11) or warfarin (Koch-Weser J., 1970; Ref. 12). The clinical relevance of these studies is uncertain (particularly the interaction study with warfarin). However, based on precautionary principle, and taking into consideration that these drugs can easily be replaced by others, it is accepted that these interactions are included in the SmPC.

For that concerns the PK/PD relationship, according to PK/PD model developed, dasiglucagon dose was the only predictor in the model with clinically significant impact on D20. Other covariates had limited impact on D20. Model-based simulations indicated that the average time to achieve glucose cfb of at least 20 mg/dL was shorter for 0.6 mg compared to 0.3 mg for all body weight group. Increasing dasiglucagon doses predominantly benefit patients with relatively slower responses, while typical patients and patients with already rapid responses benefit less.

2.5.4. Conclusions on clinical pharmacology

Dasiglucagon PK and PD were mainly investigated in adult and paediatric patients with T1DM. Overall, clinical pharmacology studies provided adequately characterized dasiglucagon PK and PD.

2.5.5. Clinical efficacy

2.5.5.1. Main studies

The primary efficacy evaluation is derived from three pivotal, placebo-controlled trials: two trials in adult patients with T1DM (trials 16137 and 17145), and one trial in a paediatric patient population with T1DM (trial 17086).

An overview of these 3 pivotal efficacy trials is provided in the next table.

Trial ID Phase	Trial description	No. of patients randomized and dosed (FAS)	Dosing of dasiglucagon
Pivotal trials in adults			
Trial 16137 Phase 3 1st pivotal	A randomized, double-blind, parallel-group trial to confirm the clinical efficacy and safety of dasiglucagon for the treatment of hypoglycemia in patients with T1DM compared to placebo and with GlucaGen® HypoKit® (in the following referred to as 'GlucaGen') as a reference treatment group.[a] Patients were randomized 2:1:1 to either dasiglucagon, placebo or GlucaGen.	168 T1DM patients	• 0.6 mg SC (Single doses, administered via pre-filled syringe).
Trial 17145 Phase 3 2nd pivotal	A randomized, double-blind, parallel-group trial to confirm the clinical efficacy and safety of dasiglucagon for the treatment of hypoglycemia in patients with T1DM compared to placebo. Patients were randomized 3:1 to either dasiglucagon or placebo.	44 T1DM patients	• 0.6 mg SC (Single doses, administered via auto-injector)
Pivotal trial in pediatric patients			
Trial 17086 Phase 3 Pediatric pivotal	A randomized, double-blind, 3-arm, parallel-group trial to confirm the clinical efficacy and safety of dasiglucagon for the treatment of hypoglycemia in pediatric patients with T1DM aged 6 to <18 years. The trial evaluated the efficacy, PK/PD and safety of dasiglucagon. Patients were randomized 2:1:1 to either dasiglucagon, placebo or GlucaGen.	41 pediatric T1DM children: 16 patients aged 6 to 11 years, and 25 patients aged 12 to 17 years, (inclusive).	• 0.6 mg SC (Single doses, administered via pre-filled syringe)

***Abbreviations**: FAS: full analysis set; SC: subcutaneous; T1DM: type 1 diabetes mellitus.*
***Note**: [a] No formal statistical comparison to dasiglucagon was done*

Methods

Study Participants

The phases 2 and 3 trials of the development program comprised patients with T1DM. Patients with T2DM were not included in the development program due to the potential confounding effect of endogenous insulin production on endpoint assessment (in accordance with feedback from BfArM, FDA and MHRA). Guidelines for treatment of severe hypoglycemia do not distinguish between treatment of patients with T1DM and T2DM.

The two pivotal trials 16137 and 17145 comprised male and female patients with T1DM aged between 18 and 75 years. The age range 18-75 years was defined to ensure that patients above 65 years were included. Patients were required to have been diagnosed with T1DM for at least one year, with diagnostic criteria as defined by the American Diabetes Association. Patients were furthermore to have been treated with insulin for at least one year and be on a stable insulin treatment regimen prior to screening. Hemoglobin A1c (HbA1c) was required to be <10% to include patients across a wide range of glycaemic control.

Specifically covering the paediatric age range, the paediatric pivotal trial 17086 comprised T1DM patients aged between 6 and 17 years (both inclusive). No hemoglobin A1c criterion was imposed for this trial, but patients were required to weigh ≥20 kg.

The phase 3 clinical development program was conducted at sites across Europe and North America to ensure regional representativeness.

Main inclusion criteria

Broad inclusion criteria were defined for the three pivotal efficacy trials 16137, 17145 and 17086 in order to reflect the intended adult and paediatric target populations of patients with diabetes:

- Patients with T1DM receiving insulin
- Trials 16137 and 17145: treated with insulin for T1DM for at least 1 year and with stable insulin treatment
- Trial 17086: T1DM for at least 1 year, and receiving daily insulin and in good and stable medical condition
- Age criterion: between 18 and 75 years (both inclusive) in pivotal trials 16137 and 17145, and ≥6 years and <18.0 years in paediatric pivotal trial 17086
- Hemoglobin A1C <10% (not an inclusion criterion for paediatric pivotal trial 17086)
- Paediatric pivotal trial 17086 only: Body weight ≥20 kg

Main exclusion criteria

Exclusion criteria for the pivotal efficacy trials 16137, 17145 and 17086 are summarized below. In addition to ensure independent outcomes, exclusion criteria were defined to exclude any patients who had previously been treated with dasiglucagon or who had previously participated in dasiglucagon clinical trials for treatment of hypoglycemia.

- Known presence of insulinoma (i.e., insulin secreting pancreas tumor).
- Atypically strenuous exercise within 4 days prior to dosing.
- Not fasting from 22:00 hours the evening prior to dosing, apart from water (small amounts of carbohydrates of up to 20 g to prevent hypoglycemia were allowed in pivotal trials 16137 and 17145).
- 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. For the paediatric pivotal trial 17086, the above time thresholds were 72, 48 and 22 hours, respectively.
- Use of any short acting (bolus) insulin within 6 hours prior to dosing, except insulin glulisine (Apidra®). For the paediatric pivotal trial 17086, the time threshold was 12 hours.
- Plasma glucose value <50 mg/dL (2.8 mmol/L) within the last 24 hours or plasma glucose value <60 mg/dL (3.3 mmol/L) within the last 5 hours prior to initiation of the hypoglycemic procedure (pivotal trials 16137 and 17145) or within the last 5 hours prior to admission to the clinic (paediatric pivotal trial 17086).

Treatments

In all three pivotal efficacy trials 16137, 17145 and 17086, both dasiglucagon and comparator treatment(s) were administered as a single subcutaneous (SC) injection following a controlled induction of hypoglycemia using intravenous (IV) administration of insulin.

For safety reasons the procedure differed between adult and pediatric patients as outlined below.

Pivotal trials 16137 and 17145 (adult patients)

To avoid interference from the patients' prior insulin therapy, insulin was initially washed out by ensuring adherence to the criteria outlined in Table 1 6. Hypoglycemia was then gradually induced by an IV infusion of fast-acting insulin glulisine (Apidra®, 100 U/mL). The infusion liquid was prepared by mixing 15 U of Apidra® with 49 mL saline and 1 mL of the patient's blood or plasma. To achieve a controlled decline in plasma glucose targeting a plasma glucose level of 55 mg/dL (3.1 mmol/L), the initial infusion rate was set to correspond to 150% of the patient's usual basal insulin requirement. This could subsequently be increased or decreased within the range of 0% to 200% or more as judged necessary by the investigator.

After start of insulin infusion, plasma glucose was measured approximately every 10 minutes while plasma glucose was above 110 mg/dL and approximately every 5 minutes once plasma glucose was at or below 110 mg/dL. When the glucose concentration had declined to <60 mg/dL (3.3 mmol/L), the insulin infusion was stopped. During this phase, plasma glucose concentrations were measured using the glucose analyzer YSI 2300 (Yellow Springs Instruments, Yellow Springs, OH) or the Super GL analyzer, Dr. Müller Gerätebau GmbH, Freital, Germany (the latter used for trial 16137 only).

Plasma glucose concentration was measured using the glucose analyzer at 5 minutes after stopping the insulin infusion procedure, and baseline laboratory samples for plasma glucose, dasiglucagon, GlucaGen and insulin concentrations were obtained. Investigational product was to be administered no more than 2 minutes thereafter, provided that plasma glucose was ≥45 mg/dL and <60 mg/dL (2.5-3.3 mmol/L). If plasma glucose was <45 mg/dL (2.5 mmol/L) or >60mg/dL (3.3 mmol/L), sufficient IV glucose solution or insulin was administered to adjust plasma glucose to within the 45-60 mg/dL target range, and baseline sampling was repeated. Investigational product was not to be administered earlier than 5 and 10 minutes after discontinuing insulin and glucose infusion, respectively. If plasma glucose was not within target range after the second attempt, the patient was to be rescheduled for a new treatment visit within 7 days (+ 2 days).

Trial 17086 (pediatric patients)

The hypoglycemic clamp procedure in paediatric pivotal trial 17086 was similar to that of trials 16137 and 17145, but a more conservative approach was taken due to the paediatric setting, discontinuing insulin IV infusion when plasma glucose level declined to below 80 mg/dL. Also, the lower blood glucose threshold for administering IV glucose was raised from <45 mg/dL to <54 mg/dL, with investigational product being administered when plasma glucose was ≥54 mg/dL and <80 mg/dL (3.0-4.4 mmol/L).

Injection sites comprised abdomen, buttock, thigh, and deltoid, with permitted injection sites varying from trial to trial.

The dose of dasiglucagon was fixed (i.e. a single dose was used in all patients) and was the same in all three pivotal efficacy trials 16137, 17145 and 17086; it was 0.6 mg, which was determined as the optimal dose based on data from the phase 2, dose-finding trial 15126. As the tested aqueous formulation of dasiglucagon contained 1 mg dasiglucagon per mL, the injected volume was 0.6 mL (and similarly for placebo).

Where GlucaGen was included as an active comparator (pivotal trial 16137 and paediatric pivotal trial 17086), the GlucaGen dose level was 1.0 mg in accordance with product label, with reconstitution and administration procedures also according to product label.

Dasiglucagon and placebo were administered via a pre-filled syringe in all phase 3 trials, except for the 2nd adult pivotal trial, 17145, for which administration was via auto-injector (in which the pre filled syringe was mounted). In trials including active comparator (GlucaGen), this was administered using the injection kit provided with the product.

The table below shows the active treatment used in the 3 pivotal trials and the comparators.

Table 21- Investigational product dosing in trials supporting efficacy evaluation

Trial	dasiglucagona	Comparators	Injection site
Pivotal efficacy trials			

Trial	dasiglucagona	Comparators	Injection site
16137 1st pivotal	0.6 mg SC (single doses).	Placebo; 1.0 mg GlucaGen	Abdomen, buttock, or thigh
17145 2nd pivotal	0.6 mg SC (single doses)	Placebo	Buttock or deltoid
17086 Pediatric pivotal	0.6 mg SC (single doses)	Placebo; 1.0 mg GlucaGenb	Abdomen or thigh

Objectives

Trial 16137 – 1st adult pivotal
Trial title: A phase 3, randomized, double-blind, parallel trial to confirm the clinical efficacy and safety of dasiglucagon in the rescue treatment of hypoglycemia in subjects with type 1 diabetes mellitus (T1DM) compared to placebo and with reference to GlucaGen.
Objectives:

- Primary: to demonstrate superiority of dasiglucagon relative to placebo following a single subcutaneous (SC) 0.6 mg dose administered to patients with T1DM with insulin-induced hypoglycemia.
- Secondary: to compare the glycemic response observed after dasiglucagon with that of GlucaGen.

Trial 17145 – 2nd adult pivotal
Title: A randomized, double-blind, parallel-group trial to confirm the clinical efficacy and safety of dasiglucagon in the rescue treatment of hypoglycemia in subjects with type 1 diabetes
Objectives:

- Primary: to demonstrate superiority of dasiglucagon compared to placebo following a single subcutaneous (SC) 0.6 mg dose administered to patients with T1DM with insulin induced hypoglycemia.
- Secondary: to evaluate the safety, immunogenicity and PK of dasiglucagon following a single SC dose administered to patients with T1DM with insulin induced hypoglycemia as compared to placebo.

Trial 17086 – pediatric pivotal
Trial title: A phase 3, randomized, double-blind, placebo- and active-controlled, parallel arm trial to assess the efficacy, safety, and pharmacokinetics of dasiglucagon relative to placebo and GlucaGen when administered as a rescue therapy for severe hypoglycemia in children with T1DM treated with insulin
Objectives:

- Primary: to demonstrate superiority of dasiglucagon relative to placebo following a single subcutaneous (SC) 0.6 mg dose administered to pediatric patients with T1DM with insulin-induced hypoglycemia.
- Secondary: the secondary objectives comprised confirmation of comparability between dasiglucagon and reconstituted glucagon (GlucaGen) and assessment of the safety and PK profile of dasiglucagon in pediatric patients with T1DM.

Outcomes/endpoints

For all the three pivotal trials, the primary endpoint was time to plasma glucose recovery, with plasma glucose recovery defined as first increase in plasma glucose of ≥20 mg/dL (1.1 mmol/L) from baseline without administration of rescue intravenous (IV) glucose.
For the two adult pivotal trials (16137 and 17145) the (key) secondary endpoints were similar:

- Plasma glucose recovery within 30 minutes, within 20 minutes, within 15 minutes and within 10 minutes after investigational product injection without administration of rescue IV glucose.
- Plasma glucose change from baseline at 30, 20, 15 and 10 minutes, after investigational product injection or at the time of rescue.
- Time to first plasma glucose concentration ≥70 mg/dL (3.9 mmol/L) without administration of rescue IV glucose.
- Plasma glucose response as area under the effect curve (AUE) above baseline from time zero to 30 minutes (AUE0-30min).

For the paediatric trial (17086) the key secondary endpoints were:

- Plasma glucose recovery within 30, 20, 15, and 10 minutes after investigational medicinal product (IMP) injection without administration of IV glucose.
- Plasma glucose changes from baseline at 30, 20, 15, and 10 minutes after IMP injection or at the time of rescue IV glucose.

Among the exploratory endpoints, in the adult trials there was the measure of plasma glucose concentration ≥70 mg/dL (3.9 mmol/L) or increase of ≥20 mg/dL (1.1 mmol/L) within 30 minutes after investigational product injection without administration of rescue IV glucose.

Sample size

Trial 16137 – 1st pivotal

From phase 2, the median time to an increase of 20 mg/dL of the 0.6 mg dose was approximately 10 minutes. With a 2:1:1 randomization ratio for the three treatments, dasiglucagon 0.6 mg, placebo, and GlucaGen 1 mg, and assuming an exponential time-to-recovery distributions with median times of 10 and at least 20 minutes for dasiglucagon and placebo, respectively, a two-sided log-rank test was able to detect a difference between dasiglucagon and placebo with 90% power with a follow-up time of 45 minutes at a 5% significance level with 78 subjects treated with dasiglucagon and 39 subjects with placebo. The median time-to-recovery for placebo was expected to be longer than 20 minutes, which means that the power was greater than 90%. A total number of 156 subjects with T1DM were expected to complete the treatment visit (Visit 2). To qualify as completed, the subject must be dosed and have blood drawn for the PK and PD assessments described in the protocol. For these reasons, it was expected that up to 176 subjects should have been randomized to have 156 subjects completing Visit 2 (78 subjects in the dasiglucagon group and 39 subjects in each of the placebo and GlucaGen groups).

Trial 17145 – 2nd pivotal

From phase 2, the median time to an increase in plasma glucose of 20 mg/dL (1.1 mmol/L) of the 0.6 mg dose was approximately 10 minutes. For placebo-treated subjects, we assume that the median time to recovery will be more than 50 minutes. Assuming an exponential time-to-recovery distribution with median times of 10 and 50 minutes, a two-sided log-rank test will be able to detect a difference between dasiglucagon and placebo with 92% power with a follow-up time of 45 minutes at a 5% significance level with 40 subjects randomized 3 to 1 between active and placebo. Since it was expected to have completing the dosing visit (Visit 2), a total of 46 randomized subjects were planned.

Trial 17086 – paediatric pivotal

The primary comparison is between the dasiglucagon and placebo treatment arms. From phase 2 results, the median time to an increase of 20 mg/dL of the 0.6 mg dose was approximately 10 minutes. For placebo-treated patients, the median time to recovery is assumed to be more than 50 minutes. Assuming an exponential time-to-recovery distribution with median times of 10 and 50 minutes, a 2-sided log-rank test will be able to detect a difference between dasiglucagon 0.6 mg and placebo with 90% power with a follow-up time of 45 minutes at a 5% significance level with 20 patients randomized to the dasiglucagon arm and 10 patients to placebo. GlucaGen® is included as a reference to compare the responses and AE profile to dasiglucagon with those to a marketed product. It is expected that 10 patients in the GlucaGen® group will suffice for the comparison. A total of 40 children ≥6 years and <18 years of age with T1DM were planned to be randomized into the trial (2:1:1 dasiglucagon/placebo/GlucaGen®) and stratified by age intervals: 6 years to <12 years, and 12 years to <18 years and by injection site (abdomen/thigh). A minimum of 16 patients enrolled into each age group, including a minimum of 8 patients in either of age groups on dasiglucagon treatment arm were requested.

Randomisation and Blinding (masking)

Trial 16137 – 1st pivotal

Subjects who have given written informed consent and meet all inclusion and none of the exclusion criteria were randomized in a 2:1:1 ratio to either 0.6 mg dasiglucagon (n=78), placebo (n=39), or 1 mg GlucaGen (n=39) via an Interactive Web Response System (IWRS). Randomization continued until 156 subjects have completed Visit 2. Subjects with previous exogenic glucagon exposure will not be excluded from the trial, but the information on previous glucagon administration was recorded, to enable subgroup analyses. Randomization was performed using a fixed-block randomization scheme. The randomization scheme was generated prior to the initiation of the study by an independent statistician/programmer who was not be a member of the study team. Randomization was stratified by treatment group and by injection site (abdomen, buttocks, or thigh).

Trial 17145 – 2nd pivotal

Subjects who had given written informed consent were provided with a subject number. Once assigned, the subject number was used throughout the trial and had not been reused by any other subject. Subjects who met all inclusion and none of the exclusion criteria or dosing day exclusion criteria were assigned a subject randomization number and randomized in a 3:1 ratio to 0.6 mg dasiglucagon (n=30) or placebo (n=10) via an Interactive Web Response System (IWRS). This randomization also determined whether the subject was to be injected in the buttock or deltoid. Randomization continued until a total of 40 subjects had completed Visit 2. Randomization was stratified by treatment group and by injection site (abdomen/thigh).

Trial 17086 – paediatric pivotal

Randomization was performed using a central, dynamic variance minimization randomization method using an interactive web response system (IWRS) that randomized a patient to one of the 3 treatment arms and then assigned dispensing unit numbers to that patient. An unblinded statistician/programmer who was separate from the trial team generated the randomized kit list before the initiation of the trial. Treatment assignment was kept strictly confidential and accessible only to authorized persons until after data base lock. Randomization was stratified by age intervals (6 years to <12 years, and 12 years to <18 years) and by injection site (abdomen/thigh).

Blinding

All the three pivotal trials were conducted in a double-blinded manner to increase trial validity and to reduce bias during evaluation of the treatments. However, in trial 16137 and 17086, since dasiglucagon and GlucaGen were not identical in appearance (liquid formulation and powder for reconstitution, respectively), unblinded trial personnel were responsible for handling, preparing (according to the prescription from the IWRS), and administering the trial product, and the syringes used for administration were wrapped in aluminum foil to maintain the blinding at bedside. To maintain double-blind conditions, all trial assessments at the trial center have been done by blinded trial personnel not involved in the administration of trial medications. Treatment assignment was kept strictly confidential and accessible only to authorized persons until after the time of unblinding. Codes with treatment assignment were, however, be readily available in the IWRS to the blinded personnel in case of an emergency.

Statistical methods

There were no global amendments for trials 17145 and 17086, while the global amendment 2 (04-Apr-2018) of trial 16137 included just some additional explanations, as per FDA recommendations, without affect the statistical analysis plan.

No interim analyses were planned for any of the three pivotal trials.

Efficacy analyses

For the confirmatory analyses, the primary and key secondary endpoints for the dasiglucagon 0.6 mg and placebo treated subjects were compared. The primary and secondary endpoints were evaluated on the full analysis set (FAS), including all randomized subjects who received at least one dose of trial medication. The primary endpoint was summarized using Kaplan-Meier (KM) estimates for each treatment group and stratification factor. The stratified log-rank test was applied to compare the dasiglucagon 0.6 mg treatment group to the placebo group. The same method was applied to compare dasiglucagon 0.6 mg with GlucaGen® in trials 16137 and 17086. The primary endpoint was additionally analyzed using a Cox proportional hazards time to event statistical model.

For trials 16137 and 17145, the key secondary endpoints of plasma glucose recovery within 30, 20, 15 and 10 minutes were compared between treatment groups using Fisher's exact test. Plasma glucose change from baseline at 30, 20, 15 and 10 minutes were analyzed using an Analysis of Covariance (ANCOVA) model.

For trial 17086, the secondary endpoints of 10-, 15-, 20- and 30- minute recovery rates of 2 treatment groups were compared by a Cochran-Mantel-Haenszel test stratified by age group and injection site. If due to small or zero cell counts the Cochran-Mantel- Haenszel test fails, non-stratified Fisher's exact tests will be applied instead. Plasma glucose changes from baseline at 10, 15, 20 and 30 minutes after trial product injection were analyzed in an analysis of variance with factors treatment (3 levels) age group (2 levels) and injection site (2 levels) for each endpoint.

In the primary analysis, those subjects who require rescue IV glucose were censored at the time to plasma glucose recovery. In the sensitivity analysis, the time to plasma glucose recovery were analyzed without censoring those subjects who require rescue IV glucose before 45 minutes. All sensitivity analyses were based upon the Per protocol (PP) set, including all subjects of the FAS for whom no relevant protocol deviations were documented.

Type I error and multiplicity control

The treatment group difference between dasiglucagon and placebo for primary and key secondary endpoints were tested in a pre-specified hierarchical order to control he type 1 error rate across the planned multiple comparisons. The primary endpoint and key secondary endpoints were inferentially evaluated in the following order, where inference proceeded at the two-sided 0.05 criterion significance level until the first failure to reject the null hypothesis for a dasiglucagon versus placebo comparison:

- Primary: Time to plasma glucose recovery

- Key secondaries 1-4: Plasma glucose recovery within 30 minutes, within 20 minutes, within 15 minutes, and within 10 minutes after study drug injection without administration of rescue IV glucose.

- Key secondaries 5-8: Plasma glucose changes from baseline (CFB) within 30 minutes, within 20 minutes, within 15 minutes, and within 10 minutes after study drug injection or at the time of rescue.

In trials 16137 and 17086, the GlucaGen versus placebo comparisons was not included in the inferential testing hierarchy, since the efficacy of GlucaGen was previously established, and these comparisons were intended to support the validity of the study for the dasiglucagon versus placebo comparisons.

Analogous supportive sensitivity analyses were conducted in the PP set, but without inference intent.

Subgroup analyses

Summary tables describing the primary endpoint were created based on age, sex, race/ethnicity or other demographic characteristic(s).

Safety analyses

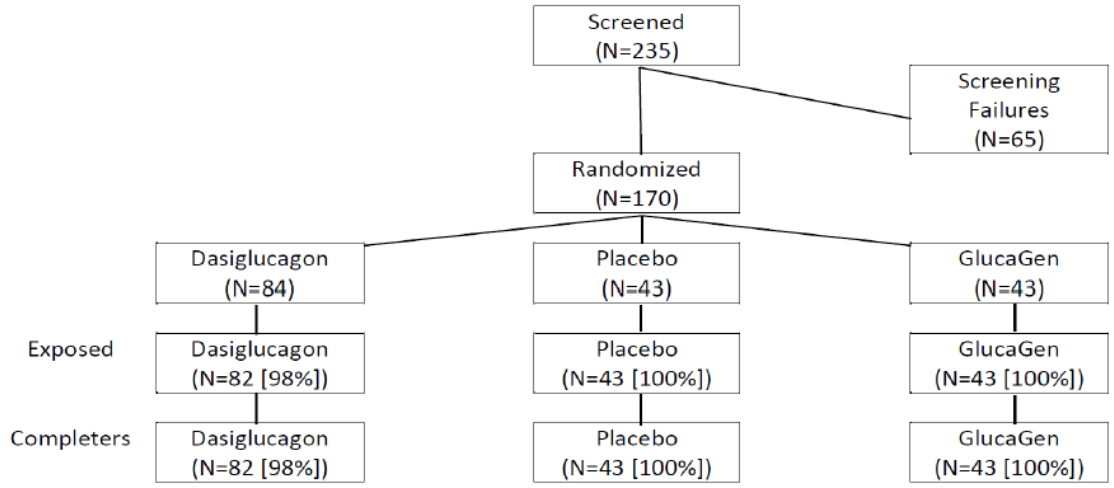
The safety analyses included by-treatment-group descriptive summaries of drug exposure, clinical laboratory assessment (including immunogenicity incidence), rescue IV glucose (incidence and amount of glucose infused), and adverse events. All safety analyses were based upon the Safety analysis set

(SAS), including all randomized subjects who received at least one dose of trial medication.

Results

Participant flow

Trial 16137
The following figure shows the patients' disposition.



Source: Post-text Table 14.1.1
Calculation of percent based on number of randomized patients.

Two patients in the dasiglucagon group discontinued prior to being treated. The primary reason for not completing the trial was patient withdrawal of consent for one patient and AE for another patient an AE of ventricular extrasystoles prior to investigational product administration; this patient also withdrew consent).

Trial 17145
The following figure shows the patients' disposition.

Table 10-1 Subject Disposition: All Randomized Subjects (N=45)

Disposition, n (%)	Treatment		
	0.6 mg Dasiglucagon (N=34)	Placebo (N=11)	All Subjects (N=45)
Randomized	34	11	45
Completed	34 (100.0)	10 (90.9)	44 (97.8)
Withdrew[1]	0 (0.0)	1 (9.1)	1 (2.2)
Reasons for Withdrawal			
Safety concern at discretion of the investigator (includes AE)	0 (0.0)	0 (0.0)	0 (0.0)
Withdrawal of consent by subject	0 (0.0)	1 (9.1)	1 (2.2)
Study discontinuation by sponsor	0 (0.0)	0 (0.0)	0 (0.0)
Randomization by Site			
Randomized at site 101, n (%)	15 (44.1)	6 (54.5)	21 (46.7)
Randomized at site 105, n (%)	14 (41.2)	4 (36.4)	18 (40.0)
Randomized at site 106, n (%)	5 (14.7)	1 (9.1)	6 (13.3)
Source data: Table 14.1.1 [1] Subject [redacted] withdrew consent prior to IMP administration and was not treated.			

Of the 45 subjects randomized in the study, 44 subjects (97.8%) completed the study (i.e. completed the 28 days follow-up visit or the last scheduled procedure). All subjects but one completed Visit 3. One (1) subject randomized to placebo did not wish to continue with the study and voluntarily withdrew consent from the study on Day 1 prior to IMP administration.

Trial 17086

The following figure shows the patients' disposition.

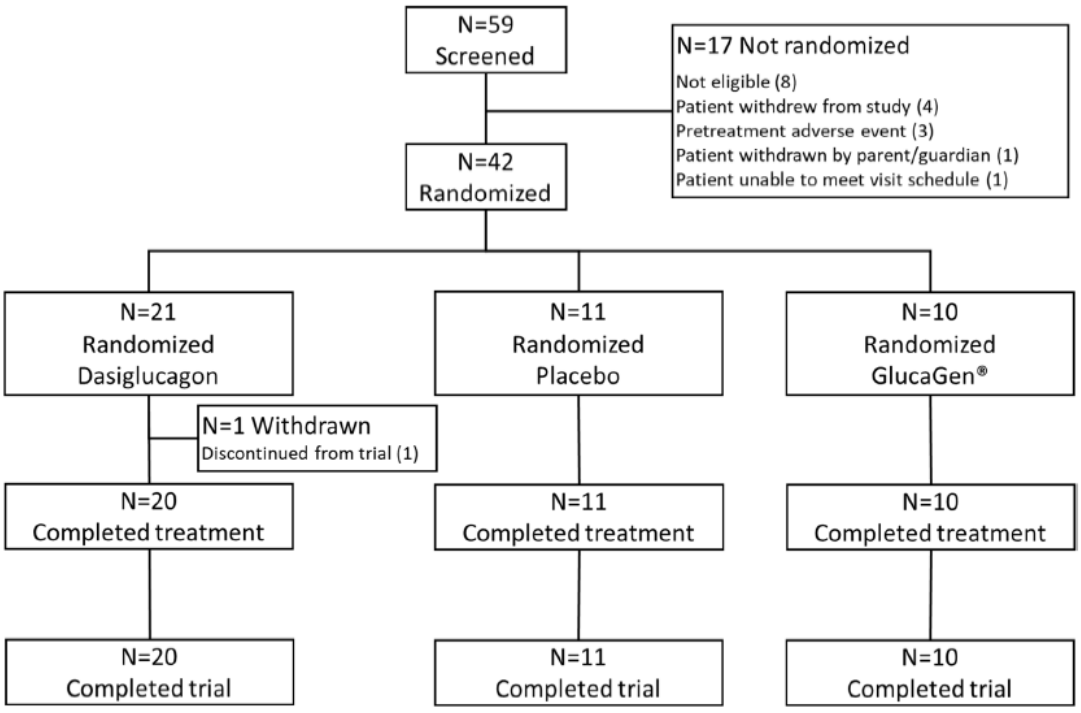


Figure 10.1: Disposition of trial patients

Source: Table 14.1.3, Table 14.1.4, and Listing 16.2.1.2

A total of 42 patients were randomly assigned to the treatment groups, and 41 patients received IMP. The reason for withdrawal of 1 patient after randomization but before treatment was that the patient did not agree to rescheduling the dosing visit after having failed to reach the required pre-dose plasma glucose range at the initial dosing visit. All 41 patients who were treated completed the trial.

The analysis sets were defined and composed as described below:

Trial 16137

The safety analysis set and FAS included all randomized patients who received trial medication: 82 patients in the dasiglucagon group, 43 patients in the placebo group and 43 patients in the GlucaGen group.

The PP set included all patients of the FAS for whom no protocol deviations were documented that may significantly impact efficacy evaluation. Two treated patients were excluded from the PP set. One patient was excluded because pre-dose PD and glucagon laboratory samples were not collected at the site. The second patient had a blood sugar value of 2.6 mmol using a regular blood meter on Day -1, which was not retested at the site. If the patient's blood sugar had been confirmed as less than 2.8 mmol, this would have fulfilled dosing day exclusion criterion #9.

Trial 17145

A total of 45 subjects were randomized, 34 to dasiglucagon and 11 to placebo. One subject was randomized to placebo but withdrew on Day 1 before treatment and was not included in any analysis population.

The Safety and FAS population consisted of all randomized subjects who received at least one dose of the trial drug and included 34/34 subjects (100.0%) randomized to dasiglucagon and 10/11 subjects (90.9%) randomized to placebo.

The PP population consisted of all subjects in the FAS for whom no relevant major protocol deviations were documented. One subject was randomized and treated with dasiglucagon, but was excluded from the PP population, as this subject did meet the dosing day exclusion criterion #9, which was a plasma glucose reading < 50 mg/dL. The PP population therefore included 33/34 subjects (97.1%) randomized to dasiglucagon and 10/11 subjects (90.9%) randomized to placebo.

Trial 17086

The analysis of the primary endpoint was based on the FAS. A secondary analysis of the primary endpoint was based on the PPS. Safety analysis was based on the safety analysis set.

One patient was randomized to receive dasiglucagon but was not treated. Three patients, 1 from each treatment group, were not included in the PPS due to irregularities with plasma glucose sampling. Thus, a total of 92.7% (38 of 41) of the patients in the FAS were included in the PPS.

Recruitment

16137: Trial period: 07 December 2017 – 25 May 2018.

17145: First Subject Visit: 01-Nov-2018; Last Subject Visit: 11-Mar-2019.

17086: Trial period: 28 September 2018 to 28 June 2019.

Conduct of the study

Trial 16137

For Trial 16137, some protocol amendments were done in order to clarify better some methodological issues. A total of four major protocol deviations were recorded for three patients as follows:

Two patients were included into the trial in spite of meeting the exclusion criterion of diastolic blood pressure ≥90 mmHg. Another patient had blood sugar of 2.6 mmoL not confirmed at the site. If the blood sugar value had been confirmed as <2.8 mmoL, Dosing Day Exclusion Criterion #9 would have been met.

Trial 17145 and Trial 17086

There were some protocol deviations mainly regarding the timing of plasma glucose measure (few minutes out of the planned window).

Baseline data

Trial 16137

The following table shows the baseline characteristics.

Table 10-2: Demographic and Baseline Characteristics (Safety Analysis Set)

Parameter	Category / Statistic	Dasiglucagon (N=82)	Placebo (N=43)	GlucaGen® (N=43)	Total (N=168)
Sex	Female	32 (39%)	16 (37%)	15 (35%)	63 (38%)
	Male	50 (61%)	27 (63%)	28 (65%)	105 (63%)
Race	Asian	3 (4%)	2 (5%)	0	5 (3%)
	Black or African American	1 (1%)	1 (2%)	2 (5%)	4 (2%)
	Native Hawaiian or Other Pacific Islander	0	0	1 (2%)	1 (1%)
	White	76 (93%)	39 (91%)	39 (91%)	154 (92%)
	Other	1 (1%)	1 (2%)	0	2 (1%)
	Multiple	1 (1%)	0	1 (2%)	2 (1%)
Ethnicity	Hispanic or Latino	2 (2%)	2 (5%)	3 (7%)	7 (4%)
	Not Hispanic or Latino	80 (98%)	41 (95%)	40 (93%)	161 (96%)
ADA Tested		82	0	43	125
Baseline ADA (1)	Positive	0	0	0	0
	Negative	82 (100%)	0	43 (100%)	125 (74%)
Age (years)	N	82	43	43	168
	Mean (SD)	39.2 (12.1)	38.0 (13.1)	40.2 (11.5)	39.1 (12.2)
	Median	37.0	36.0	38.0	38.0
	Min, Max	18, 71	18, 65	23, 66	18, 71
Weight (kg)	N	82	43	43	168
	Mean (SD)	78.3 (13.5)	79.6 (13.0)	80.7 (15.1)	79.2 (13.7)
	Median	77.8	81.6	80.0	78.2
	Min, Max	49.9, 119.2	48.6, 108.6	54.0, 126.2	48.6, 126.2
Height (cm)	N	82	43	43	168
	Mean (SD)	173.1 (9.44)	174.2 (9.15)	175.9 (9.71)	174.1 (9.45)
	Median	174.0	175.0	175.0	175.0
	Min, Max	151.0, 193.5	156.0, 191.0	160.0, 194.0	151.0, 194.0
BMI (kg/m^2)	N	82	43	43	168
	Mean (SD)	26.1 (4.13)	26.1 (3.34)	25.9 (3.42)	26.1 (3.75)

Parameter	Category / Statistic	Dasiglucagon (N=82)	Placebo (N=43)	GlucaGen® (N=43)	Total (N=168)
	Median	25.0	25.7	26.0	25.8
	Min, Max	18.6, 38.4	19.7, 34.2	19.4, 35.0	18.6, 38.4
HbA1c (%)	**N**	82	43	43	168
	Mean (SD)	7.52 (0.95)	7.17 (0.74)	7.41 (0.97)	7.40 (0.91)
	Median	7.4	7.1	7.4	7.3
	Min, Max	5.2, 9.7	6.0, 9.2	5.4, 8.9	5.2, 9.7

Source: Post-text Table 14.1.3
Abbreviations: ADA = anti-drug antibody; BMI = body mass index; HbA1C = glycated hemoglobin; max = maximum; min = minimum; SD = standard deviation
Calculation of percent based on number of patients included in each treatment group.
(1) Number of patients whose baseline samples were tested for ADA.

The mean (SD) duration of diabetes was slightly higher in the dasiglucagon group (22.6 [12.32] years) relative to the patients in the placebo and GlucaGen groups (19.8 [11.17] and 19.4 [11.01] years, respectively).

Trial 17145
The following table shows the baseline characteristics.

Table 10-4 Demographic and Baseline Characteristics: Safety Population (N=44)

Characteristics	Treatment		
	0.6 mg Dasiglucagon (N=34)	**Placebo (N=10)**	**All Subjects (N=44)**
Sex, n (%)			
Male	16 (47.1)	9 (90.0)	25 (56.8)
Female	18 (52.9)	1 (10.0)	19 (43.2)
Age (years), Mean (SD)	42.4 (13.49)	36.5 (12.80)	41.0 (13.42)
Race, n (%)			
American Indian or Alaska Native	0 (0.0)	0 (0.0)	0 (0.0)
Asian	0 (0.0)	0 (0.0)	0 (0.0)
Black or African American	0 (0.0)	1 (10.0)	1 (2.3)
Native Hawaiian or Other Pacific Islander	0 (0.0)	1 (10.0)	1 (2.3)
White	34 (100.0)	7 (70.0)	41 (93.2)
Multiple	0 (0.0)	1 (10.0)	1 (2.3)
Ethnicity, n (%)			
Hispanic or Latino	4 (11.8)	3 (30.0)	7 (15.9)
Not Hispanic or Latino	30 (88.2)	7 (70.0)	37 (84.1)
Weight (kg), Mean (SD)	84.49 (20.102)	83.69 (10.812)	84.30 (18.295)
Height (cm), Mean (SD)	172.2 (8.86)	173.3 (6.38)	172.5 (8.30)
Body Mass Index (kg/m^2), Mean (SD)	28.41 (5.802)	27.92 (3.975)	28.30 (5.402)
Duration of Diabetes (years), Mean (SD)	22.5 (13.82)	21.2 (13.42)	22.2 (13.59)
Source data: Modified from Table 14.1.4 - One subject had multiple races: "White" and "Native Hawaiian or Other Pacific Islander."			

Medical history for the Safety population is summarized by treatment group in the table below.

Table 10-5 Medical History: Safety Population (N=44)

System Organ Class, n (%)	Treatment		
	0.6 mg Dasiglucagon (N=34)	Placebo (N=10)	All Subjects (N=44)
Subjects with Any Medical History	33 (97.1)	9 (90.0)	42 (95.5)
Surgical and medical procedures	16 (47.1)	5 (50.0)	21 (47.7)
Metabolism and nutrition disorders	12 (35.3)	6 (60.0)	18 (40.9)
Injury, poisoning and procedural complications	11 (32.4)	2 (20.0)	13 (29.5)
Psychiatric disorders	11 (32.4)	1 (10.0)	12 (27.3)
Vascular disorders	6 (17.6)	4 (40.0)	10 (22.7)
Endocrine disorders	8 (23.5)	1 (10.0)	9 (20.5)
Immune system disorders	9 (26.5)	0 (0.0)	9 (20.5)
Nervous system disorders	8 (23.5)	1 (10.0)	9 (20.5)
Eye disorders	6 (17.6)	1 (10.0)	7 (15.9)
Gastrointestinal disorders	5 (14.7)	2 (20.0)	7 (15.9)
Infections and infestations	4 (11.8)	2 (20.0)	6 (13.6)
Respiratory, thoracic and mediastinal disorders	5 (14.7)	1 (10.0)	6 (13.6)
Musculoskeletal and connective tissue disorders	4 (11.8)	1 (10.0)	5 (11.4)
Skin and subcutaneous tissue disorders	4 (11.8)	0 (0.0)	4 (9.1)
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	3 (8.8)	0 (0.0)	3 (6.8)
Reproductive system and breast disorders	3 (8.8)	0 (0.0)	3 (6.8)
General disorders and administration site conditions	2 (5.9)	0 (0.0)	2 (4.5)
Blood and lymphatic system disorders	1 (2.9)	0 (0.0)	1 (2.3)
Congenital, familial and genetic disorders	1 (2.9)	0 (0.0)	1 (2.3)
Ear and labyrinth disorders	1 (2.9)	0 (0.0)	1 (2.3)
Hepatobiliary disorders	1 (2.9)	0 (0.0)	1 (2.3)
Renal and urinary disorders	1 (2.9)	0 (0.0)	1 (2.3)
Social circumstances	1 (2.9)	0 (0.0)	1 (2.3)
Source data: Modified from Table 14.1.5			

-The table is sorted by descending frequency of the Overall System Organ Class.
-System Organ Class (SOC) was coded with MedDRA, version 22.0.

Concomitant medications by anatomic therapeutic chemical classification for the Safety population are summarized by treatment group in the table below.

Table 10-6 Concomitant Medications: Safety Population (N=44)

ATC Level 2[1], n (%)	Treatment		
	0.6 mg Dasiglucagon (N=34)	Placebo (N=10)	All Subjects (N=44)
Subject with Any Concomitant Medication	34 (100.0)	10 (100.0)	44 (100.0)
Drugs Used in Diabetes	34 (100.0)	10 (100.0)	44 (100.0)
Lipid Modifying Agents	12 (35.3)	4 (40.0)	16 (36.4)
Agents Acting on The Renin-Angiotensin System	9 (26.5)	4 (40.0)	13 (29.5)
Thyroid Therapy	7 (20.6)	1 (10.0)	8 (18.2)
Drugs for Functional Gastrointestinal Disorders	6 (17.6)	0 (0.0)	6 (13.6)
Psychoanaleptics	5 (14.7)	1 (10.0)	6 (13.6)
Vitamins	5 (14.7)	1 (10.0)	6 (13.6)
Antithrombotic Agents	4 (11.8)	0 (0.0)	4 (9.1)
Drugs for Acid Related Disorders	4 (11.8)	0 (0.0)	4 (9.1)
Analgesics	3 (8.8)	0 (0.0)	3 (6.8)
Antianemic Preparations	3 (8.8)	0 (0.0)	3 (6.8)
Antiemetics And Antinauseants	3 (8.8)	0 (0.0)	3 (6.8)
Calcium Channel Blockers	2 (5.9)	1 (10.0)	3 (6.8)
Other Gynecologicals	3 (8.8)	0 (0.0)	3 (6.8)
Antiinflammatory And Antirheumatic Products	2 (5.9)	0 (0.0)	2 (4.5)
General Nutrients	2 (5.9)	0 (0.0)	2 (4.5)
Psycholeptics	2 (5.9)	0 (0.0)	2 (4.5)
Sex Hormones and Modulators Of The Genital System	1 (2.9)	1 (10.0)	2 (4.5)
Anti-Parkinson Drugs	1 (2.9)	0 (0.0)	1 (2.3)
Antibacterials For Systemic Use	1 (2.9)	0 (0.0)	1 (2.3)
Antidiarrheals, Intestinal Antiinflammatory/Antiinfective Agents	1 (2.9)	0 (0.0)	1 (2.3)
Antifungals for Dermatological Use	1 (2.9)	0 (0.0)	1 (2.3)
Antihistamines for Systemic Use	1 (2.9)	0 (0.0)	1 (2.3)
Antivirals for Systemic Use	1 (2.9)	0 (0.0)	1 (2.3)
Digestives, Incl. Enzymes	1 (2.9)	0 (0.0)	1 (2.3)
Drugs for Obstructive Airway Diseases	0 (0.0)	1 (10.0)	1 (2.3)
Drugs for Treatment Of Bone Diseases	1 (2.9)	0 (0.0)	1 (2.3)
Immunosuppressants	1 (2.9)	0 (0.0)	1 (2.3)
Mineral Supplements	1 (2.9)	0 (0.0)	1 (2.3)
Ophthalmological and Otological Preparations	1 (2.9)	0 (0.0)	1 (2.3)
Ophthalmologicals	1 (2.9)	0 (0.0)	1 (2.3)
Urologicals	1 (2.9)	0 (0.0)	1 (2.3)

Source data: Modified from Table 14.1.6

[1] Therapeutic subgroup (ATC level 2).

-ATC = Anatomic Therapeutic Chemical Classification using B3 WHO Drug Global - Mar 2019.

-Concomitant Medications were medications received on or after the date of the first dose of randomized study medication.

-Subjects were counted only once in each ATC level 2 category.

Trial 17086
An overview of the key demographic characteristics and body measurements of the trial population is given in the next table.

Table 10.2: Baseline characteristics (safety analysis set)

Demography		Dasiglucagon (N=20)	Placebo (N=11)	GlucaGen® (N=10)	Total (N=41)
Age (years)	Mean (±SD)	12.3 (±3.42)	12.8 (±3.25)	12.4 (±3.50)	12.5 (±3.32)
	Min-max	7-17	7-17	7-17	7-17
Age groups	6-11 years (%)	8 (40.0)	4 (36.4)	4 (40.0)	16 (39.0)
	12-17 years (%)	12 (60.0)	7 (63.6)	6 (60.0)	25 (61.0)
Sex	Male (%)	10 (50.0)	5 (45.5)	8 (80.0)	23 (56.1)
	Female (%)	10 (50.0)	6 (54.5)	2 (20.0)	18 (43.9)
Race	White (%)	19 (95.0)	10 (100.0)	10 (100.0)	39 (97.5)
	Multiple (%)	1 (5.0)	0	0	1 (2.5)
	Missing (%)	0	1	0	1
Ethnicity	Hisp/Lat (%)	4 (20.0)	2 (20.0)	1 (10.0)	7 (17.5)
	Not Hisp/Lat (%)	16 (80.0)	8 (80.0)	9 (90.0)	33 (82.5)
	Missing (%)	0	1	0	1
Body measurements					
Height (cm)	Mean (±SD)	154.6 (±18.32)	161.1 (±19.45)	158.5 (±20.10)	157.3 (±18.78)
	Min-max	124-183	130-186	128-183	124-186
Weight (kg)	Mean (±SD)	51.54 (±22.202)	54.95 (±21.404)	48.81 (±14.992)	51.79 (±20.106)
	Min-max	21.2-117.0	23.0-91.7	25.0-67.1	21.2-117.0
Body mass index (kg/m^2)	Mean (±SD)	20.74 (±6.057)	20.39 (±4.885)	18.92 (±2.617)	20.20 (±5.050)
	Min-max	13.8-35.3	13.3-28.2	15.0-24.0	13.3-35.3
Body Mass Index class	<25 kg/m^2 (%)	17 (85.0)	9 (81.8)	10 (100.0)	36 (87.8)
	25- <30 kg/m^2 (%)	1 (5.0)	2 (18.2)	0	3 (7.3)
	30- <35 kg/m^2 (%)	1 (5.0)	0	0	1 (2.4)
	≥35 kg/m^2 (%)	1 (5.0)	0	0	1 (2.4)

Abbreviation: Hisp=Hispanic; Lat=Latino; Max=maximum; Min=minimum
Source: Table 14.1.5 and Table 14.1.7.2

In the next tables the baseline diabetes characteristics are shown.

Table 10.3: Time since diagnosis of diabetes in years (safety analysis set)

Time (years)	Dasiglucagon (N=20)	Placebo (N=11)	GlucaGen® (N=10)	Total (N=41)
Mean (±SD)	6.13 (±3.577)	5.44 (±3.843)	6.09 (±4.402)	5.94 (±3.771)
Median	5.59	5.21	4.64	5.33
Min-max	1.8-12.9	1.1-14.1	1.2-16.3	1.1-16.3

Abbreviations: Max=maximum; Min=minimum
Source: Table 14.1.7.1

Table 10.4: Hemoglobin A1c at screening (safety analysis set)

Hemoglobin A1c [%]	Dasiglucagon (N=20)	Placebo (N=11)	GlucaGen® (N=10)	Total (N=41)
Mean (±SD)	7.6200 (±1.13860)	7.7727 (±1.18751)	7.8100 (±1.17516)	7.7073 (±1.13433)
Median	7.5500	7.8000	7.5000	7.6000
Min-max	6.000-11.200	5.300-9.900	6.900-10.700	5.300-11.200

Abbreviations: Max=maximum; Min=minimum

Source: Table 14.3.5.2.1

Numbers analysed

Trial 16137: Total of 170 patients were eligible and randomized to dasiglucagon, placebo or GlucaGen treatment. One hundred and sixty-eight (168) patients completed the trial.

Trial 17145: The study enrolled 68 subjects, 45 of which were randomized to either dasiglucagon (34 subjects) or placebo (11 subjects). Of these, 44 subjects were included in the Safety population (34 subjects randomized to dasiglucagon and 10 subjects randomized to placebo), 44 subjects were included in the Full Analysis Set (FAS) (34 subjects randomized to dasiglucagon and 10 subjects randomized to placebo), and 43 subjects were included in the Per-Protocol (PP) population (33 subjects randomized to dasiglucagon and 10 subjects randomized to placebo).

Trial 17086: Analyzed (full analysis set): 41.

Outcomes and estimation

Trial 16137 Results

The primary endpoint of plasma glucose recovery was defined as first increase in plasma glucose of ≥20 mg/dL (1.1 mmol/L) from baseline without administration of rescue IV glucose.

As summarized in table below for the FAS, the median time to plasma glucose recovery within 45 minutes was statistically significantly less in the dasiglucagon group (10 minutes [95% CI: 10, 10]) than in the placebo group (40 minutes [95% CI: 30, 40]; $p<0.001$ log-rank test). In the GlucaGen group, the median time to plasma glucose recovery was 12 minutes (95% CI: 10, 12).

Table 11-1: Time to Plasma Glucose Recovery (Full Analysis Set)

Plasma Glucose Recovery within 45 min (min)	Dasiglucagon (N=82)	Placebo (N=43)	GlucaGen® (N=43)
No. of subjects in the analysis, n	82	43	43
No. of subjects with event, n (%)[1]	82 (100.0)	31 (72.1)	43 (100.0)
No. of censored subjects, n (%)[2]	0 (0.0)	12 (27.9)	0 (0.0)
Median Duration of PG Recovery (95% CI)[3]	10 (10, 10)	40 (30, 40)	12 (10, 12)
Log-rank test 2-sided p-value[3]	<0.001***		
Wilcoxon test 2-sided p-value[4]	<0.001***		
K-M estimates, (95% CI)			
0 min	1.000 (1.000, 1.000)	1.000 (1.000, 1.000)	1.000 (1.000, 1.000)
6 min	0.927 (0.844, 0.966)	1.000 (1.000, 1.000)	0.953 (0.827, 0.988)
8 min	0.720 (0.609, 0.804)	1.000 (1.000, 1.000)	0.767 (0.611, 0.867)
10 min	0.341 (0.241, 0.444)	1.000 (1.000, 1.000)	0.512 (0.355, 0.648)
12 min	0.134 (0.071, 0.217)	1.000 (1.000, 1.000)	0.140 (0.057, 0.259)
15 min	0.012 (0.001, 0.059)	0.977 (0.846, 0.997)	0.047 (0.008, 0.139)
17 min	0.012 (0.001, 0.059)	0.907 (0.771, 0.964)	0.023 (0.002, 0.106)
20 min	0.012 (0.001, 0.059)	0.860 (0.716, 0.935)	0.023 (0.002, 0.106)
25 min	0.000 (NE, NE)	0.767 (0.611, 0.867)	0.000 (NE, NE)
30 min	0.000 (NE, NE)	0.535 (0.376, 0.670)	0.000 (NE, NE)
40 min	0.000 (NE, NE)	0.279 (0.156, 0.416)	0.000 (NE, NE)
45 min	0.000 (NE, NE)	0.279 (0.156, 0.416)	0.000 (NE, NE)
Hazard ratio (95% CI)[5]	111.9 (37.9, 330.4)		72.0 (24.2, 214.4)
Treatment effect p-value in CPH [5]	<0.001***		<0.001***
Injection site effect p-value in CPH [6]	0.154		

Source: Post-text Table 14.2.4.7

Abbreviations: CI = Confidence interval; IV = intravenous; K-M = Kaplan-Meier; NE = Not Evaluable; PG = Plasma Glucose.

[1] Recovery is defined as first increase in plasma glucose of ≥20 mg/dL (1.1 mmol/L) from baseline within 45 minutes during the hypoglycemic clamp procedure without administration of rescue IV glucose.

[2] Patients who receive rescue IV glucose or did not recover at 45 minutes after dosing are censored 45 minutes after dosing.

[3] Kaplan-Meier estimate with 95% CI, p-value based on treatment group difference between dasiglucagon and placebo using a two-sided log-rank test stratified by injection site. Treatment groups without censoring utilized a distribution free method to compute the confidence interval for median time.

[4] As supportive analysis, dasiglucagon compared to placebo using a Wilcoxon test stratified by injection site.

[5] The hazard ratios (95% CIs) and two-sided p-values based on the treatment effect (Dasiglucagon compared with Placebo, GlucaGen® compared with Placebo) in a discrete-time Cox proportional hazards (CPH) model with treatment group and injection site modeled as categorical effects, and baseline plasma glucose modeled as a continuous covariate.

[6] Type 3 test for the effect of injection site in the Cox proportional hazards (CPH) model.

The Kaplan Meier estimates of time to plasma glucose recovery are displayed in the next figure.

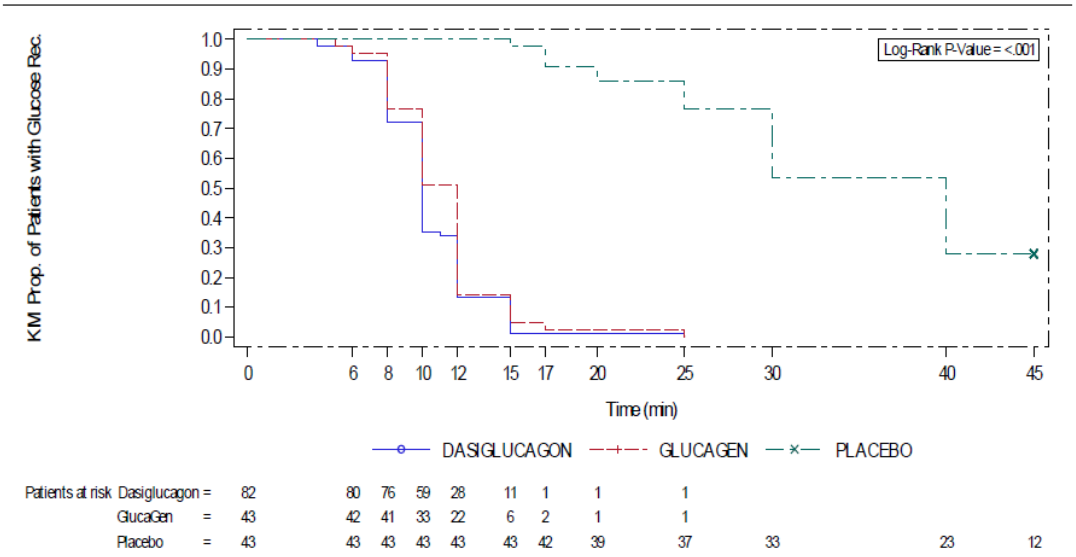


Figure 11-2: Kaplan Meier Estimates of Time to Plasma Glucose Recovery

Source: Post-test Figure 14.2.4.1
Prop. = proportion. Rec = Recovery. P-value based on treatment group difference between dasiglucagon and placebo using a two-sided log-rank test stratified by injection site.

Key Secondary Endpoint: the percentages of patients in the FAS who had plasma glucose recovery within 30, 20, 15 and 10 minutes were significantly higher in the dasiglucagon group than in the placebo group (p<0.001 at each time point; next table).

Table 11-2: Time to Plasma Glucose Recovery Within Defined Time Points (Full Analysis Set)

Plasma Glucose Recovery (min)[1]	Dasiglucagon (N=82)	Placebo (N=43)	p-value[2]	GlucaGen® (N=43)	p-value[3]
PG Recovery w/in 30 minutes, n (%)[4]	82 (100.0)	20 (46.5)	<0.001	43 (100.0)	<0.001
PG Recovery w/in 20 minutes, n (%)[4]	81 (98.8)	6 (14.0)	<0.001	42 (97.7)	<0.001
PG Recovery w/in 15 minutes, n (%)[4]	81 (98.8)	1 (2.3)	<0.001	41 (95.3)	<0.001
PG Recovery w/in 10 minutes, n (%)[4]	53 (64.6)	0	<0.001	21 (48.8)	<0.001

Source: Post-text Table 14.2.7.1
Abbreviations: IV = intravenous; PG = plasma glucose
[1] Events are defined as first increase in plasma glucose of ≥20 mg/dL (1.1 mmol/L) from baseline within 45 minutes during the hypoglycemic clamp procedure without administration of rescue IV glucose.
[2] Pairwise test of independent binomial proportions with Fisher's Exact test comparing Dasiglucagon versus Placebo.
[3] Pairwise test of independent binomial proportions with Fisher's Exact test comparing GlucaGen® versus Placebo.
[4] Plasma glucose recovery will follow an a priori defined hierarchical inferential test order, proceeding until the first failure to reject the null hypothesis comparing Dasiglucagon versus Placebo.

Key Secondary Endpoint: Plasma Glucose Changes from Baseline at 30, 20, 15 and 10 minutes were significantly larger in the dasiglucagon group than in the placebo group (p<0.001 at each time point).

Exploratory endpoint: for the endpoint of plasma glucose concentration ≥70 mg/dL or an increase of ≥20 mg/dL within 30 minutes, the endpoint was achieved by all patients in the dasiglucagon and GlucaGen groups versus 67.4% in the placebo group (p<0.001 and p=0.001, respectively).

Trial 17145 Results

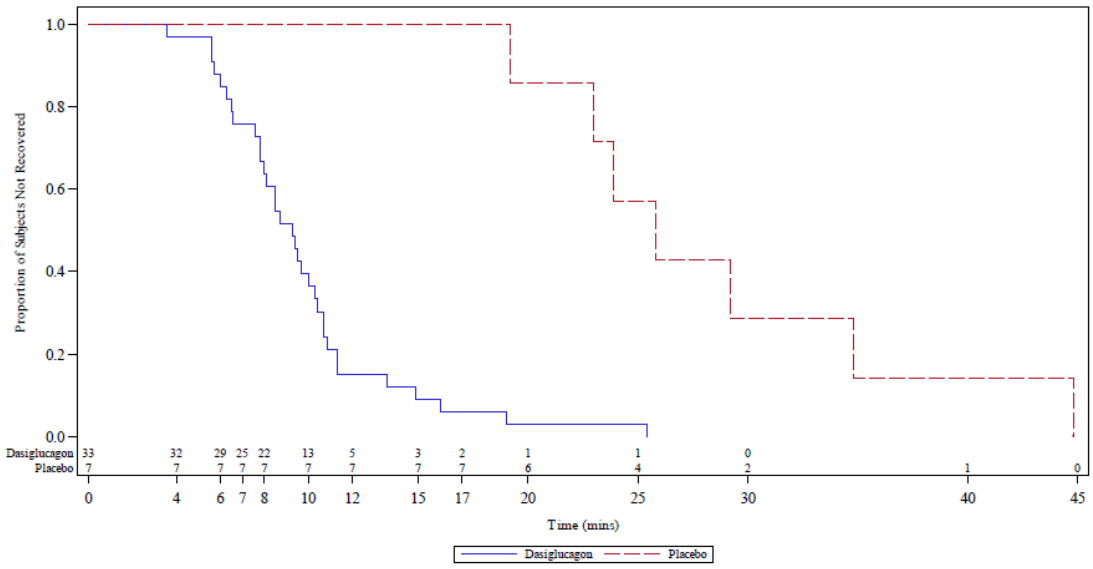
Primary efficacy endpoint

The primary efficacy endpoint (time to plasma glucose recovery, defined as first increase in plasma glucose of ≥20 mg/dL from baseline without administration of rescue IV glucose) showed a median (95% CI) plasma glucose recovery time of 10 (8.0, 12.0) min for subjects who received dasiglucagon and 35 (20.0, NC) min for subjects who received placebo. There was a significant treatment group difference between dasiglucagon and placebo (pairwise two-sided log-rank test: p<0.0001; Wilcoxon test stratified by injection sites: p<0.0001).

One (n=1) subject who received dasiglucagon was rescued within 30 minutes after drug injection (three subjects in Placebo did not recover). However, at a subsequent evaluation, the blood glucose level after dasiglucagon administration increased, from baseline, of 20 mg/dL at 10 minutes. Nevertheless, as rescue treatment and plasma glucose sampling at 10 minutes were performed simultaneously, this subject was disqualified from having achieved plasma glucose recovery.

The following figure shows the KM figures for the primary endpoint.

Figure 11-2 Kaplan-Meier Plot of Time to Plasma Glucose Recovery- Estimated Actual Time: FAS Population (N=44)



Source data: Figure 14.2.1.8

Key Secondary endpoint: within 30, 20, 15, and 10 minutes after IMP injection, 33 (97.1%), 32 (94.1%), 30 (88.2%) and 21 (61.8%) subjects who received dasiglucagon had recovered, and 5 (50%), 1 (10%), 0 (0%) and 0 (0%) subjects who received placebo had recovered. There was a significant treatment group difference between dasiglucagon and placebo (all p≤0.0012) (see next table).

Table 11-3 Plasma Glucose Recovery After Drug Injection Without Rescue IV Glucose: FAS Population (N=44)

Subject Status, n (%)	Treatment	
	0.6 mg Dasiglucagon (N=34)	Placebo (N=10)
Within 30 mins		
Subjects Recovered	33 (97.1)	5 (50.0)

Subject Status, n (%)	Treatment	
	0.6 mg Dasiglucagon (N=34)	Placebo (N=10)
Subjects not Recovered	1 (2.9)	5 (50.0)
p-value[1]	0.0012	--
Within 20 mins		
Subjects Recovered	32 (94.1)	1 (10.0)
Subjects not Recovered	2 (5.9)	9 (90.0)
p-value[1]	<.0001	--
Within 15 mins		
Subjects Recovered	30 (88.2)	0 (0.0)
Subjects not Recovered	4 (11.8)	10 (100.0)
p-value[1]	<.0001	--
Within 10 mins		
Subjects Recovered	21 (61.8)	0 (0.0)
Subjects not Recovered	13 (38.2)	10 (100.0)
p-value[1]	0.0006	--

Source data: Modified from Table 14.2.2.1
- Subjects who were rescued were considered as failed to recover. Subject [redacted] in dasiglucagon treatment group was rescued.
[1] P-value from Fisher's exact test.

The median (95% CI) time to first plasma glucose concentration ≥70 mg/dL (3.9 mmol/L) from baseline was 9.0 (8.0, 10.0) minutes for subjects who received dasiglucagon and 27.5 (12.0, 40.0) minutes for subjects who received placebo (p<0.0001).

Thirty-three (33) (97.1%) subjects who received dasiglucagon and 6 (60%) subjects who received placebo had plasma glucose ≥70 mg/dL (3.9 mmol/L) or increase of ≥20 mg/dL (1.1 mmol/L) within 30 minutes after trial drug injection without rescue.

At all timepoints, the plasma glucose change from baseline was statistically significantly greater for dasiglucagon relative to placebo (p<0.0001 for all pair-wise comparisons).

Trial 17086

Primary endpoint

The time to plasma glucose recovery is presented in the table below. All patients in dasiglucagon or GlucaGen groups recovered within 45 minutes of dosing without receiving rescue glucose. Four patients (36.4%) in the placebo group were censored (they failed to recover within 45 minutes of dosing).

Superiority with respect to the primary endpoint of time to plasma glucose recovery was shown for dasiglucagon relative to placebo, with a median time to plasma glucose recovery of 10 minutes (95% CI, 8 to 12) versus 30 minutes (95% CI, 20 to [upper limit not estimable]; p<0.001 for the primary

analysis). No difference between dasiglucagon and GlucaGen groups was evident for time to plasma glucose recovery.

Table 11.1: Time to plasma glucose recovery, summary (full analysis set)

	Dasiglucagon (N=20)	Placebo (N=11)	GlucaGen® (N=10)	p-value
Status				
Recovered[1] (%)	20 (100.0)	7 (63.6)	10 (100.0)	
Censored[2] (%)	0	4 (36.4)	0	
Time to recovery (minutes)				
25th percentile	8.00	30.00	10.00	
Median (95% CI)	10.00 (8.00-12.00)	30.00 (20.00-)[4]	10.00 (8.00-12.00)	
75th percentile	12.00		12.00	
Dasiglucagon versus placebo				
P-value (log-rank test) - stratified[3]				<0.001
P-value (log-rank test) - non-stratified				<0.001
P-value (Wilcoxon test) - stratified[3]				<0.001
Dasiglucagon versus GlucaGen				
P-value (log-rank test) - stratified[3]				0.430
P-value (log-rank test) - non-stratified				0.787

Abbreviation: IV=intravenous

[1] Increase of plasma glucose of 20 mg/dL within 45 minutes without IV glucose

[2] Did not recover within 45 minutes or received IV glucose before 45 minutes (all censored at 45 minutes)

[3] Stratified by age group and injection site

[4] Upper limit not estimable

Source: Table 14.2.1.1

A KM plot showing the estimated probability of not having recovered with each treatment, based on the FAS, is shown in the next figure.

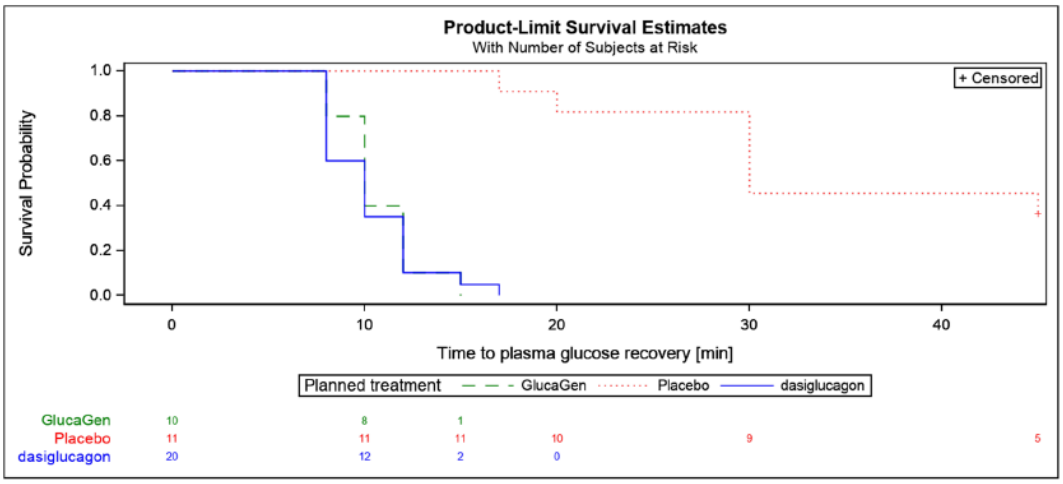


Figure 11.1: Time to plasma glucose recovery, Kaplan-Meier plot (full analysis set)

Abbreviation: min=minutes

Source: Table 14.2.1.2

The per-protocol analysis showed similar results.

Results for the primary endpoint of time to plasma glucose recovery are presented by age group (6 to 11 years and 12 to 17 years) in the next table and they suggest efficacy with respect to the primary endpoint is maintained for dasiglucagon relative to placebo across age groups.

Table 11.4: Time to plasma glucose recovery by age group, summary (full analysis set)

Age group	Dasiglucagon (N=20)	Placebo (N=11)	GlucaGen® (N=10)	p-value
6-11 years				
Recovered[1] (%)	8 (100.0)	3 (75.0)	4 (100.0)	
Censored[2] (%)	0	1 (25.0)	0	
12-17 years				
Recovered[1] (%)	12 (100.0)	4 (57.1)	6 (100.0)	
Censored[2] (%)	0	3 (42.9%)	0	
Time to recovery (minutes)				
6-11 years				
Median (95% CI)	9.00 (8.00-12.00)	25.00 (17.00-)[4]	10.00 (8.00-10.00)	
12-17 years				
Median (95% CI)	10.00 (8.00-12.00)	45.00 (30.00-)[4]	12.00 (8.00-15.00)	
Dasiglucagon versus placebo				
P-value (log-rank test) - stratified[3]				<0.001
P-value (Wilcoxon test) - stratified[3]				<0.001

[1] Increase of plasma glucose of 20 mg/dL within 45 minutes without IV glucose

[2] Did not recover within 45 minutes or received IV glucose before 45 minutes (all censored at 45 minutes)

[3] Stratified by age group

[4] Upper limit not estimable

Source: Table 14.2.1.12

Secondary endpoint: Plasma glucose recovery within 30, 20, 15, and 10 minutes after IMP injection was calculated for the FAS. Summary statistics are presented in the next table. For each time point, the proportion of patients who had recovered was substantially greater in the dasiglucagon group than in the placebo group, and the difference between the dasiglucagon and placebo groups in plasma glucose recovery was statistically significant for all time points. On the contrary, results were similar between dasiglucagon and GlucaGen groups.

Table 11.7: Plasma glucose recovery within 30, 20, 15, and 10 minutes after investigational medicinal product injection (full analysis set)

Plasma glucose recovery within		Dasiglucagon (N=20)	Placebo (N=11)	GlucaGen® (N=10)	p-value[1] dasiglucagon versus placebo
10 minutes post-dose	no (%)	7 (35.0)	11 (100.0)	4 (40.0)	0.0005
	yes (%)	**13 (65.0)**	**0**	**6 (60.0)**	
	95% CIs[2]	44.1-85.9		29.6-90.4	
15 minutes post-dose	no (%)	1 (5.0)	11 (100.0)	0	<.0001
	yes (%)	**19 (95.0)**	**0**	**10 (100.0)**	
	95% CIs[2]	85.4-100.0		100.0-100.0	
20 minutes post-dose	no (%)	0	9 (81.8)	0	<.0001
	yes (%)	**20 (100.0)**	**2 (18.2)**	**10 (100.0)**	
	95% CIs[2]	100.0-100.0	0.0; 41.0	100.0-100.0	
30 minutes post-dose	no (%)	0	5 (45.5)	0	0.0073
	yes (%)	**20 (100.0)**	**6 (54.5)**	**10 (100.0)**	
	95% CIs[2]	100.0-100.0	25.1; 84.0	100.0-100.0	

[1] p-values calculated using a Cochran-Mantel-Haenszel test stratified by age group and injection site

[2] CIs for response rate (category 'yes')

Source: Table 14.2.2.1

The changes from baseline in plasma glucose at each time point (as above), was greater in the dasiglucagon group than in the placebo group, and the difference in the mean change from baseline in plasma glucose between the dasiglucagon and placebo groups was statistically significant. Furthermore, at each time point, the mean increase from baseline in plasma glucose was similar for dasiglucagon and GlucaGen (table below).

Table 11.8: Plasma glucose changes from baseline at 30, 20, 15, and 10 minutes after investigational medicinal product injection (full analysis set)

Change from baseline (mg/dL)[1]		Dasiglucagon (N=20)	Placebo (N=11)	GlucaGen® (N=10)	p-value[2] LS-mean (95% CI) dasiglucagon versus placebo
10 minutes post-dose	n	20	10	10	
	Mean (±SD)	27.225 (13.6768)	-3.405 (8.0276)	20.919 (6.7227)	
	Median	25.495	-3.423	20.991	
	Min-max	-3.06-61.98	-16.04-10.99	6.85-29.01	<.0001
	LS-mean	27.239	-3.378	20.431	30.617
	95% CI (LS-mean)	22.792-31.686	-9.751-2.996	14.159-26.703	21.995-39.239
15 minutes post-dose	n	20	11	10	
	Mean (±SD)	45.342 (15.0860)	0.835 (11.1276)	40.631 (9.7317)	
	Median	46.577	0.000	40.090	
	Min-max	17.12-76.04	-14.95-17.12	28.11-54.05	<.0001
	LS-mean	45.437	1.166	40.283	44.272
	95% CI (LS-mean)	39.842-51.033	-6.371-8.702	32.390-48.176	33.773-54.770
20 minutes post-dose	n	20	11	10	
	Mean (±SD)	65.369 (15.2461)	7.322 (13.3543)	58.000 (10.5297)	
	Median	60.631	5.946	59.099	
	Min-max	36.04-90.27	-14.05-27.03	41.08-73.15	<.0001
	LS-mean	65.351	7.511	57.509	57.839
	95% CI (LS-mean)	59.375-71.327	-0.538-15.560	49.080-65.938	46.628-69.051
30 minutes post-dose	n	20	11	10	
	Mean (±SD)	98.459 (19.6527)	17.510 (15.6313)	85.225 (12.5052)	
	Median	101.622	21.081	88.649	
	Min-max	58.02-130.27	-14.05-36.94	65.23-104.14	<.0001
	LS-mean	98.150	17.303	84.442	80.847
	95% CI (LS-mean)	90.398-105.902	6.862-27.745	73.507-95.377	66.302-95.391

Abbreviations: LS-mean=least square mean; Max=maximum; Min=minimum

[1] If rescue IV glucose was administered before 10, 15, 20, or 30 minutes, respectively, the patient's plasma glucose result was determined from the latest central plasma glucose value prior to rescue IV glucose administration

[2] p-values were calculated using an analysis of variance with factors treatment, age group and injection site

Source: Table 14.2.2.3

The mean glucose concentrations versus time are shown in the next figure.

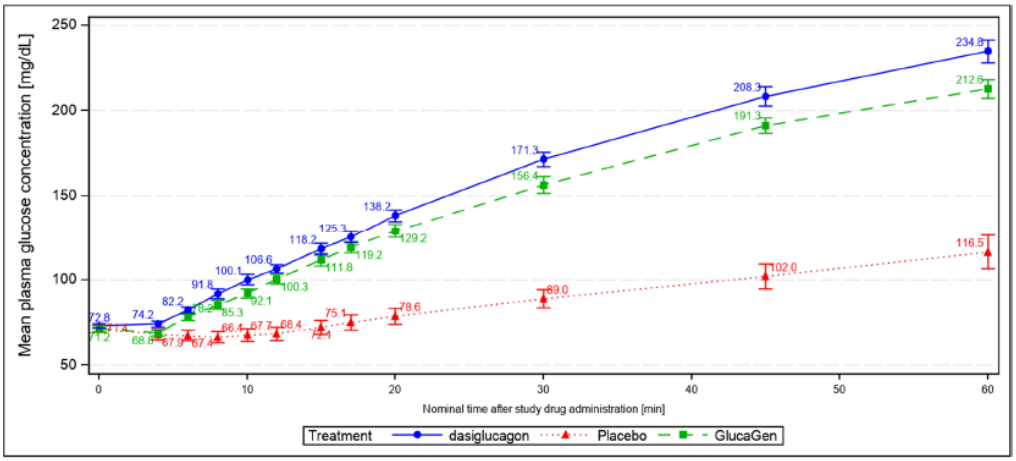


Figure 11.7: Mean glucose concentrations versus time - original scale (full analysis set)

Abbreviation: min=minutes

Source: Table 14.4.2.2

Ancillary analyses

Post-hoc analyses comparing dasiglucagon vs GlucaGen (trial 16137)

Plasma AUC0-90min was similar in the dasiglucagon group and GlucaGen group, with an LSM ratio of 0.910 (95% CI: 0.801, 1.033; p=0.144) for glucagon:dasiglucagon. In a post hoc analysis, plasma AUC0-120min was statistically significantly higher in the dasiglucagon group than in the GlucaGen group, with an LSM ratio of 0.844 (95% CI: 0.749, 0.951; p= 0.006) for glucagon:dasiglucagon.

Cmax was significantly lower in the dasiglucagon group than in the GlucaGen group, with an LSM ratio of 1.158 (95% CI: 1.010, 1.328; p=0.036) for glucagon:dasiglucagon.

The maximum plasma concentration was reached around 40 minutes after injection in the dasiglucagon group and around 20 minutes after the injection in the GlucaGen group. The Tmax LSM ratio for glucagon:dasiglucagon was 0.700 (95% CI: 0.663, 0.739; <0.001).

Non-inferiority of dasiglucagon vs GlucaGen for the primary endpoint

For both pivotal trials with an active comparator (trial 16137 and 17086), post hoc analyses showed dasiglucagon to be non-inferior to GlucaGen with respect to the primary endpoint; in both trials, the upper 95% confidence limit was well below a 3-minute margin (see table below).

Table 4-5 Non-inferiority of dasiglucagon vs GlucaGen for the primary endpoint - FAS

Trial	Treatment difference (min) (Dasiglucagon – GlucaGen)	95% CI (two-sided)
16137	-0.68	-1.83 to 0.48 min
17086	-0.40	-2.32 to 1.52 min

For further details, see M 2.7.3, Section 3.2.1.1 and 3.2.1.2.

Summary of main efficacy results

The following table summarises the efficacy results from the main studies supporting the present application. These summaries should be read in conjunction with the discussion on clinical efficacy as well as the benefit risk assessment (see related sections).

Table 4-6 Primary and key secondary endpoint results – pivotal efficacy trials – full analysis set

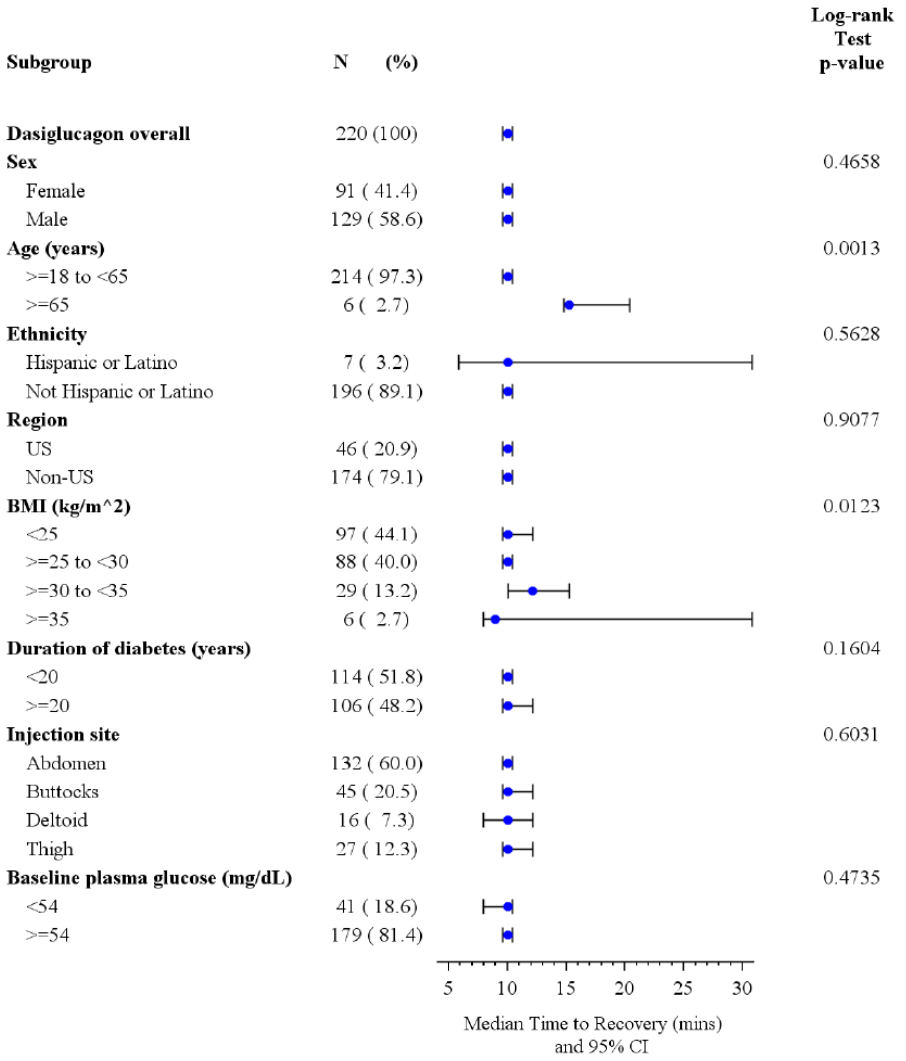
	1st pivotal trial 16137			2nd pivotal trial 17145		Pediatric pivotal trial 17086		
	Dasiglucagon N=82	Placebo N=43	GlucaGen N=43	Dasiglucagon N=34	Placebo N=10	Dasiglucagon N=20	Placebo N=11	GlucaGen N=10
Primary endpoint								
Time to recovery[1] (minutes); median (95% CI)	10 (NE)	40 (30; 40)	12 (10;12)	10 (8; 12)	35 (20, NE)	10 (8; 12)	30 (20; NE)	10 (8; 12)
	p<0.0001[2]			p<0.0001[2]		p<0.001[2]		
Key secondary endpoints								
Proportion of patients achieving plasma glucose recovery without administration of rescue IV glucose								
- within 30 minutes	100%	47%	100%	97%	50%	100%	55%	100%
- within 20 minutes	99%	14%	98%	94%	10%	100%	18%	100%
- within 15 minutes	99%	2%	95%	88%	0%	95%	0%	100%
- within 10 minutes	65%	0%	49%	59%	0%	65%	0%	60%
	p<0.0001 for all tests[2]			P<0.005 for all tests[2]		p<0.01 for all tests[2]		
Mean[3] plasma glucose change from baseline at predefined time points after trial drug injection or at the time of rescue (mg/dL)								
- at 30 minutes	91.1	19.1	88.3	85.4	15.55	98.15	17.3	84.4
- at 20 minutes	59.8	8.7	58.3	53.0	10.0	65.4	7.5	57.5
- at 15 minutes	43.6	5.1	44.0	41.7	5.3	45.4	1.2	40.3
- at 10 minutes	24.0	-0.1	21.8	24.55	0.7	27.2	-3.4	20.4
	p<0.0001 for all tests[2]			p<0.0001 for all tests[2]		p<0.0001 for all tests[2]		

Abbreviations: NE: not estimable. [1] defined as first increase in plasma glucose of ≥ 20 mg/dL (1.1 mmol/L) from baseline without administration of rescue IV glucose (censoring at 45 min); [2] test relative to placebo. [3] least square mean. M 2.7.3, Tables 3.5, 3.6, 3.7, 3.8, 3.9 and 3.10

2.5.5.2. Analysis performed across trials (pooled analyses and meta-analysis)

An exploratory pooled subgroup analysis (comprising the pivotal trials 16137 and 17145, dose-finding trial 15126 and bridging trial 17084) was conducted for the adult patient population to increase sensitivity to detect any subgroup effects. The effect of intrinsic and extrinsic factors on efficacy was investigated for the primary endpoint of time to plasma glucose recovery, with plasma glucose recovery defined as first increase in plasma glucose of ≥20 mg/dL (1.1 mmol/L) from baseline without administration of rescue IV glucose.

A forest plot for the pooled, dasiglucagon-treated population and by subgroups is shown for the primary endpoint of time to plasma glucose recovery in the next figure.

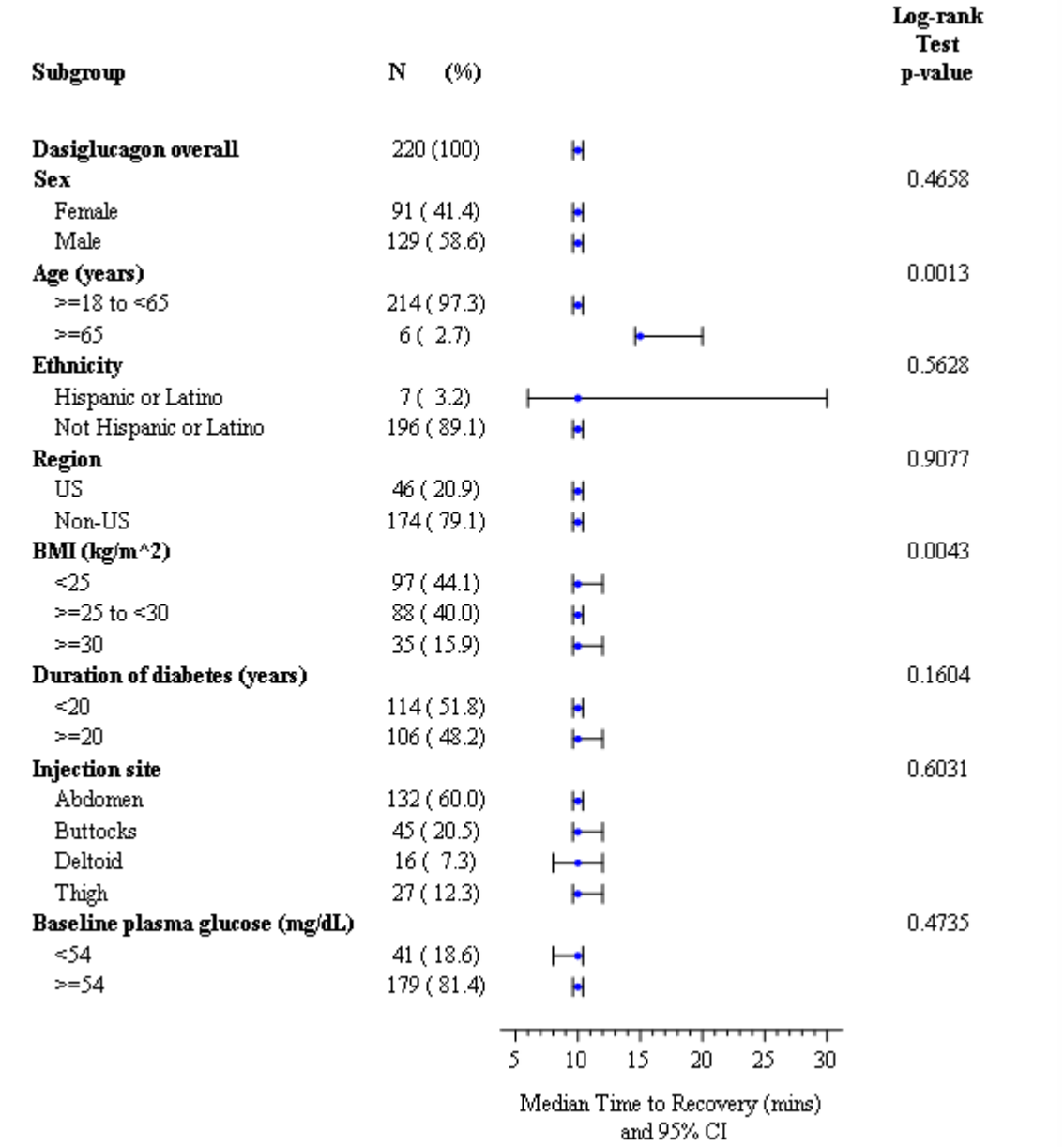


Abbreviations: BMI: body mass index. **Notes**: p-values pertain to log-rank test of equality over strata. Subgroup categories containing <5 subjects are excluded. Non-US: Europe; US: non-Europe; the distribution of doses by injection site is shown for all treatment groups in Appendix 6.2, Table 1.4.
Cross-reference: Appendix 6.3, Figure 2.31 (in Figure 2.3.1, "Non-Europe" is presented as "US", and "Europe" as "non-US")

Figure 3-6 Forest plot of median time to plasma glucose recovery with dasiglucagon by subgroup – adult efficacy pool - full analysis set

The figure below shows the same plot as above, but two BMI categories have been added to give the subgroup BMI >30 kg/m^2.

Figure 21 Forest plot of median time to plasma glucose recovery by subgroup - efficacy pool - full analysis set



Abbreviations: BMI: body mass index.
Notes: p-values pertain to log-rank test of equality over strata. Subgroup categories containing <5 subjects are excluded. Non-US: Europe; US: non-Europe; the distribution of doses by injection site is shown for all treatment groups in M2.7.3, Appendix 6.2, Table 1.4.
Cross-reference: Appendix 2, Figure 1.0.1

2.5.5.3. Supportive study(ies)

Trial 17084
A phase 3b, randomized, double-blind, crossover trial to compare the efficacy and safety of two different batches of subcutaneous dasiglucagon in patients with type 1 diabetes mellitus (see figure below for the design).

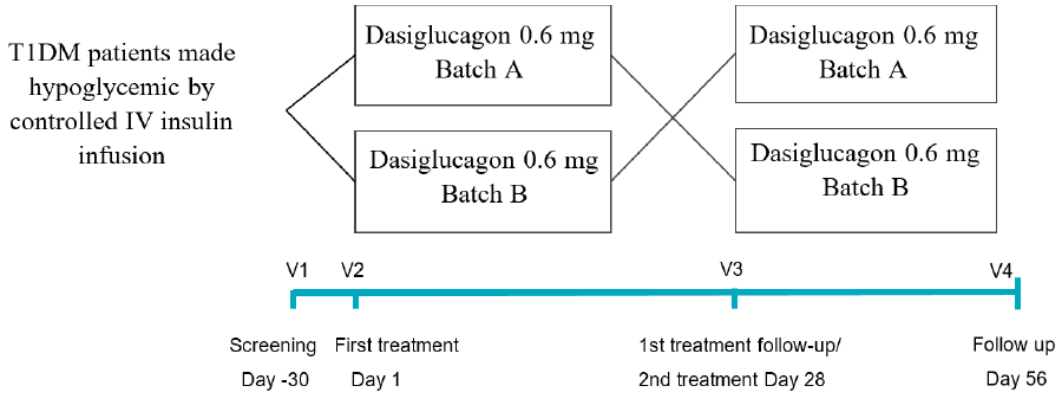


Figure 9.1 Schematic representation of the trial design
Abbreviations: IV=intravenous; T1DM=type 1 diabetes mellitus; V=visit
Source: Trial protocol final version 3, dated 05 February 2019 (Appendix 16.1.1)

Dasiglucagon is intended to be physically and chemically stable in a ready-to-use aqueous formulation for the acute treatment of severe hypoglycemia in patients with diabetes mellitus. For a product intended for a rescue indication, a room temperature shelf life of a minimum of 6 months would increase the usability, and thereby potentially public health benefits. Therefore, the Applicant compared the performance of a batch kept at the dual storage intended conditions which includes a period at room temperature (Batch B) versus a batch kept refrigerated (Batch A).

The primary objective was to show non-inferiority of the efficacy of a single SC dose of dasiglucagon Batch B relative to that of dasiglucagon Batch A for treatment of hypoglycemia in patients with T1DM. The secondary objective was to evaluate the safety, immunogenicity, and pharmacokinetics (PK) of the 2 different batches of dasiglucagon following a single SC dose administered to patients with T1DM with insulin-induced hypoglycemia.

In order to avoid bias in patient selection and in the evaluation of clinical assessments, patients were randomly assigned 1:1 to either dasiglucagon Batch A or dasiglucagon Batch B as their initial dose and the other as the second dose. To avoid bias in the evaluation of clinical assessments, the trial was conducted in a double-blinded manner.

The trial recruited adult male and female patients with T1DM for at least 1 year, hemoglobin A1c level of <10% at screening, and stable antidiabetic treatment for at least 30 days before screening.

Number of patients: Planned: 88; Screened: 109; Randomized: 92; Completed the trial: 83; Analyzed (full analysis set): 90.

Primary endpoint: Time to plasma glucose recovery. Plasma glucose recovery is defined as the first increase in plasma glucose of ≥20 mg/dL (1.1 mmol/L) from baseline without administration of rescue (IV) glucose.

Main Secondary endpoint: Plasma glucose change from baseline at 30, 20, 15, and 10 minutes after investigational medicinal product (IMP) injection or at the time of rescue.

Treatments

- dasiglucagon Batch A: 0.6 mg; aqueous formulation, 1 mg/mL in pre-filled syringes containing 0.6 mL. This batch had been stored at refrigerated conditions.

- dasiglucagon Batch B: 0.6 mg; aqueous formulation, 1 mg/mL in pre-filled syringes containing 0.6 mL. This batch had been stored at the intended dual storage conditions.

Results

Primary endpoint

Time to plasma glucose recovery: the difference of Batch B versus Batch A was 0.40 minutes (95% CI: -0.08 to 0.88), equivalent to 24 seconds (95% CI: -5 to 53). The non-inferiority margin was 2 minutes and the upper limit of the confidence interval for the difference was well below 2 minutes. Thus, non-inferiority was shown for the primary analysis. No statistically significant period effect or sequence (carry-over) effect was found (see tables below).

Table 11.1: Time to plasma glucose recovery — interpolated (per protocol set)

Time to plasma glucose recovery (minutes) - interpolated	Batch A (N=82)	Batch B (N=82)
n	82	82
Mean (±SD)	9.2 (±2.34)	9.6 (±2.89)
Median	9.0	9.0
Min-max	5-15	5-18

Abbreviations: max=maximum; min=minimum

Source: Table 14.2.1.1

Table 11.2: Time to plasma glucose recovery — inferential statistics (per protocol set)

Planned Treatment/ Comparison	N	LS-adjusted mean	95% CI (2-sided)	Non-inferiority margin	Decision on non-inferiority	p-value (2-sided)
Batch A	82	9.211	8.871; 9.551			
Batch B	82	9.614	9.274; 9.954			
Difference (Batch B versus Batch A)		0.403	-0.078; 0.884	2 minutes	Non-inferiority met	
Period effect						0.6185
Sequence (carry-over) effect						0.2431

Abbreviations: LS=least-square; max=maximum; min=minimum

Note: For those patients who received rescue intravenous glucose prior to recovery or who did not reach recovery, time to plasma glucose recovery was set to missing. Derived from an analysis of variance model with the factors sequence, visit, treatment, and patients nested within the sequence.

Source: Table 14.2.1.2

Other *secondary endpoints* were in line with the primary one. In particular, the differences of the least-square means between the change in plasma glucose from baseline for all measured time points of Batch B versus Batch A were between -1.0 and 1.1 mg/dL, with associated 95% Cis: -3.9 and 4.9 mg/dL.

2.5.6. Discussion on clinical efficacy

Dasiglucagon is intended for "the treatment of severe hypoglycaemia in adults, adolescents, and children aged 6 years and over with diabetes mellitus".

Severe hypoglycemia (also known as Level 3 hypoglycemia) is defined as a hypoglycemic episode that requires the assistance of another person to actively administer carbohydrate, glucagon, or to perform

other resuscitative actions. Therefore, dasiglucagon will be administered mostly by caregivers that are not healthcare professionals. This was the rationale that guided the development of this formulation of glucagon that is ready to use, with no reconstitution phase needed.

The primary efficacy evaluation is based upon three pivotal, placebo-controlled trials, two in adults (trials 16137 and 17145) and one in children (17086).

Design and conduct of clinical studies

All pivotal trials were placebo-controlled; one of the adult trials (16137) and the paediatric trial (17086) also included an arm with an already approved active comparator, GlucaGen (a glucagon formulation); no formal pre-specified statistical analysis was performed against the active comparator; however, a post-hoc non-inferiority analysis was provided. The placebo control allows measuring the absolute effect of dasiglucagon, while the two arms with the active comparator allow a comparison with a glucagon formulation already available.

Regarding the main inclusion/exclusion criteria, all patients enrolled were affected by T1DM to avoid a possible confounding effect of residual endogenous insulin in T2DM. T2DM patients are older, frail and they usually concomitantly take several other therapies; therefore, enrolling at least a small number of T2DM patients would have been informative to confirm the efficacy in this sub-population that is included in the intended indication. However, the pathophysiology of hypoglycaemia in both forms of diabetes is the same (indeed, the relevant guidelines do not make differences between type 1 and type 2 DM), therefore a similar response might be expected. It is not clear to what extent the exclusions of some patients (for instance those with malignancy and with heart failure) could have limited the generalizability of the trial results (since elder patients often are affected by these disorders).

Patients were enrolled within an age range of 18-75 years in the two adult trials; however very few patients were >65 years old (and no one was >74 years), and this information is reflected in the SmPC. The age range for enrolment in the paediatric pivotal trial 17086 was ≥6 - <18.0 years (but the youngest child enrolled was 7-years old). Hemoglobin A1C was required to be <10% in the adult trials, whereas in the paediatric pivotal trial 17086 a body weight ≥20 kg was required. Patients with known insulinoma were excluded due to the risk of stimulating an exaggerated insulin release from the insulinoma, with subsequent hypoglycaemia.

Dasiglucagon was administered via subcutaneous (SC) injection at a fixed dose of 0.6 mg (for both adult and paediatric patients), while the active comparator GlucaGen (when provided) was administered at the dose of 1.0 mg. Dasiglucagon and placebo were administered via a pre-filled syringe in all phase 3 trials, except for the 2nd adult trial 17145 for which administration was via auto-injector (in which the pre-filled syringe was mounted). In trials including active comparator (GlucaGen), this was administered using the injection kit provided with the product.

The trials were performed with the hypoglycemic clamp (insulin infusion) procedure, a reproducible technique which induces a hypoglycemic condition in a controlled manner. This also means that dasiglucagon was not tested in a "real-world" setting in any of the pivotal trials, nor was any data submitted about the real-world use. From one side this can raise concern regarding the real-world use of dasiglucagon (even more considering that it will be used by caregivers who are not healthcare professionals), but from the other side the clamp technique allows an objective and precise evaluation of the pharmacodynamic and efficacy properties of dasiglucagon. Therefore, the procedure used is considered appropriate to evaluate the efficacy of dasiglucagon. However, supportive evidence from a "real-world" setting would have been very useful to support the effectiveness of dasiglucagon in the target population.

It is important to note that for safety reasons, dasiglucagon was not administered if the plasma glucose was <45 mg/dL. Therefore, the effect of dasiglucagon on more severe hypoglycaemia has not been studied.

The primary objectives of the 3 pivotal trials were basically identical and aimed to demonstrate superiority of dasiglucagon relative to placebo following a single SC 0.6 mg dose administered to T1DM patients with insulin-induced hypoglycemia. The secondary objectives provided important complementary information, such as a comparison against the active comparator. The objectives were overall in line with the general aim of assessing the efficacy of dasiglucagon.

For all the three pivotal trials, the primary endpoint was time to plasma glucose recovery, with "plasma glucose recovery" defined as first increase in plasma glucose of ≥20 mg/dL (1.1 mmol/L) from baseline without administration of rescue intravenous (IV) glucose. The primary endpoint is deemed appropriate to answer the primary objective of the studies. The secondary endpoints allow gathering more information on the proportion of subjects reaching plasma glucose recovery at several time intervals. The exploratory endpoint of the adult trials "plasma glucose concentration ≥70 mg/dL or increase of ≥20 mg/dL within 30 minutes after investigational product injection without administration of rescue IV glucose" is considered important since it could allow an indirect comparison given that it has been used as primary endpoints in other similar products containing glucagon approved in EU. The time to plasma recovery was measured "from injection", that is the appropriate way to assess objectively the efficacy of dasiglucagon. However, given the route of administration and the intended use (by caregivers), complementary data about the efficacy using "time from decision" would have allowed evaluating the probable real-life time required for patient recovery.

Statistical methods were well reported and can be considered appropriate for all the three pivotal trials, as well as sample size calculations, randomization and blinding/unblinding procedures. The overall type I error over the primary endpoints and the key secondary endpoint was controlled at 5% (two-sided). No deviations from the statistical analysis plan seems to have occurred.

There were some protocol deviations with few study subjects, mainly regarding glucose measuring out of the planned time window by few minutes; from the information provided it seems unlikely that such deviations have had a significant impact on the interpretation of results.

Efficacy data and additional analyses

The baseline characteristics were overall well balanced among the arms of the pivotal trials. In adult trial 16137 the time from diabetes diagnosis was higher in the dasiglucagon compared to the other two arms. Some small unbalances are seen in the use of other medicines in the adult trial 17145; the number of trial subjects is small, and therefore it is difficult to draw meaningful conclusions. For the paediatric trial (17086), the lowest age of enrolled patients was 7 years, whereas the sought indication is from 6 years above (see PK section for extrapolation data).

The number of subjects analysed can be considered acceptable, since a strong difference in the pharmacodynamic endpoint is expected compared to placebo if dasiglucagon works.

Results

Trial 16137 (adult)

The primary endpoint of plasma glucose recovery (first increase in plasma glucose of ≥20 mg/dL from baseline without administration of rescue IV glucose) showed a median time to plasma glucose recovery within 45 minutes statistically significantly shorter in the dasiglucagon group (10 min) than in the placebo group (40 min; $p<0.001$) and numerically slightly shorter than the GlucaGen group (12 min, although there was no formal statistical comparison planned between dasiglucagon and GlucaGen). The Kaplan-Meyer curves for time to plasma glucose recovery showed a similar profile between dasiglucagon and GlucaGen.

Regarding the secondary endpoints, the percentages of patients with plasma glucose recovery within 30, 20, 15 and 10 minutes were significantly higher in the dasiglucagon group than in the placebo group and were similar to GlucaGen. A numerically higher proportion of subjects experienced glucose recovery within 10 min with dasiglucagon compared to GlucaGen (64.6 vs 48.8%). All key secondary endpoints were met.

The exploratory endpoint of plasma glucose concentration ≥70 mg/dL or an increase of ≥20 mg/dL within 30 minutes, was achieved by all patients in the dasiglucagon and GlucaGen groups versus 67.4% in the placebo group ($p<0.001$).

Trial 17145 (adult)

The primary endpoint showed a median (95% CI) plasma glucose recovery time of 10 min (8.0, 12.0) for subjects who received dasiglucagon and 35 min (20.0, NC) for subjects who received placebo ($p<0.0001$).

The key secondary endpoints showed that at any time interval, more subjects in dasiglucagon recovered compared to placebo: within 30, 20, 15, and 10 minutes after drug injection, respectively 33 (97.1%), 32 (94.1%), 30 (88.2%) and 21 (61.8%) subjects who received dasiglucagon had recovered, vs 5 (50%), 1 (10%), 0 (0%) and 0 (0%) subjects who received placebo (all $p\leq0.0012$). Other secondary/exploratory endpoints yielded consistent results, and all key secondary endpoints were met.

Trial 17086 (pediatric)

The primary endpoint in the paediatric trial (time to plasma glucose recovery after dasiglucagon administration) showed the superiority of dasiglucagon relative to placebo, with a median time to plasma glucose recovery of 10 minutes versus 30 minutes, respectively ($p<0.001$). The performance of dasiglucagon was numerically identical to that of GlucaGen. There seemed to be no difference between the two age groups analysed (6-11 and 12-17 years).

Additional analyses and considerations

In the trials "time from injection" was used for the endpoints (even when GlucaGen, that needs reconstitution, was used as active comparator) and no data are available regarding recovery time measured from “time from decision”. Since a similar recovery time (from injection) was observed between dasiglucagon and GlucaGen, and since Glucagen requires extra steps for reconstitution prior to injection, it is likely that dasiglucagon would yield at least a non-longer recovery time if measured “from decision”.

In other glucagon-based products, the SmPC contains instructions on the possibility to administer a second dose of the product in case the first one is not able to induce a recovery of the clinical conditions within 15 min. Even if this use has not been studied in dasiglucagon clinical trials, considering the seriousness of the underlying condition, the applicant has been asked to discuss this point. From PK/PD data, it seems that a second administration of dasiglucagon is expected to be safe. Thus, the possibility of a second administration of dasiglucagon has been added to the SmPC.

A post-hoc analysis showed the non-inferiority of dasiglucagon with respect to the active comparator GlucaGen for the primary endpoint (first increase in plasma glucose of ≥20 mg/dL from baseline) in both adults and children.

A pooled analysis comprising the adult pivotal trials 16137 and 17145, dose-finding trial 15126 and bridging trial 17084 for subgroups was performed; overall, there seems to be no major differences among the several subgroups analysed except for BMI and age; the median time to glucose recovery is consistently around 10 minutes. In the subgroup of patients with BMI between 30 and 35 kg/m^2 a longer recovery time was observed; however, there were only 29 patients in this subgroup and thus it is not easy to infer meaningful conclusions. In the subgroup of patients aged >65 years (n=6) a longer recovery time was observed as well; again, the very small number makes it difficult to draw any meaningful conclusions.

Efficacy when stored at room temperature

Since the product is intended for emergency use, it is probable that it will be kept at room temperature for prolonged periods of time. The Applicant thus tested the efficacy of dasiglucagon when stored according to a so-called "dual storage" method, versus the refrigerated storage, in the trial 17084. This was a double-blind crossover trial in which patients were randomized to receiving two doses of dasiglucagon, 28 days apart: one dose from a Batch A (kept refrigerated) and the other one from Batch B (stored at room temperature up to 25°C); the randomization was to the order of administration. The primary endpoint showed that the time to plasma glucose recovery was 9.2 min in Batch A vs 9.6 min in Batch B; the difference was 24 seconds that was within the pre-specified non-

inferiority margin of 2 minutes, showing that no clinically relevant difference was seen when the batch was stored according to the "dual storage" conditions versus the refrigerated method. For further details see the Quality section.

Device use

The two human factor studies showed some errors with the use of the syringe or the auto-injector (mostly with the former); however, the applicant provided further information from which the potential impact seems low (see the quality section).

2.5.7. Conclusions on the clinical efficacy

The efficacy of dasiglucagon for the treatment of severe hypoglycemia in adults and children >6 years with diabetes mellitus was established since dasiglucagon proved superior to placebo in the time required to increase plasma glucose during insulin-induced hypoglycemia (the median time to recovery was about 10 minutes vs 30-40 min in placebo). At several time points analysed, a greater proportion of patients experienced plasma glucose recovery with dasiglucagon compared to placebo. The data presented in this application showed overall similar numerical results between the performance of dasiglucagon and the active comparator GlucaGen (but no pre-specified analysis was performed), and non-inferiority with the latter was shown in a post-hoc analysis.

Some uncertainties about efficacy in the elder population are raised since very few patients >65 years were enrolled. No real-life studies were performed.

Even if not tested in clinical studies, a provision has been added to the SmPC allowing the administration of a second dose in case the first dose fails to obtain a recovery in 15 minutes.

2.5.8. Clinical safety

The safety evaluation is primarily based on safety data from the dasiglucagon clinical development program (9 trials) for the treatment of severe hypoglycemia in paediatric and adult patients with diabetes aged ≥6 years (see Table 1 1 for an overview of trial designs):

Table 1-1 Overview of Zealand Pharma-sponsored clinical trials of dasiglucagon for the treatment of severe hypoglycemia

Trial ID Phase Trial population	**Description**	**Design** (glycemic status at dosing)	**Dosing** (randomization)	**Number of subjects exposed**
Trial 14013 **First in man** Phase 1 Healthy subjects (Part 1), Adults with T1DM (Part 2)	A randomized, double-blind trial of single ascending doses of dasiglucagon administered SC or IM to healthy subjects (Part 1) and a single dose of dasiglucagon administered IM to hypoglycemic T1DM patients (Part 2) to evaluate the safety, tolerability, PK and PD of dasiglucagon as compared to an active comparator Follow-up period: 21 days (Part 1) 7 days (Part 2)	Part 1: Ascending dose (Euglyc[a]) Part 2: Crossover[b] (Hypo[c])	Part 1: Single SC doses of 0.01, 0.1, 0.3, 1.0, or 2.0 mg dasiglucagon or 1.0 mg GlucaGen (3:1). Single IM doses of 0.3, 1.0 or 2.0 mg dasiglucagon or 1.0 mg GlucaGen (3:1) Part 2: Single IM dose of 0.7 mg dasiglucagon and 1.0 mg GlucaGen	Part 1: Each dasiglucagon SC and IM dose group: n=6: Total: SC: n=30; IM: n=18 1.0 mg GlucaGen: SC: n=10; IM: n=6 Part 2: n=20 (exposed to both dasiglucagon and GlucaGen)
Trial 15007 **Multiple ascending doses** Phase 1b Healthy subjects	A randomized, placebo-controlled, double-blind trial of multiple ascending doses of dasiglucagon administered to healthy subjects to evaluate the safety, tolerability, PK and PD of dasiglucagon Follow-up period: 23 days	Ascending Dose (Euglyc[a])	5 consecutive single SC daily doses of 0.1, 0.3 or 1.0 mg dasiglucagon or placebo (3:1)	Each dasiglucagon dose group: n=6[d], Total: n=18 Placebo: n=6[d]

Trial 17144 **IV/QTc** Phase 1 Healthy subjects	A randomized, double-blind, placebo-controlled, dose-escalation trial in healthy subjects to evaluate the safety and tolerability of a single IV administration of dasiglucagon and the bioavailability of dasiglucagon following SC compared to IV dosing administration. Follow-up period: 28 days	Ascending dose (Euglyc[a])	Single IV dose of 0.03, 0.1, 0.3, 0.6, or 1.5 mg dasiglucagon or placebo, or single SC dose of 0.6 mg dasiglucagon	0.03 mg dasiglucagon IV: n=6 0.1 mg dasiglucagon IV: n=6 0.3 mg dasiglucagon IV: n=6 0.6 mg dasiglucagon IV: n=12 1.5 mg dasiglucagon IV: n=6 0.6 mg dasiglucagon SC: n=6 Placebo IV: n=18
Trial 15126 **Dose-finding** Phase 2 Adults with T1DM	A randomized, double-blind trial of single doses of dasiglucagon administered SC to hypoglycemic patients with T1DM to enable dose-finding and to describe the PK/PD of dasiglucagon vs. GlucaGen. Follow-up period: 21 days	Parallel/ crossover[e] (Hypo[c])	Single SC dose of 0.1, 0.3, 0.6 or 1.0 mg dasiglucagon and 0.5 or 1.0 mg GlucaGen	0.1 mg dasiglucagon: n=6 0.3 mg dasiglucagon: n=16 0.6 mg dasiglucagon: n=17 1.0 mg dasiglucagon: n=16 0.5 mg GlucaGen: n=17 1.0 mg GlucaGen: n=34
Trial 16137 **1st pivotal** Phase 3 Adults with T1DM	A randomized, double-blind, parallel-group trial to confirm the efficacy and safety of dasiglucagon in the treatment of hypoglycemia in patients with T1DM compared to placebo and with GlucaGen as a reference treatment arm. Follow-up period: 28 days	Parallel (Hypo[c])	Single SC dose of 0.6 mg dasiglucagon, placebo or 1.0 mg GlucaGen (2:1:1)	0.6 mg dasiglucagon: n=82 Placebo: n=43 1.0 mg GlucaGen: n=43
Trial 17145 **2nd pivotal** Phase 3 Adults with T1DM	A randomized, double-blind, parallel-group trial in patients with T1DM to confirm the efficacy and safety of dasiglucagon vs. placebo in the treatment of hypoglycemia. Follow-up period: 28 days	Parallel (Hypo[c])	Single SC dose of 0.6 mg dasiglucagon or placebo (3:1)	0.6 mg dasiglucagon: n=34 Placebo: n=10

Trial ID Phase Trial population	**Description**	**Design** (glycemic status at dosing)	**Dosing** (randomization)	**Number of subjects exposed**
Trial 17084 **Bridging** Phase 3 Adults with T1DM	A randomized, double-blind, crossover trial in patients with T1DM evaluating the efficacy and safety of single doses of two dasiglucagon batches. The trial compared a dasiglucagon batch reflecting storage under the intended dual storage conditions (Batch B) with a batch stored under refrigerated conditions (Batch A; representative of dasiglucagon tested in the rest of the clinical program). Follow-up period: 28 days	Crossover[f] (Hypo[c])	Single SC dose of 0.6 mg dasiglucagon (Batch A) and 0.6 mg dasiglucagon (Batch B) (1:1)	0.6 mg dasiglucagon: Batch A: n=45 Batch B: n=45
Trial 16136 **Immunogenicity** Phase 3 Adults with T1DM	A phase 3, randomized, double-blind, parallel group safety trial in patients with T1DM to evaluate the immunogenicity of dasiglucagon and GlucaGen administered SC. Follow-up period: 104 days	Parallel (Euglyc[a])	Three single SC doses (given 7±1 days apart) of 0.6 mg dasiglucagon or 1 mg GlucaGen (1:1)	0.6 mg dasiglucagon: n=57 1.0 mg GlucaGen: n=54
Trial 17086 **Pediatric pivotal** Phase 3 Children with T1DM	A randomized, double-blind, 3-arm, parallel-group trial in pediatric patients with T1DM aged 6 to <18 years to evaluate the efficacy, PK/PD and safety of dasiglucagon. Follow-up period: 28 days	Parallel (Hypo[c])	Single SC dose of 0.6 mg dasiglucagon, placebo or 1.0 mg GlucaGen (2:1:1)	Children 6 to 11 years: Dasiglucagon: n=8 Placebo: n=4 GlucaGen: n=4 Children 12 to 17 years: Dasiglucagon: n=12 Placebo: n=7 GlucaGen: n=6

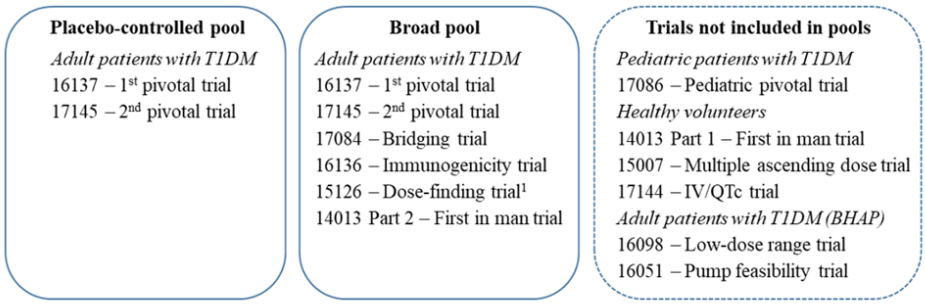
Abbreviations: PK: pharmacokinetic; PD: pharmacodynamic; IM: intramuscular; IV: intravenous; QTc: QT interval corrected for pulse rate; SC: subcutaneous; T1DM: type 1 diabetes mellitus. **Footnotes:** [a] Euglyc: euglycemic conditions; [b] Randomized, crossover design (7±3 days between doses); [c] hypo: hypoglycemic clamp; [d] all subjects received 5 consecutive daily doses; [e] Randomized, double-blind, parallel group design for 0.1 mg dasiglucagon and 1.0 mg GlucaGen and crossover design for 0.3, 0.6 or 1.0 mg dasiglucagon with 0.5 or 1.0 mg GlucaGen (7±3 days between doses); [f] Randomized, crossover design (28 days between doses).

Safety data from the two Zealand Pharma-sponsored trials within the indication of chronic dasiglucagon therapy via a bihormonal (insulin and dasiglucagon) artificial pancreas (BHAP) contribute as supportive.

Table 1-2 Overview of Zealand Pharma-sponsored clinical trials of dasiglucagon in patients with T1DM for the BHAP indication

Trial ID Phase Indication	Description	Design	Dosing (randomization)	Number of subjects exposed
Trial 16098 **Low-dose range trial** Phase 2 BHAP	A single center, randomized, 4-period, complete crossover, double-blind trial in patients with T1DM comparing dasiglucagon to active comparator (Lilly Glucagon[a]). Tests a concentrated formulation of dasiglucagon (4 mg/dL) intended for pump use. Follow-up period: 21 days	Crossover[b]	Multiple SC doses of dasiglucagon (0.03, 0.08, 0.2, and 0.6 mg)[c] and Lilly Glucagon (0.03, 0.08, and 0.2 mg).	Exposed to dasiglucagon (n=22), Exposed to Lilly Glucagon (n=23). N=17 received all planned doses of dasiglucagon (2× 0.03 mg, 2× 0.08 mg, 2× 0.2 mg and 2× 0.6 mg) and Lilly Glucagon (1× 0.03 mg, 1× 0.08 mg and 1× 0.2 mg
Trial 16051 **Pump feasibility trial** Phase 2 BHAP	A single-center, open-label, randomized, crossover trial in patients with T1DM testing the safety and tolerability of the bionic pancreas using the iPhone platform when used with dasiglucagon. Follow-up period: 25 days	Crossover[d]	Two 1-day treatment arms in which dasiglucagon[c] or Lilly Glucagon delivered via pump for up to 8 hours (not to exceed a total dose of 1 mg).	Dasiglucagon (n=10) Lilly Glucagon (n=12)

Abbreviations: BHAP: bihormonal (insulin and dasiglucagon) artificial pancreas; SC: subcutaneous; SOC: standard of care; T1DM: type 1 diabetes mellitus.
Footnotes: [a] Glucagon for Injection (Eli Lilly); [b] Double-blind, 4-period trial where single doses of 0.03, 0.08, 0.2 and 0.6 mg dasiglucagon were given on consecutive days under euglycemic (Day 1) and hypoglycemic (Day 2) conditions. On Day 1, doses of 0.03, 0.08, and 0.2 mg dasiglucagon given in a randomized, crossover design with 0.03, 0.08, and 0.2 mg Lilly Glucagon (≥5 hours between doses); [c] tests a 4 mg/mL dasiglucagon formulation intended for pump use; [d] randomized, crossover design (1–21 days between doses).



Abbreviations: BHAP: Bihormonal artificial pancreas indication; T1DM: type 1 diabetes mellitus. **Footnotes:**
[1] Patients exposed to dasiglucagon doses <0.6 mg (n=22) and GlucaGen doses of 0.5 mg (n=17) are not included in the broad pool.

Figure 1-2 Pooling of trials and data

For the broad pool, patients exposed to dasiglucagon doses of 0.6 mg or higher are combined into one group (i.e., ≥0.6 mg dasiglucagon), as a conservative approach for the broad pool safety evaluation.

For pooling purposes, data from the crossover bridging trial 17084 are handled as repeated single dose exposures to dasiglucagon. Also the patient data from each single dose in the crossover periods of trials 14103 Part 2 and 15126 are handled as independent data.

2.5.8.1. Patient exposure

Within the development program for severe hypoglycemia, 466 subjects have been exposed to dasiglucagon, 88 to placebo, and 194 to GlucaGen. Of the 466 subjects exposed to dasiglucagon, 358 were patients with type 1 diabetes mellitus (T1DM; 338 adult and 20 paediatric patients) and 108 were healthy subjects. In addition, 32 adult patients with T1DM have been exposed to dasiglucagon and 35 to Lilly Glucagon in the two supportive trials (BHAP).

Table 1-6 Exposure – overview for pools and individual trials – adult patients

	dasiglucagon				
	0.6 mg N (doses)	>0.6 mg N (doses)	Total ≥0.6 mg N (doses)	placebo N (doses)	Glucagen 1 mg N (doses)
Adults					
Placebo-controlled pool	116 (116)		116 (116)	53 (53)	43 (43)
Broad pool	280 (470)	36 (36)	316 (506)	53 (53)	151 (252)
16137	82 (82)		82 (82)	43 (43)	43 (43)
17145	34 (34)		34 (34)	10 (10)	
17084	90 (174)		90 (174)		
16136	57 (163)		57 (163)		54 (155)
15126	17 (17)	16 (16)	33 (33)		34 (34)
14013 Part 2		20 (20)	20 (20)		20 (20)

Abbreviations: N: number of patients exposed; doses: total number of doses. **Notes:** Placebo-controlled pool: trials 16137 and 17145; Broad pool: trials 16137, 17145, 17084, 16136, 15126 (does not include patients exposed to dasiglucagon doses <0.6 mg and GlucaGen dose of 0.5 mg) and 14013 Part 2; Data from the crossover bridging trial 17084 are handled as repeated single-dose exposures to dasiglucagon (see Section 1.1.7.2 for further details).

The duration of the observation period for the majority of patients in the broad pool (and also placebo-controlled pool) was <35 days, with the number of patients observed for ≥35 days attributable to the longer follow up period of 104 days in trial 16136.

Table 1-9 Duration of the observation period – broad pool

	dasiglucagon ≥0.6 mg		placebo		GlucaGen 1 mg	
	n	(%)	n	(%)	n	(%)
Safety analysis set (N)	316		53		151	
Patient days of observation	15115		1689		7539	
Duration of observation period						
>= 1 day	316	(100)	53	(100)	151	(100)
>= 7 days	289	(91.5)	53	(100)	126	(83.4)
>= 14 days	280	(88.6)	53	(100)	115	(76.2)
>= 28 days	208	(65.8)	30	(56.6)	83	(55.0)
>= 35 days	81	(25.6)	11	(20.8)	56	(37.1)
>= 60 days	57	(18.0)	1	(1.9)	54	(35.8)
>= 100 days	42	(13.3)	0		39	(25.8)

Abbreviations: N: number of patients exposed; n: number of patients in the duration category; %: percentage of patients in the duration category. **Notes:** Broad pool: trials 16137, 17145, 17084, 16136, 15126 (does not include patients exposed to dasiglucagon doses <0.6 mg and GlucaGen dose of 0.5 mg) and 14013, Part 2.

Overall, the subject's characteristics were similar across the groups, the majority were European patients, with a very limited number of subjects >65 years old. Mean BMI was similar across treatment group (around 26 kg/m2). The mean duration of diabetes was approximately 20 years and mean HbA1c at baseline was around 7.3%. Exposure to males was approximately twice that of females in all treatment groups apart from the dasiglucagon group of the placebo-controlled pool where exposure to males and females was closer to parity.

In total, 20 pediatric patients have been exposed to dasiglucagon, 11 to placebo, and 10 to GlucaGen. Mean age was 12−13 years, with a similar proportion of patients in the two age groups across treatment groups, with approximately 40% of the patients in the age group of 6−11 years and 60% in the age group of 12−17 years.

The overall exposure can be considered sufficient to establish a safety profile for the dasiglucagon for the proposed population and indication.

2.5.8.2. Adverse events

The applicant approach was that an analysis based on naïvely pooled data does not take into account the potential bias caused by differences between trials including different randomization ratios (Simpson's paradox). To account for this, AE summary tables for pooled data present adjusted incidences using the Cochran Mantel Haenszel weighting method. As a result, even though the patient count is the same, different AEs in the same pooled treatment group can have different adjusted percentages if the AEs come from different trials.

Common adverse events are presented for the:

- Entire observation period (placebo-controlled pool and broad pool)
- Period within 12 hours of dosing (placebo-controlled pool)

Information from this period is used for the adverse reactions section of the product label prescribing information. The 12-hour time frame is considered appropriate for labelling purposes due to the short half-life of dasiglucagon (approximately 30 minutes) and its intended use as a single dose rescue treatment. AEs reported during this period are more likely to be associated with glucagon receptor agonism than those that occur later in the observation period.

Table 2-5 Adverse events – overview – placebo-controlled pool

	dasiglucagon 0.6 mg			placebo			GlucaGen 1 mg		
	n	%	E	n	%	E	n	%	E
Safety analysis set (N)	116			53			43		
Adverse events	90	(78.2)	228	17	(32.0)	25	32	(74.4)	62
Serious adverse events	0			0			0		
Severity									
Mild	79	(68.9)	181	16	(30.2)	21	27	(62.8)	51
Moderate	27	(23.0)	45	4	(7.7)	4	7	(16.3)	9
Severe	2	(1.4)	2	0			1	(2.3)	2
Possibly/probably related events	73	(63.0)	128	4	(7.7)	7	27	(62.8)	47
AEs leading to withdrawal	0			0			0		
Outcome									
Recovered	89	(77.3)	222	15	(28.4)	23	32	(74.4)	61
Recovering	6	(5.4)	6	2	(3.6)	2	0		
Recovered with Sequelae	0			0			0		
Not Recovered	0			0			1	(2.3)	1
Fatal	0			0			0		
Unknown	0			0			0		

Abbreviations: AEs: adverse events; N: number of patients in the safety analysis set; n: number of patients experiencing at least one event; %: percentage of patients experiencing at least one event (Cochran-Mantel-Haenszel adjusted); E: number of events. **Notes:** Placebo-controlled pool: trials 16137 and 17145; MedDRA Version 22.0.

No deaths or other SAEs were reported, and there were no AEs leading to withdrawal in the placebo-controlled pool. The majority of AEs were mild or moderate in severity and had the outcome 'resolved'. In total, 4 severe AEs were reported: 2 events with dasiglucagon and 2 with GlucaGen.

A similar percentage of patients experienced at least one AE in the dasiglucagon and GlucaGen groups (78.2% and 74.4% of patients, respectively) with a lower percentage in the placebo group (32.0%). A similar pattern was seen for the individual trials of the placebo-controlled pool.

Table 2-8 Adverse events – overview – broad pool

	dasiglucagon 20.6 mg			placebo			GlucaGen 1 mg		
	n	%	E	n	%	E	n	%	E
Safety analysis set (N)	316			53			151		
Adverse events	238	(75.0)	1263	17	(32.0)	25	106	(70.5)	696
Serious adverse events	1	(0.4)	1	0			0		
Severity									
Mild	211	(66.7)	1038	16	(30.2)	21	93	(61.7)	621
Moderate	99	(32.7)	219	4	(7.7)	4	37	(24.2)	73
Severe	6	(1.8)	6	0			1	(0.7)	2
Possibly/probably related events	201	(63.8)	469	4	(7.7)	7	83	(55.3)	155
AEs leading to withdrawal	5	(1.9)	5	0			0		
Outcome									
Recovered	236	(74.4)	1250	15	(28.4)	23	103	(68.6)	680
Recovering	9	(2.5)	9	2	(3.6)	2	2	(1.3)	2
Recovered with Sequelae	0			0			0		
Not Recovered	4	(1.5)	4	0			9	(5.9)	14
Fatal	0			0			0		
Unknown	0			0			0		

Abbreviations: AEs: adverse events; N: number of patients in the safety analysis set; n: number of patients experiencing at least one event; %: Percentage of patients experiencing at least one event (Cochran-Mantel-Haenszel adjusted); E: Number of events. **Notes:** Broad pool: trials 16137, 17145, 17084, 16136, 15126 (does not include patients exposed to dasiglucagon doses <0.6 mg and GlucaGen dose of 0.5 mg) and 14013 Part 2; MedDRA Version 22.0.

As noted for the placebo-controlled pool, nausea, and vomiting were among the most frequently reported AEs with active treatment. Other more frequently reported AEs were hypoglycemia, headache, dizziness, nasopharyngitis, diarrhoea, and injection site erythema. All other AEs were reported for 4 or fewer patients in any one treatment group, with no apparent treatment-related differences or clustering.

Table 2-7 Common adverse events in ≥5% patients within 12 hours post dose by SOC and preferred term – placebo-controlled pool

	dasiglucagon 0.6 mg			placebo			GlucaGen 1 mg		
	n	%	E	n	%	E	n	%	E
Safety analysis set (N)	116			53			43		
All events	74	(64.0)	122	4	(7.7)	5	26	(60.5)	40
GASTROINTESTINAL DISORDERS	73	(63.0)	109	2	(4.1)	3	25	(58.1)	35
Nausea	66	(56.5)	67	2	(4.1)	2	23	(53.5)	23
Vomiting	29	(24.6)	36	1	(1.8)	1	9	(20.9)	11
Diarrhoea	6	(5.1)	6	0			1	(2.3)	1
NERVOUS SYSTEM DISORDERS	13	(11.2)	13	2	(3.6)	2	5	(11.6)	5
Headache	13	(11.2)	13	2	(3.6)	2	5	(11.6)	5

Abbreviations: N: number of patients in the safety analysis set; n: number of patients experiencing at least one event; %: Percentage of patients experiencing at least one event (Cochran-Mantel-Haenszel adjusted); E: number of events. **Notes:** Common AEs in ≥5% of patients are defined as events (preferred terms) occurring in ≥5% of patients in the dasiglucagon treatment group of the placebo-controlled pool; Placebo-controlled pool: trials 16137 and 17145: MedDRA version 22.0.

Table 2-10 Adverse events within 12 hours post dose – pediatric patients

	Dasiglucagon			Placebo			GlucaGen		
	n	(%)	E	n	(%)	E	n	(%)	E
Overall									
Safety analysis set (N)	20			11			10		
AEs	14	(70.0)	30	3	(27.3.)	3	6	(60.0)	10
Age 6–11 years									
Safety analysis set (N)	8			4			4		
AEs	3	(37.5)	4	0	(0.0)	0	4	(100.0)	8
Age 12–17 years									
Safety analysis set (N)	12			7			6		
AEs	11	(91.7)	26	3	(42.9)	3	2	(33.3)	2

Abbreviations: N: number of patients in the safety analysis set; n: number of patients experiencing at least one event; %: percentage of patients experiencing at least one event; E: number of events.

No deaths or other SAEs were reported in the paediatric trial, and there were no AEs leading to withdrawal. AEs were reported for 75.0% of patients (15/20 patient; 36 events) following dasiglucagon administration, 63.6% of patients (7/11 patients; 23 events) following placebo, and 90.0% of patients (9/10 patients; 15 events) following GlucaGen. All AEs were mild or moderate in severity and had an outcome of 'resolved'.

Table 2-11 Common adverse events in ≥5% patients within 12 hours post dose by preferred term – pediatric patients

	Dasiglucagon			Placebo			GlucaGen		
	n	(%)	E	n	(%)	E	n	(%)	E
Overall									
Safety analysis set (N)	20			11			10		
Nausea	13	(65.0)	14	0	(0.0)	0	3	(30.0)	3
Vomiting	10	(50.0)	13	0	(0.0)	0	1	(10.0)	1
Headache	2	(10.0)	2	0	(0.0)	0	1	(10.0)	1
Injection site pain	1	(5.0)	1	0	(0.0)	0	0	(0.0)	0
Age 6–11 years									
Safety analysis set (N)	8			4			4		
Nausea	2	(25.0)	2	0	(0.0)	0	2	(50.0)	2
Vomiting	2	(25.0)	2	0	(0.0)	0	1	(25.0)	1
Age 12–17 years									
Safety analysis set (N)	12			7			6		
Nausea	11	(91.7)	23	0	(0.0)	0	1	(16.7)	1
Vomiting	8	(66.7)	11	0	(0.0)	0	0	(0.0)	0
Headache	2	(16.7)	2	0	(0.0)	0	0	(0.0)	0
Injection site pain	1	(8.3)	1	0	(0.0)	0	0	(0.0)	0

Abbreviations: N: number of patients in the safety analysis set; n: number of patients experiencing at least one event; %: Percentage of patients experiencing at least one event; E: Number of events. **Notes:** Common AEs in ≥5% of patients are defined as events (preferred terms) occurring in ≥5% of patients in the dasiglucagon treatment group.

Nausea and, to a lesser extent, vomiting – both common side-effects of glucagon-receptor agonists – were among the most frequently-reported AEs with active treatment in adult and paediatric patients.

Hypoglycemia, a common side-effect of insulin treatment, was also relatively frequently reported. For both adult and paediatric patients, AEs of hypoglycemia occurred throughout the entire observation period, with all events evaluated by the investigator as unrelated to the investigational product.

For the broad pool, other more frequently reported AEs were headache, dizziness, nasopharyngitis, diarrhoea, and injection site erythema.

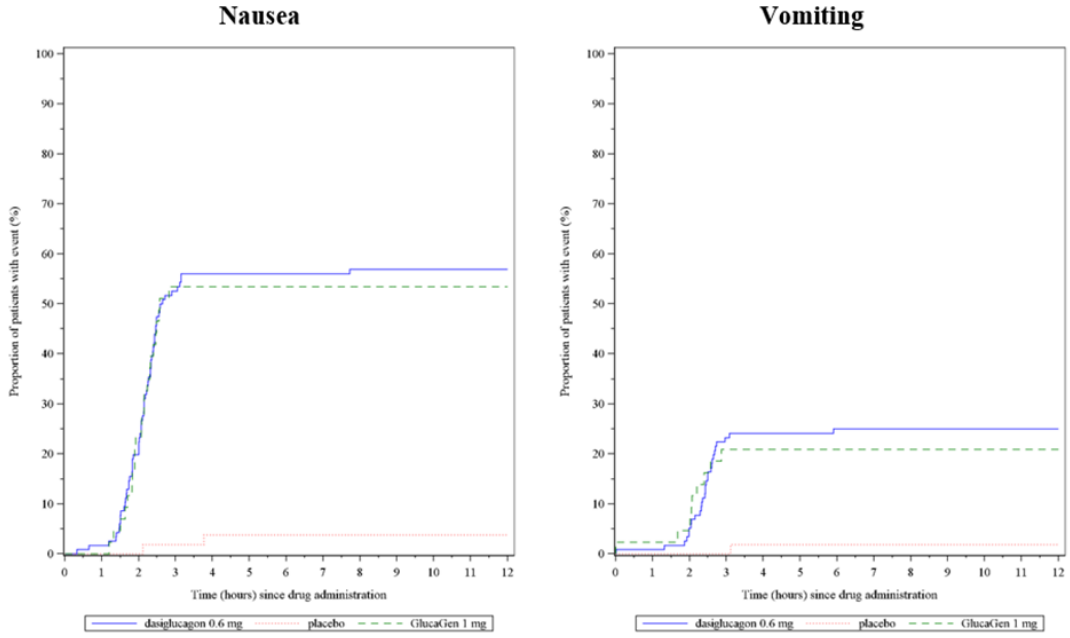
Gastrointestinal events (predominantly nausea and vomiting) are common side effects following administration of glucagon products. These transient gastrointestinal side effects are thought to be partly due to diminished intestinal motility resulting from an inhibitory effect of glucagon on gastrointestinal contractions.

The vast majority of all reported gastrointestinal AEs (98.7%; 149/151 events) occurred within 12 hours of dosing.

Table 2-24 Gastrointestinal events within 12 hours post dose by preferred term – placebo-controlled pool

	dasiglucagon 0.6 mg			placebo			GlucaGen 1 mg		
	n	%	E	n	%	E	n	%	E
Safety analysis set (N)	116			53			43		
All events	76	(65.6)	144	8	(15.9)	13	29	(67.4)	49
GASTROINTESTINAL DISORDERS	73	(63.0)	109	2	(4.1)	5	25	(58.1)	35
Nausea	66	(56.5)	67	2	(4.1)	2	23	(53.5)	23
Vomiting	29	(24.6)	36	1	(1.8)	1	9	(20.9)	11
Diarrhoea	6	(5.1)	6	0			1	(2.3)	1
Dyspepsia	0			1	(2.3)	1	0		
Hypoaethesia oral	0			1	(1.8)	1	0		

Abbreviations: N: number of patients in the safety analysis set; n: number of patients experiencing at least one event; %: Percentage of patients experiencing at least one event (Cochran-Mantel-Haenszel adjusted); E: Number of events. **Notes:** Placebo-controlled pool: trials 16137 and 17145; MedDRA version 22.0.



Notes: One minus the Kaplan-Meier survival probability plotted for the time to the first occurrence of nausea (left-hand panel) and vomiting (right-hand panel). One patient had an event of vomiting with onset on same day as dosing with missing onset time; for this event, the onset time is imputed as the dosing time.
Cross-reference: Appendix 3, Figure 3.38 and 3.40

Figure 2-3 Time to first nausea and vomiting within 12 hours post dose – placebo-controlled pool

Overall for the broad pool, nausea was reported for 58% of patients (182/316 patients; 253 events) following administration of dasiglucagon and 44.1% of patients (66/151 patients; 74 events) following GlucaGen; vomiting was reported for 28.7% of patients (89/316 patients; 125 events) following dasiglucagon and 13.6% of patients (20/151; 23 events) following GlucaGen; diarrhoea was reported for 4.6% of patients (15/316 patients; 18 events) following dasiglucagon and 2% of patients (3/151; 3 events) following GlucaGen.

It was notable that nausea and vomiting were reported for a higher percentage of patients (78.9% nausea and 42.2% vomiting) following dosing with dasiglucagon in trial 17084 (an uncontrolled, crossover trial comparing 2 batches of dasiglucagon) than the other trials of the broad pool.

Pediatric patients

For both nausea and vomiting, a higher percentage of patients experienced these events with dasiglucagon than GlucaGen (see Table 2-11 with Common AE in pediatric patients above). This is attributed to nausea and vomiting being more frequently reported for dasiglucagon in the age group of 12−17 years: nausea was reported in 91.7% (11/12) patients in dasiglucagon group and in 16.7% (1/6) in GlucaGen group; vomiting was reported in 66.7% (8/12) in dasiglucagon and none in GlucaGen group. No treatment-related imbalance was apparent in the age group from 6−11 years. The higher incidence of nausea and vomiting in the 12−17-year age group was not due to higher dasiglucagon exposure.

Figure 8.23 Time to first nausea within 12 hours post dose - trial 17086

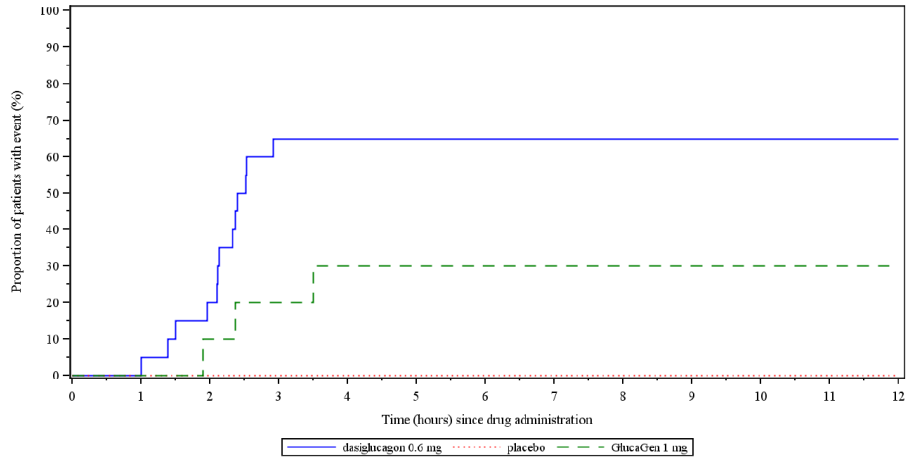
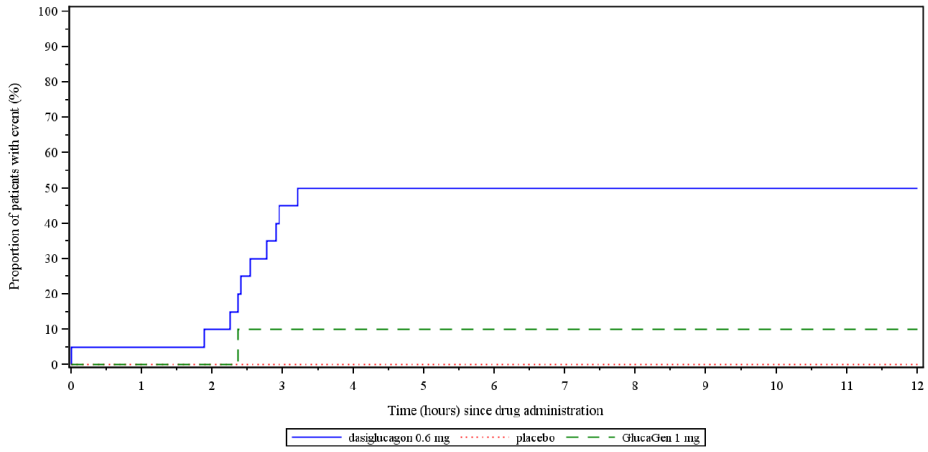


Figure 8.27 Time to first vomiting within 12 hours post dose - trial 17086



Nervous system disorders

Table 2-16 Adverse events in nervous system disorders SOC by preferred term – broad pool

SOC	Dasiglucagon ≥ 0.6 mg			Placebo			GlucaGen 1.0 mg		
Safety analysis set (N)	316			53			151		
	n	%	E	n	%	E	n	%	E
Nervous system disorders	53	(17.3)	65	2	(3.6)	2	23	(15.2)	25
Headache	42	(13.6)	49	2	(3.6)	2	16	(10.6)	17
Dizziness	9	(3.2)	10	0	(0.0)	0	6	(4.0)	6
Burning sensation	2	(0.6)	2	0	(0.0)	0	1	(0.6)	1
Dysgeusia	1	(0.4)	1	0	(0.0)	0	0	(0.0)	0
Orthostatic intolerance	1	(0.4)	1	0	(0.0)	0	0	(0.0)	0
Somnolence	1	(0.4)	1	0	(0.0)	0	0	(0.0)	0
Presyncope	1	(0.2)	1	0	(0.0)	0	0	(0.0)	0
Syncope	0	(0.0)	0	0	(0.0)	0	1	(0.6)	1

Abbreviations: N: number of patients in the safety analysis set; n: number of patients experiencing at least one event; %: percentage of patients experiencing at least one event (Cochran-Mantel-Haenszel adjusted); E: number of events. **Notes:** Broad pool: trials 16137, 17145, 17084, 16136, 15126 (does not include patients exposed to dasiglucagon doses <0.6 mg and GlucaGen dose of 0.5 mg) and 14013, Part 2; MedDRA Version 22.0.

Table 2-17 Adverse events in nervous system disorders SOC by preferred term – pediatric patients

SOC	Dasiglucagon 0.6 mg			Placebo			GlucaGen 1.0 mg		
Safety analysis set (N)	20			11			10		
	n	%	E	n	%	E	n	%	E
Nervous system disorders	2	(10.0)	2	1	(9.1)	1	1	(10.0)	1
Headache	2	(10.0)	2	1	(9.1)	1	1	(10.0)	1

Abbreviations: N: number of patients in the safety analysis set; n: number of patients experiencing at least one event; %: percentage of patients experiencing at least one event.

Due to the mild, transient freezing absences observed in rats following repeated dosing of dasiglucagon or glucagon, a medical review of the AEs reported in the SOC 'nervous system disorders' was conducted. The review did not identify any AEs in healthy subjects, adult patients or paediatric patients corresponding to the findings in rats.

For both adult trials (broad pool) and paediatric trial the most common AE from the SOC "nervous system disorders" was headache: 13.6% in dasiglucagon group vs 3.6% placebo vs 10.6% GlucaGen (adult broad pool); in children the frequencies around 10%, balanced between the arms.

2.5.8.3. Serious adverse event/deaths/other significant events

No deaths have been reported in any clinical trials of dasiglucagon.

One SAE of 'hypoglycemia' occurred in trial 16136 in an adult patient treated with dasiglucagon and was considered unrelated to the investigational product.

In total, 5 cases of AEs leading to withdrawal from trial (all with dasiglucagon) were reported in two non-placebo controlled trials (16136 –immunogenicity trial and 17084 – bridging trial). All of them were mild or moderate with variable time of onset and were due to the common AE (vomiting, headache). However, one withdrawal was due to the event "ECG QT prolonged" of moderate severity.

Cardiac adverse events (hemodynamic events and QT prolongation)

Due to the non-clinical evidence and known glucagon effects, signs of changes in blood pressure and heart rate ('hemodynamic events')– were pre specified as AEs of special interest (AESIs):

- Post-dose clinical signs or measured vital signs indicating a clinically significant drop in blood pressure including signs of orthostatic hypotension, vasovagal responses, or bradycardia.
- Post-dose change in pulse or blood pressure considered an event of hypo- or hypertension as judged by the investigator.

Most events related to a change in blood pressure (increase or decrease) and occurred 1.5−2 hours after administration of investigational product and approximately half of the hemodynamic events were associated with nausea. The events were transient and vital signs normalized within 2 hours.

During the period of 12 hours post-dose (broad pool) in dasiglucagon group 2.3% of patients experienced at least one hemodynamic AE vs 0% in placebo and 5.9% of patients in GlucaGen group. All events were mild (1.9% dasiglucagon arm vs 4.6% GlucaGen arm) or moderate (0.5% dasiglucagon vs 1.3% GlucaGen) and all events recovered without withdrawing from the trial. Almost all were considered related to the treatment (except one in GlucaGen arm). The time to the first occurrence of hemodynamic AEs was comparable between to active treatment groups, about 1.5−2 hours after administration of investigational product. The events were transient and vital signs normalized within 2 hours. No hemodynamic events were reported in the active treatment groups of the pediatric trial.

Table 2-19 Hemodynamic events within 12 hours post dose – overview – broad pool

	dasiglucagon ≥0.6 mg			placebo			GlucaGen 1 mg		
	n	%	E	n	%	E	n	%	E
Safety analysis set (N)	316			53			151		
Adverse events	7	(2.3)	7	0			9	(5.9)	11
Serious adverse events	0			0			0		
Severity									
Mild	5	(1.9)	5	0			7	(4.6)	9
Moderate	2	(0.5)	2	0			2	(1.3)	2
Severe	0			0			0		
Possibly/probably related events	7	(2.3)	7	0			8	(5.2)	10
AEs leading to withdrawal	0			0			0		
Outcome									
Recovered	7	(2.3)	7	0			9	(5.9)	11
Recovering	0			0			0		
Recovered with Sequelae	0			0			0		
Not Recovered	0			0			0		
Fatal	0			0			0		
Unknown	0			0			0		

Abbreviations: N: number of patients in the safety analysis set; n: number of patients experiencing at least one event; %: percentage of patients experiencing at least one event (Cochran-Mantel-Haenszel adjusted); E: number of events. AE: adverse events; MedDRA: Medical Dictionary for Regulatory Activities.
Notes: Broad pool: trials 16137, 17145, 17084, 16136, 15126 (does not include patients exposed to dasiglucagon doses <0.6 mg and GlucaGen dose of 0.5 mg) and 14013 Part 2; MedDRA version 22.0.

Table 2-20 Hemodynamic events within 12 hours post dose by system organ class and preferred term – broad pool

	dasiglucagon ≥ 0.6 mg			placebo			GlucaGen 1 mg		
	n	%	E	n	%	E	n	%	E
Safety analysis set (N)	316			53			151		
All events	7	(2.3)	7	0			9	(5.9)	11
VASCULAR DISORDERS	3	(1.1)	3	0			6	(3.9)	8
Hypotension	2	(0.7)	2	0			2	(1.3)	3
Hypertension	1	(0.4)	1	0			2	(1.3)	3
Orthostatic hypotension	0			0			1	(0.7)	1
Circulatory collapse	0			0			1	(0.6)	1
CARDIAC DISORDERS	2	(0.6)	2	0			1	(0.6)	1
Palpitations	1	(0.4)	1	0			0		
Bradycardia	1	(0.2)	1	0			0		
Tachycardia	0			0			1	(0.6)	1
NERVOUS SYSTEM DISORDERS	2	(0.6)	2	0			1	(0.6)	1
Orthostatic intolerance	1	(0.4)	1	0			0		
Presyncope	1	(0.2)	1	0			0		
Syncope	0			0			1	(0.6)	1
INVESTIGATIONS	0			0			1	(0.6)	1
Blood pressure decreased	0			0			1	(0.6)	1

Abbreviations: N: number of patients in the safety analysis set; n: number of patients experiencing at least one event; %: percentage of patients experiencing at least one event (Cochran-Mantel-Haenszel adjusted); E: number of events. **Notes:** Broad pool: trials 16137, 17145, 17084, 16136, 15126 (does not include patients exposed to dasiglucagon doses <0.6 mg and GlucaGen dose of 0.5 mg) and 14013. Part 2: MedDRA version 22.0.

Torsade de Pointes/ QT prolongation

A 53-year-old male patient in trial 16136 (dosed with 0.6 mg dasiglucagon) experienced prolonged QT interval in the ECG during the first dosing day of dasiglucagon. The adverse event was assessed as probably related to investigational product by the investigator, and the investigator decided to withdraw the patient from further dose administrations. At screening and baseline, QTc values were 462 and 455 ms and remained at this level for 45 min after dosing. At 60-, 237- and 240-min post-dose, QTc was prolonged to 470, 471 and 480 ms, respectively. The ECG results obtained at screening and 37 days after dosing were normal. The patient had a cardiology consultation 43 days after dosing and underwent a coronary angiography. The cardiologist's conclusions list an angiographic report of atypical angina, normal left ventricle systolic function and mild coronary artery disease. The Applicant argues that at screening and baseline, QTc was relatively long, and the observed change of the QTc interval is consistent with the overall observation of a secondary effect on the QTc interval and is not of clinical concern.

Adverse events in patients with clinically significant changes in ECG

A 24-year-old male patient with T1DM and no other relevant medical history, had T wave inversion in the 12-lead ECGs recorded at 20-, 35-, and 45-min post dose during the second treatment sequence, after administration of dasiglucagon (Batch A). No ECG abnormalities were observed during the first treatment sequence (Batch B). The T wave inversion was very discrete with STT depression at slight negativity at baseline and with very small changes post-dose, which were not associated with any symptoms suggestive of myocardial ischemia. The reported AE was mild and non-serious and was considered possibly related to investigation product.

Vital signs and ECG findings

The administration of single doses of 0.6 mg dasiglucagon SC does not appear to be associated with any clinically relevant cardiac safety concern, similar to GlucaGen. Transient and mild increases or decreases in blood pressure may occur, often in association with nausea. On the dosing day, a mean increase in pulse of approximately 6-7 bpm was observed in the placebo-controlled pool, following administration of dasiglucagon and GlucaGen; in placebo group a mean increase was 4 bpm. In other trials in all treatment groups small or no increases in pulse were observed.

Hypoglycemia

Table 2-27 Episodes of hypoglycemia – overview for trials of the broad pool

	dasiglucagon >=0.6 mg			placebo			GlucaGen 1 mg		
Safety analysis set	N			N			N		
16137	82			43			43		
17145	34			10			-		
17084	90			-			-		
16136	57			-			54		
15126	33			-			34		
14013, Part 2	20			-			20		
Hypoglycaemia - entire observation period									
	n	%	E	n	%	E	n	%	E
16137	23	(28.0)	39	5	(11.6)	6	9	(20.9)	10
17145	6	(17.6)	29	2	(20.0)	3	-		
17084	18	(20.0)	28	-			-		
16136	28	(49.1)	581	-			29	(53.7)	447
15126	4	(12.1)	9	-			7	(20.6)	11
14013, Part 2	0			-			3	(15.0)	3
Hypoglycaemia - within 12 hours of dosing									
	n	%	E	n	%	E	n	%	E
16137	1	(1.2)	1	0			1	(2.3)	1
17145	1	(2.9)	1	2	(20.0)	2	-		
17084	1	(1.1)	2	-			-		
16136	6	(10.5)	10	-			10	(18.5)	18
15126	0			-			5	(14.7)	6
14013, Part 2	0			-			3	(15.0)	3

Notes: Data are for episodes of 'hypoglycemia' (preferred term) captured by the standardized MedDRA query. MedDRA version 22.0. **Abbreviations:** N: number of patients in the safety analysis set; n: number of patients experiencing at least one event; %: percentage of patients experiencing at least one event; E: number of events. Trial 15126 does not include patients exposed to dasiglucagon doses <0.6 mg and GlucaGen dose of 0.5 mg; Data from the crossover bridging trial 17084 are handled as repeated single-dose exposures to dasiglucagon (see Section

Across the trials of the broad pool, there was one SAE of 'hypoglycemia' (the AE was also severe; see below); all other AEs were non-serious. All hypoglycemia AEs were evaluated by the investigator as unrelated or unlikely related to investigational product, apart from 3 events in the GlucaGen group which were considered possibly or probably related. All AEs had the outcome 'resolved'. For all treatment groups, the majority of AEs (89−94% across the 3 treatment groups) were mild in severity. There were two severe AEs of 'hypoglycemia', one of which was also an SAE; both events were evaluated as unlikely related to investigational product by the investigator.

In the paediatric patients (≥6 to <18 years), in total, 20 non-serious AEs of hypoglycemia were captured by the standardized MedDRA query (all preferred term 'hypoglycemia'): hypoglycemia was reported for 10%, 36.4% and 20% of patients in the dasiglucagon, placebo, and GlucaGen groups, respectively. All the events were evaluated by the investigator as unlikely or not related to investigational product, were mild or moderate in severity, and had an outcome of 'resolved'.

Injection site reactions

For all trials of the broad pool, the injection site had to be assessed for skin reactions for up to 360 minutes after dosing and at the follow-up visit. The following skin reactions were assessed according to a dedicated eCRF and, if identified, reported as an AE: spontaneous pain, pain on palpation, itching, redness, oedema, induration/infiltration, other.

Table 2-30 Injection site reactions - overview - broad pool

	dasiglucagon ≥0.6 mg			placebo			GlucaGen 1 mg		
	n	%	E	n	%	E	n	%	E
Safety analysis set (N)	316			53			151		
Adverse events	10	(3.1)	14	2	(3.6)	2	9	(6.0)	11
Serious adverse event	0			0			0		
Severity									
Mild	10	(3.1)	13	2	(3.6)	2	9	(6.0)	11
Moderate	1	(0.2)	1	0			0		
Severe	0			0			0		
Possibly/probably related events	10	(3.1)	14	2	(3.6)	2	9	(6.0)	11
AEs leading to withdrawal	0			0			0		
Outcome									
Recovered	10	(3.1)	14	2	(3.6)	2	9	(6.0)	11
Recovering	0			0			0		
Recovered with Sequelae	0			0			0		
Not Recovered	0			0			0		
Fatal	0			0			0		
Unknown	0			0			0		

Abbreviations: N: number of patients in the safety analysis set; n: number of patients experiencing at least one event; %: Percentage of patients experiencing at least one event (Cochran-Mantel-Haenszel adjusted); E: Number of events. **Notes:** Broad pool: trials 16137, 17145, 17084, 16136, 15126 (does not include patients exposed to dasiglucagon doses <0.6 mg and GlucaGen dose of 0.5 mg) and 14013, Part 2; MedDRA version 22.0.

Similar to the results from the broad pool, 10 injection site AEs occurred within 12 hours of dosing in the placebo-controlled pool, with a low percentage of patients experiencing injection site reactions in all treatment groups (dasiglucagon: 3.2%; placebo: 3.6%; GlucaGen: 7.0%). The majority of injection site reactions (8/10) had an onset within 1 hour of dosing and lasted less than 2 hours. All events were evaluated by the investigator as related to investigational product treatment, the majority (9/10) were mild in severity (1 AE in the dasiglucagon group was of moderate severity), and all had an outcome of 'resolved'. The most frequently occurring injection site reaction was 'injection site erythema' (6 AEs), with a similar percentage of patients experiencing this AE with active treatment and placebo. Other injection site AEs were 'injection site pain' (3 patients, all in the dasiglucagon group) and 'injection site oedema' (1 patient in the GlucaGen group).

Table 2-6 Injection site reactions by site of administration (Broad pool, Safety Analysis Set)

Site of administration Preferred term	Dasiglucagon >=0.6mg (N=316) n (%)	E	Placebo (N=53) n (%)	E	Glucagen 1mg (N=151) n (%)	E
Number of injections						
Abdomen		398		13		204
Buttock		45		21		13
Deltoid muscle		16		4		0
Thigh		47		15		35
Any injection site reactions	10 (3.1)	14	2 (3.6)	2	9 (6.0)	11
Abdomen	7 (2.5)	10	1 (1.8)	1	7 (4.5)	9
Injection site erythema	5 (1.7)	5	1 (1.8)	1	4 (2.6)	5
Injection site oedema	3 (1.1)	3	0 (0.0)	0	2 (1.2)	2
Injection site pruritus	2 (0.8)	2	0 (0.0)	0	1 (0.6)	1
Injection site urticaria	0 (0.0)	0	0 (0.0)	0	1 (0.6)	1
Buttock	0 (0.0)	0	0 (0.0)	0	2 (1.5)	2
Injection site erythema	0 (0.0)	0	0 (0.0)	0	1 (0.7)	1
Injection site oedema	0 (0.0)	0	0 (0.0)	0	1 (0.7)	1
Deltoid muscle	2 (0.3)	3	0 (0.0)	0	0 (0.0)	0
Injection site erythema	1 (0.2)	1	0 (0.0)	0	0 (0.0)	0
Injection site pain	2 (0.3)	2	0 (0.0)	0	0 (0.0)	0
Thigh	1 (0.2)	1	1 (1.8)	1	0 (0.0)	0
Injection site erythema	0 (0.0)	0	1 (1.8)	1	0 (0.0)	0
Injection site pain	1 (0.2)	1	0 (0.0)	0	0 (0.0)	0

In the paediatric patients, in total, 7 injection site AEs were captured by the MedDRA query. AEs were reported for 5.0% of patients (1/20 patients; 1 event) following dasiglucagon administration and 40.0% of patients (4/10 patients; 6 events) following GlucaGen; no events occurred in the placebo group. Other characteristics were similar to ISRs of adult subjects.

Allergic reactions

A standardized MedDRA query (Hypersensitivity [narrow scope]) was performed for the broad pool to capture AEs related to hypersensitivity.

Overall, 5 AEs related to hypersensitivity were captured by the search: 0.6% of patients (2/316 patients; 2 events) in the dasiglucagon group and 1.9% of patients (3/151 patients; 3 events) in the GlucaGen group. There were no events with placebo.

The 2 AEs reported in the dasiglucagon group were ‘dermatitis contact’ (a reaction to adhesive) and ‘infusion site rash’ (associated with IV infusion of insulin/glucose during the hypoglycemic clamp procedure). Both events were mild, considered not related to investigational product by the investigator and had the outcome ‘resolved’. These results do not indicate a risk of hypersensitivity following dasiglucagon administration.

Other findings

Patients who had a screening AST or ALT value >2.5× upper limit of normal range (ULN) or a bilirubin value >1.5× ULN were to be excluded from participation in all phase-3 trials.

Patients who had an estimated glomerular filtration rate (eGFR) value of <30 mL/min/1.73 m2 at screening were to be excluded from participation in all phase-3 trials.

No hepatic or renal AEs were identified across the trials (both adults and children).

No AEs related to medication errors or accidental overdose were reported in trials with adult patients, paediatric patients, or healthy subjects.

Adverse reactions

AEs occurring within 12 hours of dosing (short half-life (30 minutes) and its intended use as a single dose rescue treatment) with a plausible causal relationship to the medicinal product based on a medical evaluation that took into account the pharmacology of the drug, time of onset, incidences higher than expected for a T1DM population, and the occurrence of events typical of drug induced adverse reactions. Based on the above criteria, the adverse reactions identified in the placebo-controlled pool (in which a total of 116 patients were administered dasiglucagon) were initially proposed in the Table of the Section 4.8 of the SmPC.

Other adverse reactions identified in trials outside of the placebo-controlled pool include two patients who experienced hypotension, and hypertension, palpitations and orthostatic intolerance (once case of each in separate patients).

The table in section 4.8 of the SmPC (based on the broad pool data) is the following:

Table 2-7 Tabulated list of adverse reactions associated with dasiglucagon

System Organ Class	Very common (≥1/10)	Common (≥1/100 to <1/10)	Uncommon (≥1/1,000 to <1/100)
Nervous system disorders		Headache (9.6%) Dizziness (2.9%)	Presyncope (0.3%)
Cardiac disorders			Palpitations (0.6%) Bradycardia (0.3%)
Vascular disorders			Hypotension (0.8%) Hypertension (0.4%) Hot flush (0.3%)
Gastrointestinal disorders	Nausea (62.2%) Vomiting (31.6%)	Diarrhoea (2.4%)	Abdominal pain upper (0.4%)
Skin and subcutaneous tissue disorders			Hyperhidrosis (0.2%)
General disorders and administration site conditions		Injection site erythema (1.7 %)	Injection site pruritus (0.9%) Injection site pain (0.7%) Injection site oedema (0.6%) Fatigue (0.4%)

2.5.8.4. Laboratory findings

For each trial, blood samples for routine hematology and biochemistry measurements were taken at pre-specified intervals (at screening, dosing and follow up visits, that varied by the specific study). The parameters were assessed for central tendencies and outliers.

The applicant provided data from hematology, biochemistry and urinalysis form different trials with dasiglucagon without clinically relevant findings.

For all trials of the broad pool, transient increases in mean leucocyte count to or marginally above the upper limit of the normal range (ULN) were observed on the dosing day for dasiglucagon and GlucaGen – and to a lesser extent with placebo – which returned to baseline levels by the next measurement after the dosing day. For dasiglucagon, mean leukocyte counts increased from pre-dose values of 5.17−6.26×109/L to a maximum of 9.09−10.80×109/L on the dosing day. The mean increases in leukocyte count are not considered clinically relevant and have been previously noted following glucagon treatment.

When asked, also graphical representation of principal laboratory data during the study period and across the pools were presented. The glucagon and dasiglucagon arms often showed similar trend of curves for different parameters. Overall, no new clinically relevant findings were observed.

2.5.8.5. Safety in special populations

The following table presents adverse events by age:

Table 2-11 Adverse events within 12 hours post dose by age – broad pool

Meddra terms	Dasi ≥ 0.6 mg		Placebo		GlucaGen 1 mg	
	<65 yr	65-74 yr	<65 yr	65-74 yr	<65 yr	65-74 yr
Safety analysis set	308	8	52	1	147	4
Total AEs	202 (65.6)	5 (61.6)	8 (16.2)	0	87 (59.5)	3 (72.5)
Serious AEs	0	0	0	0	0	0
AEs leading to withdrawal	4 (1.5)	0	0	0	0	0
Psychiatric disorders (SOC)	0	0	0	0	0	0
Nervous system disorders (SOC)	44 (14.6)	2 (30.1)	2 (3.6)	0	21 (14.2)	0
Accidents and injuries (SMQ)	1 (0.4)	0	0	0	0	0
Cardiac disorders (SOC)	2 (0.6)	0	0	0	1 (0.7)	0
Vascular disorders (SOC)	4 (1.4)	1 (15.1)	0	0	6 (4.0)	0
Cerebrovascular disorders [a]	0	0	0	0	0	0
Infections and infestations (SOC)	0	0	1 (1.8)	0	0	0
Anticholinergic syndrome (SMQ)	10 (3.3)	1 (15.1)	0	0	5 (3.4)	0
Quality of life decreased [b]	0	0	0	0	0	0
Sum of postural hypotension, falls, black outs, syncope, dizziness, ataxia, fractures [c]	8 (2.9)	1 (15.1)	0	0	6 (4.1)	0
Other AE appearing more frequently in older patients [d]	-	-	-	-	-	-

Notes: No patients ≥75 years (yr). The % are Cochran-Mantel-Haenszel adjusted. Broad pool: trials 16137, 17145, 17084, 16136, 15126 (does not include patients exposed to dasiglucagon doses <0.6 mg and GlucaGen dose of 0.5 mg) and 14013, Part 2.

Footnotes: [a] Using central nervous system vascular disorders (SMQ); [b] using the two PTs: Quality of life decreased (PT) and Impaired quality of life (PT); [c] sum of the following PTs: orthostatic hypotension, fall, loss of consciousness, syncope, dizziness, ataxia, fracture; [d] no AEs were identified apart from those shown in the rows above.

The vast majority of patients were <65 years of age in the broad pool; only 13 patients were ≥65 years. For both active treatment groups, a similar percentage of patients experienced AEs in the two age subgroups within 12 hours of dosing, but the low number of patients in the >65 years subgroup should be taken into consideration when interpreting the results. For all treatment groups, no notable differences were observed between age subgroups with respect to the type of AEs reported (SOCs and preferred terms).

Overall, there were no patients ≥ 75 years old across the studies. In the age group 65-74 years there were 8 patients in dasiglucagon arm, 1 patient in placebo arm and 4 patients in GlucaGen arm. Total number of AEs was similar between <65y and 65-74y groups across the arms.

Some categories of AEs seem to be more frequent only in the dasiglucagon arm in the 65-74y compared to <65y group: nervous system disorders, vascular disorders, anticholinergic syndrome, Sum of postural hypotension, falls, black outs, syncope, dizziness, ataxia, fractures. The AEs were more frequent in female subjects (83.9%) respect to male subjects (57.2%) exposed to dasiglucagon, with similar trend also in GlucaGen group.

For both the dasiglucagon and GlucaGen groups, a higher percentage of patients experienced AEs in the <25 kg/m^2 subgroup compared to the other BMI subgroups within 12 hours of dosing. No clear pattern was observed for the placebo group.

No relevant differences for subgroups based on diabetes duration, renal impairment (non or mild) or region. No conclusive evaluation could be made between white patients and patients of the other race subgroups due to the low number of patients and/or low number of patients with AEs in the non-white subgroups.

Also no conclusive evaluation could be made between the two ethnic subgroups due to the low number of patients with AEs in the Hispanic and Latino subgroup.

The safety profile of dasiglucagon in paediatric patients was similar to adult patients and nausea and vomiting were reported for a greater proportion of patients receiving dasiglucagon than those receiving GlucaGen. This is attributed to nausea and vomiting being more frequently reported for dasiglucagon than GlucaGen in the 12−17 years age group were the differences between two active treatment group were relevant.

The applicant provided analysis of AEs separately for 10 subjects between 7−9 years (6 of which treated with dasiglucagon) and 6 subjects between 10−11 years (2 of them treated with dasiglucagon). Nausea was the only event reported for the dasiglucagon group in the 10−11 years subgroup. In the 7−9 years subgroup, 3 of the 4 events reported in the dasiglucagon group were gastrointestinal events (1 of nausea, 2 of vomiting). The other event (hypoglycaemia) was experienced more than 12 hours after dosing. Six gastrointestinal events were reported in 4 of the children between 7−9 years receiving GlucaGen.

Considering the small number of subjects and reported AEs in the 7−9 years vs. 10−11 years subgroups, it is concluded that the dasiglucagon safety profile is similar for the two age subgroups as well as between the dasiglucagon and GlucaGen treatment groups.

Dasiglucagon has not been studied in pregnant or lactating women; females who were pregnant (according to a positive pregnancy test), were actively attempting to get pregnant or were lactating were to be excluded from participation in all clinical trials.

One pregnancy has been reported across the dasiglucagon clinical development program: A 32-year-old female patient in the GlucaGen group of trial 16136 who became pregnant 2 months after the last administration of investigational product. The pregnancy was uneventful, and the baby was healthy.

2.5.8.6. Immunological events

Anti-drug antibody (ADA) assessments were conducted in all 9 clinical trials within the indication of severe hypoglycemia as well as in the 2 supportive trials (BHAP indication), representing a total of 498 subjects exposed to dasiglucagon (370 adult patients, 20 paediatric patients [≥6 to <18 years] and 108 healthy subjects). One of those trials (16136) in T1DM patients was a dedicated immunogenicity study with three consecutive doses and including a dedicated follow-up PK/PD assessments for ADA positive subjects.

In total, 4 subjects (3 adult patients and 1 pediatric patient) who received dasiglucagon had ADA formation, corresponding to an incidence of less than 1%.

According to the presented data, 4 subject (3 adults and 1 paediatric patient) who received dasiglucagon developed treatment-emergent ADA.

Two patients receiving a single dose of dasiglucagon had detectable ADA to dasiglucagon for at least 14 months after dosing. One ADA-positive patient receiving multiple doses of dasiglucagon had ADA with transient neutralizing activity and with cross-reactivity against native glucagon.

For the 11 years old patient from the trial 17086 with anti-dasiglucagon ADA positive on three occasions (1 month, 12 and 18 months after a single dose of dasiglucagon) and no subsequent negative test, it was decided to stop following the ADA response since the ADAs (with titer declining) had consistently been found not to cross-react with endogenous glucagon and to not have detectable neutralizing activity.

No safety or efficacy concerns were identified for any of the ADA positive patients.

A total of five patients had pre-existing antibodies. These five patients were assessed for treatment boosting of their anti-drug antibody responses. In order to be categorized as having a treatment-boosted antibody response, a fivefold increase in titer was required as defined in the protocols. None of the patients experienced a treatment boosted antibody response.

Table 3-11 Overview of anti-dasiglucagon antibodies

Trial ID/ Subject ID	Baseline	Visit day/week/months	Visit day/week/months	Visit day/week/months
15126/ [redacted]	ADA negative	Day 7: Anti-dasiglucagon antibody positive Titer ~22[a]	Day 28: Anti-dasiglucagon antibody positive Titer ~22[a]	
		Anti-glucagon antibody negative	Anti-glucagon antibody negative	
16098/ [redacted]	ADA negative	Day 24: Anti-dasiglucagon antibody positive Titer 35.4 Neutralizing activity	3.5 Months: Anti-dasiglucagon antibody positive Titer 38.8	7 Months: ADA negative
		Anti-glucagon antibody positive Titer 33.8 Neutralizing activity	Anti-glucagon antibody negative	
16137/ [redacted]	ADA negative	Day 28: Anti-dasiglucagon antibody positive Titer 24.4 No neutralizing activity	14 Months: Anti-dasiglucagon antibody positive Titer 27.7 No neutralizing activity	17 Months: ADA negative
		Anti-glucagon antibody negative	Anti-glucagon antibody negative	
17086/ [redacted]	ADA negative	Day 28: Anti-dasiglucagon antibody positive Titer 75.7 No neutralizing activity	12 Months Anti-dasiglucagon antibody positive Titer 31.1 No neutralizing activity	18 Months: Anti-dasiglucagon antibody positive Titer 30.5 No neutralizing activity
		Anti-glucagon antibody negative	Anti-glucagon antibody negative	Anti-glucagon antibody negative

Footnote: [a] The sample was not titrated but had a signal at approximately the same level as the low positive control which has a titer of 22. **Abbreviations:** ADA: anti-drug antibody.
Cross-reference: Integrated Summary of Immunogenicity [M 5.3.5.3], Table 6-4

2.5.8.7. Safety related to drug-drug interactions and other interactions

No inhibition of the cytochrome P450 enzyme system has been observed based on in vitro studies. No clinical interaction studies have been performed.

All adult subjects in the placebo-controlled pooled studies (16137 and 17145) reported 1 or more drugs for diabetes, i.e. insulins (multiple brands and forms). The following most frequently reported concomitant medications at screening belonged to the following ATC categories: 'Cardiovascular system' primarily HMG CoA reductase inhibitors (lipid-lowering agents), ACE inhibitors and angiotensin II antagonists (antihypertensive medications); Genito-urinary system and sex hormones; Thyroid hormones; Nervous system (primarily antidepressants).

During placebo-controlled adult studies the most frequently reported concomitant medication was metoclopramide (39.7% dasiglucagon vs 5.7% placebo vs 53.5% GlucaGen group), which was administered to treat adverse events of nausea and vomiting.

The list of drug interaction relevant for glucagon is also included in the SmPC for dasiglucagon: insulin (antagonizing effects), indomethacin (dasiglucagon could lose effect or even produce hypoglycaemia), warfarin (increase of anticoagulant effect), beta-blockers (transient increase in both pulse and blood pressure).

A contraindication of pheochromocytoma is also included in the SmPC as glucagon products may substantially increase blood pressure by stimulating the release of catecholamines from the tumor.

Patients 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. To prevent relapse of the hypoglycaemia, oral carbohydrates should be given to restore liver glycogen, when the patient has responded to treatment. Moreover, in patients with insulinoma, glucagon administration may directly or indirectly (through an initial rise in blood glucose) stimulate exaggerated insulin release from an insulinoma and cause hypoglycaemia. Both warnings are includede in section 4.4 of the SmPC.

2.5.8.8. Discontinuation due to adverse events

In total, 5 AEs were reported across the trials of the broad pool that led to withdrawal from trial. All 5 AEs occurred following dasiglucagon (all after the first dose in repeated single dose trials) and were considered possibly or probably related to investigational product by the investigator. There were 3 cases of withdrawal due to vomiting with nausea, one headache and in one case ECG QT prolonged (see above).

In addition, 2 patients in trial 15126 (phase 2) withdrew: one of them was in GlucaGen group (experiencing nausea, abdominal pain, vomiting) and the other in dasiglucagon 0.6 mg group (with headache, nausea, vomiting).

2.5.8.9. Post marketing experience

The applicant presented Quarterly PADERs (Periodic Adverse Drug Experiences Reports) and only one non-serious AE (burning sensation) was reported during the period March 2021-March 2023.

In addition, no new safety issue emerged in the two most recent PADER.

The applicant stated that it was not possible to estimate the actual use/ exposure in an emergency situation based on sales data, as purchased product can be stored up to 3 years and not used.

2.5.9. Discussion on clinical safety

Within the development program for severe hypoglycemia, 466 subjects have been exposed to dasiglucagon, 88 to placebo, and 194 to GlucaGen. Of the 466 subjects exposed to dasiglucagon, 358 were patients with type 1 diabetes mellitus (T1DM; 338 adult and 20 pediatric patients) and 108 were

healthy subjects. In addition, 32 adult patients with T1DM have been exposed to dasiglucagon and 35 to Lilly Glucagon in the two supportive trials (BHAP).

For the broad pool, patients exposed to dasiglucagon doses of 0.6 mg or higher are combined into one group as a conservative approach for the safety evaluation was taken. The duration of the observation period for the majority of patients in both the broad and placebo-controlled pool was <35 days. Exposure to males was approximately twice that of females in all treatment groups apart from the dasiglucagon group of the placebo-controlled pool where exposure to males and females was closer to parity. Overall, the subject's characteristics were similar across the groups, the majority were european patients, with a very limited number of subjects >65 years old.

Patients with T2DM were excluded from the development program due to the potential confounding effect of endogenous insulin production on endpoint assessment. This approach is not considered optimal for safety profile assessment, as many patients with T2DM are elderly and/or with comorbidities and were not included in the dasiglucagon development program.

Due to the short half-life of dasiglucagon, AEs reported during 12-hour post-dose time frame are considered more likely to be associated with glucagon receptor agonism than those that occur later in the observation period.

The most frequent AEs for placebo-controlled pool were nausea, vomiting, headache, diarrhoea and injection site pain. The SmPC table in section 4.8 includes all adverse reactions from all sources for which a causal relationship between the medicinal product and the adverse event is at least a reasonable possibility.

The safety profile of dasiglucagon includes the most common well-known gastrointestinal effects of glucagon treatment (nausea, vomiting) as well as headache (also observed with some glucagon products). Other less commonly observed adverse effects were diarrhea and injection site pain.

While in the placebo pool there are no relevant differences between two active treatment groups, the results from broad pool show somewhat higher frequency of nausea and vomiting that occurred within 12 hours of dosing with dasiglucagon (58% nausea and 28.7% vomiting) as compared to GlucaGen (44.1% nausea and 13.6% vomiting).

Of the 41 patients in the peadiatric trial, 16 were in the age group of 6−11 years and 25 were between 12 and 17 years of age. Although the number of peadiatric patients studied is very limited, the frequencies of gastrointestinal AEs (nausea and vomiting) seem to be much higher for dasiglucagon in respect to GlucaGen, both overall and for the age group 12-17 years. In particular, in the age group of 12−17 years nausea was reported in 91.7% (11/12) patients in dasiglucagon group and in 16.7% (1/6) in GlucaGen group; vomiting was reported in 66.7% (8/12) in dasiglucagon and none in GlucaGen group. These frequencies are also higher than those observed in studies with adult subjects. The applicant acknowledged an apparent higher incidence of gastrointestinal AEs in the paediatric patients (12−17 years) compared to the adult population and that could not be explained based on the available data. These observed differences for pediatric population are reflected in section 4.8 of the SmPC. Moreover, the high frequency of vomiting is of concern for the overall management of hypoglycaemia, occurring in close to one third and two thirds of adult and pediatric subject, respectively. The potential risk of prolonged hypoglycaemia due to vomiting may interfere with oral administration of carbohydrates in the management of hypoglycaemia. It was clarified that vomiting onset was on average much later with no reasonable effect.

Overall, the safety results were consistent across the different studies. However, considering the overall higher incidence of gastrointestinal AEs of the 17084 ("bridging") study compared to other individual studies, a detailed analysis was provided by batch and by individual patients' data after treatment with each batch. No clinically important differences were observed between the two batches with respect to gastrointestinal AEs.

No deaths have been reported in any clinical trials of dasiglucagon. One SAE of 'hypoglycemia' occurred in an adult patient treated with dasiglucagon and was considered unrelated to the investigational product.

The 5 cases of AEs leading to withdrawal from trial occurred in two non placebo-controlled trials. All of them were mild or moderate and were due to the common AEs observed across the studies: vomiting, headache. The one withdrawal due to the event "ECG QT prolonged" of moderate severity was discussed and was not considered to be clinically relevant.

Due to the non-clinical evidence and known glucagon effects, signs of changes in blood pressure and heart rate ('hemodynamic events') were pre-specified as AEs of special interest (AESIs). The administration of single doses of 0.6 mg dasiglucagon SC does not appear to be associated with any clinically relevant cardiac safety concern, similar to GlucaGen. Transient and mild increases or decreases in blood pressure may occur, often in association with nausea. There was a case of bradycardia that was clinically significant, with the short loss of consciousness; however, "bradycardia" is reported in the table of section 4.8 of the SmPC with a frequency "uncommon". The applicant provided an analysis of single haemodynamic AEs by study arm with the information on concomitant metoclopramide use and the relative discussion on potential confounding effect was considered acceptable also in view of the small numbers of haemodynamic AE reported.

A low risk of injection site reactions with dasiglucagon administration is reported and is not greater than comparator treatments. The applicant discussed how the local tolerability was assessed and provided the tabulated data. For the most part of patients in all groups, the abdomen was the most frequent site of administration and of ISR. There was no apparent treatment related pattern in the time of onset or duration of injection site AEs. Overall, the ISR were more frequent in GlucaGen compared to dasiglucagon arm, although the frequencies were generally low for across all the study arms.

Even if allergic reactions related to dasiglucagon were not reported in the clinical development program to date, it is known that hypersensitivity reactions, including anaphylactic reactions, have been reported as 'very rare' (<1/10,000 patients) with injectable glucagon. These are known medicinal product class effects of glucagon. Therefore, the applicant added an adequate paragraph indicating the potential rare class effect of hypersensitivity reactions, including anaphylactic reactions, in section 4.8 of the SmPC.

The applicant provided graphical representation of principal laboratory data during the study period and across the pools. The glucagon and dasiglucagon arms often showed similar trend of curves for different parameters and no new clinically relevant findings were observed. Regarding the hemodynamic events, in some studies a small increase in pulse was observed with dasiglucagon, GlucaGen and placebo. A decrease in diastolic blood pressure was seen in some trials with dasiglucagon and GlucaGen but not in all. Other vital signs and physical examination were without clinically relevant findings. However, this product was administered mostly as a single dose and is intended as a rescue medication. In line with this, all vital signs values returned to the pre-dose or screening level.

Across the trials of the broad pool there were only 13 patients aged ≥65 years. There were no patients ≥75 years across the studies. Although some categories of AEs seem to be more frequent only in the dasiglucagon, considering very limited sample size 65-74y (only 8 subjects exposed to dasiglucagon) it is hard to draw any firm conclusion.

The AEs were more frequent in female subjects (83.9%) respect to male subjects (57.2%) exposed to dasiglucagon, with similar trend also in GlucaGen group.

For both dasiglucagon and GlucaGen, a higher percentage of female vs. male patients experienced nausea and vomiting in the <25 kg/m^2 and ≥25 to <30 kg/m^2 subgroups. However, the potential for higher exposure in females due to the lower body mass at the recommended dose is not considered to affect the benefit-risk profile for dasiglucagon administered in the rescue indication of severe hypoglycaemia. No safety data are available with respect to the use of dasiglucagon in special populations of liver disease. As described in the SmPC (section 4.4), the use of dasiglucagon should be avoided in conditions where hepatic glycogen levels are low.

The applicant recommends the use of dasiglucagon in pregnancy by the extrapolation from glucagon and considering the animal data irrelevant for humans. This is not agreed considering the lack of data

with dasiglucagon in humans and the reproductive toxicity observed in rabbits treated with dasiglucagon at a dose which is not reassuring concerning the threshold exposure according to ICH S5 guideline. Therefore, the use of dasiglucagon in pregnancy is considered only if the expected benefit justifies the potential risk to the foetus.

Immunogenicity was studied in a dedicated trial (16136) but also in all other presented trials including the pediatric trial. 4 subject (3 adults and 1 pediatric patient) who received dasiglucagon developed treatment-emergent ADA. Two patients receiving a single dose of dasiglucagon had detectable ADA to dasiglucagon for at least 14 months after dosing. One ADA-positive patient receiving multiple doses of dasiglucagon had ADA with transient neutralizing activity and with cross-reactivity against native glucagon. No safety or efficacy concerns were identified for any of the ADA positive patients. Although the incidence (<1%) is low, the caution is important. Moreover, the potential cross-reactivity with endogenous glucagon or other glucagon products is of relevance.

The requested follow-up data on two patients resulted ADA positive were not available. More details are provided for a patient. It was decided to stop following the ADA response since the ADAs (with titer declining) had consistently been found not to cross-react with endogenous glucagon and to not have detectable neutralizing activity; there were no safety concern for the patient in question. The Applicant was asked to discuss the frequency of ADA in pediatric population. The available information is scarce and hard to draw conclusion on this issue. As the cases observed overall had low titer and transient appearance of neutralising antibodies, at present it seems to have limited clinical impact.

An adequate statement was added in the SmPC that available data, still limited, did not show any clinically relevant impact of ADA on PK/PD, efficacy or safety profile of dasiglucagon.

The potential interaction with common co-administered medicines in T2DM patients with a pharmaceutical target interfering with glucagon release, for example DPP4 inhibitors, were adequately addressed.

Regarding the human factor studies, the applicant was requested actions to put in place some actions to prevent the needle stick injuries in real life use of pre-filled syringe. In addition to information in the SmPC/PL/IFU, an administration leaflet and an instructional video are implemented. The administration leaflet and the audio-visual training materials support the appropriate use of Zegalogue.

The safety evaluation of dasiglucagon could not detect very rare adverse effects or those caused by prolonged or cumulative exposure. These limitations are less meaningful for the indication as acute potentially life-saving episodic treatment. However, in the several studies of the development for severe hypoglycemia and the supportive studies the subjects received more than one dose of dasiglucagon.

The applicant provided the most recent post marketing data available from which no new safety issue emerged. When asked, it was not possible to estimate the actual use/ exposure in an emergency situation based on sales data.

2.5.10. Conclusions on the clinical safety

Overall, the safety profile of dasiglucagon appears similar to approved glucagon products. However, the administration of dasiglucagon SC frequently leads to gastrointestinal side effects (nausea, vomiting, less frequently diarrhoea) which appear more frequent in the broad pool than those reported with GlucaGen (conventional glucagon) SC, that are well-known class side effects. These differences are even more pronounced in the paediatric patients, although mostly in the 12-17 years group. Headache was also among the most commonly reported AEs across trials.

2.6. Risk Management Plan

2.6.1. Safety concerns

Table 16 Summary of safety concerns

Summary of safety concerns	
Important identified risks	None
Important potential risks	Drug administration error leading to loss of drug benefit
Missing information	None

2.6.2. Pharmacovigilance plan

No additional pharmacovigilance activities are considered necessary for the product. Routine pharmacovigilance is considered sufficient to identify and characterise the risks of the product. A follow-up form for adverse reactions relevant to characterise the risk of "Drug administration error leading to loss of drug benefit" will be implemented.

2.6.3. Risk minimisation measures

Summary table of risk minimisation activities

Safety concern	**Risk minimisation measures**
Important potential risk of '*Drug administration error leading to loss of drug benefit'*.	**Routine risk minimisation measures:** - Information with precautions concerning the handling and use of the product to avoid inappropriate use are described in the SmPC section 4.2 (Posology and method of administration) and section 6.6 (Special precautions for disposal and other handling), in the Package leaflet (PL) Section 2 (What you need to know before you are given Zegalogue) and Section 3 (How to give Zegalogue) and in the Instructions for use (IFU). - Instructions for users to call for medical help right away after administering dasiglucagon are in the SmPC section 4.2 and the IFU and on the protective case label. - Legal status: Zegalogue is a prescription drug, requiring HCP to instruct subjects or proper use of the product before use. Instructions include the Product Information **Additional risk minimisation measures:** An administration leaflet which links to an instructional video to guide patients and caregivers how to use the device correctly to administer the full Zegalogue dose. The instructional video should be concise, focused and suitable for the use without delay in emergency situation to immediately help the patient.

2.6.4. Conclusion

The CHMP considered that the risk management plan version 0.3 is acceptable.

2.7. Pharmacovigilance

2.7.1. Pharmacovigilance system

The CHMP considered that the pharmacovigilance system summary submitted by the applicant fulfils the requirements of Article 8(3) of Directive 2001/83/EC.

2.7.2. Periodic Safety Update Reports submission requirements

The active substance is not included in the EURD list and a new entry will be required. The requirements for submission of periodic safety update reports for this medicinal product are set out in the Annex II, Section C of the CHMP Opinion. The applicant did not request an alignment of the PSUR cycle with the international birth date IBD.

2.8. Product information

2.8.1. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use.*

2.8.2. Quick Response (QR) code

An administration leaflet to patients, aiming at minimising the important potential risk of inappropriate use of the device leading to loss of drug benefit, will be given from healthcare professionals to patients upon initial prescription of Zegalogue. The administration leaflet should contain a QR code. The QR code should lead to the video that provides clear step by step instructions (simultaneously audio-video-written) relevant in emergency situation.

2.8.3. Additional monitoring

Pursuant to Article 23(1) of Regulation No (EU) 726/2004, Zegalogue (dasiglucagon) is included in the additional monitoring list as it contains a new active substance which, on 1 January 2011, was not contained in any medicinal product authorised in the EU.

Therefore, the summary of product characteristics and the package leaflet includes a statement that this medicinal product is subject to additional monitoring and that this will allow quick identification of new safety information. The statement is preceded by an inverted equilateral black triangle.

3. Benefit-Risk Balance

3.1. Therapeutic context

3.1.1. Disease or condition

Dasiglucagon is intended for the treatment of severe hypoglycaemia in adults, adolescents, and children aged 6 years and over with diabetes mellitus."

Severe hypoglycemia is characterized by severe cognitive impairment requiring assistance from another person for recovery. In addition to the well-known associations with cardiac arrhythmias and increased risk of accidents due to impaired consciousness, severe hypoglycemia has been shown to be strongly associated with increased risks of several adverse, long-term clinical outcomes including cardiovascular events and death.

Repeated hypoglycemia episodes can lead to or worsen the so-called "impaired awareness of hypoglycaemia", that is the impairment in the patient subjective perception of its own hypoglycemic state and thus the impossibility to take appropriate corrective measures. This leads to a vicious cycle that can result in increasingly worse hypoglycemic episodes.

3.1.2. Available therapies and unmet medical need

Severe hypoglycemia requires assistance from another person and a more complex approach with parental administration of glucagon (via subcutaneous or intramuscular injection) usually at the dose of 1.0 mg. Recovery usually occurs within 15-20 minutes. In medical settings, for the treatment of severe hypoglycemia, intravenous glucose is usually considered the standard therapy.

Different glucagon formulations are available in EU. Some require reconstitution before being administered to the patient; this poses some problems since glucagon is usually administered by caregivers that are not healthcare professionals and thus might be uncomfortable in preparing and performing injections. Moreover, time is required to reconstitute the glucagon formulation, potentially causing treatment delay.

Two glucagon medicinal products are ready to use formulations: Ogluo indicated for the treatment of severe hypoglycaemia in adults, adolescents, and children aged 2 years and over with diabetes mellitus is pre-filled pen and pre-filled syringe, and Baqsimi indicated for the treatment of severe hypoglycaemia in adults, adolescents, and children aged 4 years and over with diabetes mellitus is an intranasal formulation; both of them are centrally approved.

3.1.3. Main clinical studies

The primary efficacy evaluation is derived from the three pivotal, placebo-controlled trials: two trials in adult patients with T1DM (trials 16137 and 17145), and one trial in a paediatric patient population (trial 17086).

Supportive efficacy trials include a phase 2, dose-finding trial (trial 15126) to support dose selection. In addition, a phase 3, bridging trial (trial 17084) compared an active substance batch with storage under the intended dual storage conditions to an active substance batch stored under refrigerated conditions (the latter representative of the active substance tested in the rest of the clinical program) in adults.

3.2. Favourable effects

Dasiglucagon is a ready for use formulation which does not require constitution before administration to the patient; this is important as usually the product is expected to be administered in an emergency context by caregivers that are not healthcare professionals and thus might be uncomfortable to prepare and perform injections. Moreover, there will be no delay in the treatment that could potentially be caused by the reconstitution time.

In the adult pivotal trials, dasiglucagon showed a significant shorter time to plasma glucose recovery (about 10 minutes) compared to placebo (about 35 – 40 minutes, depending on the trial; $p<0.001$). At any given timepoint, more patients consistently experienced plasma glucose recovery in the dasiglucagon arm compared to placebo arm.

In the peadiatric pivotal trial, dasiglucagon showed a shorter time (about 10 minutes) to plasma glucose recovery compared to placebo (about 30 minutes, $p<0.001$).

Dasiglucagon showed numerically similar "time to plasma glucose recovery" to the active comparator GlucaGen in both adults and children (>7 years age old). A post-hoc analysis showed non-inferiority of dasiglucagon with respect to GlucaGen as to the first increase in plasma glucose of ≥20 mg/dL from baseline in both adults and children.

3.3. Uncertainties and limitations about favourable effects

In the clinical trials, "time from injection" was used for the endpoints (in order to compare objectively the product performance) but no data is presented about the efficacy using "time from decision", that is an important parameter in the real-life use of the product.

Dasiglucagon is expected to be administered mostly by caregivers (not healthcare professionals) in an emergency context where subjective variables, like anxiety levels, training in recognising the clinical condition and expertise in performing SC injections, can impact dasiglucagon effectiveness. However, no "real-life" studies have been performed.

3.4. Unfavourable effects

The most common adverse effects reported with dasiglucagon were nausea, vomiting, headache and less frequently diarrhoea and injection site pain. The safety profile of dasiglucagon includes well-known effects of glucagon treatment; all effects were transient and non-serious in nature.

Although the number of paediatric patients in the trial is very limited, the frequencies of gastrointestinal AEs (nausea and vomiting) seem to be much higher for dasiglucagon in respect to GlucaGen, both overall and for the age group 12-17 years (91.7% patients in dasiglucagon group and in 16.7% in GlucaGen group; vomiting was reported in 66.7% in dasiglucagon and none in GlucaGen group). These frequencies are also higher than those observed in studies with adult subjects.

The incidence of anti-dasiglucagon ADA (<1%) is low (studied across all trials including a dedicated one). However, in one subject both anti-dasiglucagon antibodies with neutralizing activity and anti-glucagon ADA were found, without observed effect on PK, efficacy or safety.

3.5. Uncertainties and limitations about unfavourable effects

The safety database for dasiglucagon was sufficient to adequately characterize the safety profile of dasiglucagon in the adult (mostly <65 years) and pediatric patients (all >6 years) with diabetes given the intended use of dasiglucagon, considering also safe use of the currently approved glucagon products.

However, the number of paediatric patients included in the dasiglucagon trials is limited and this hampers the conclusion on the observed higher gastrointestinal adverse events frequency in this population. Furthermore, this difference is apparently limited to 12–17-year subgroup, with no higher exposure than the 6-11-year subgroup. These effects (nausea and vomiting) are well-known common class-effects of glucagon products and were all considered mild or moderate.

Although in general no difference in adverse events in T1DM and T2DM is expected considering adverse events, many patients with T2DM are elderly and/or with comorbidities and were not included in the dasiglucagon development program. Moreover, the number of elderly patients included is very limited.

Information on dasiglucagon in human pregnancy is lacking.

No safety data have been generated for special populations such as those with liver of renal impairment (except for mild renal impairment in which no clinically relevant differences were found). However, dasiglucagon is intended to be administered as a single dose in an emergency situation. Therefore, lack of such data is considered acceptable.

Although the incidence of anti-dasiglucagon ADA is low, there are no complete follow-up data for all positive patients. Moreover, in one of them both anti-dasiglucagon antibodies with neutralizing activity and anti-glucagon ADA were found. As paediatric participants were very few in the trial program, it is not possible to draw any conclusion on the frequency of ADA formation in children and adolescents.

The safety evaluation of dasiglucagon could not detect very rare adverse effects or those caused by prolonged or cumulative exposure. These limitations are less meaningful for the indication as acute potentially life-saving episodic treatment. However, in the several studies of the development for severe hypoglycemia and the supportive studies the subjects received more than one dose of dasiglucagon.

3.6. Effects table

Effects Table for dasiglucagon in the treatment of severe hypoglycemia in adults and children >6 years with diabetes mellitus:

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
Favourable Effects						
PGR (vs placebo)	Time to glucose of ⩾ 20 mg/dL from baseline	min	10	40	p<0.001	Trial 16137
PGR (vs GlucaGen)	Time to glucose of ⩾ 20 mg/dL from baseline	min	10	12	No formal comparison	Trial 16137
PGR (vs placebo)	Time to glucose of ⩾ 20 mg/dL from baseline	min	10	35	p<0.0001	Trial 17145
PGR (vs placebo)	Time to glucose of ⩾ 20 mg/dL from baseline	min	10	30	p<0.001	Trial 17086
PGR (vs placebo)	plasma glucose concentration ⩾70 mg/dL or an increase of ⩾ 20 mg/dL	% of responders	100	67.4	p<0.001	Trial 16137
Unfavourable Effects						

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
Nausea	Incidence	%	56.5 vs 53.5[(1)] 58.4 vs 44.8[(2)] 65 vs 30[(3)]	4.1[(1)] 4.1[(2)] 0[(3)]	In general, these events were mild to moderate in severity, and infrequently led to discontinuation.	[(1)]Placebo-controlled pool (trials 16137, 17145) [(2)]Broad pool* [(3)]Pediatric trial (17086)
Vomiting	Incidence	%	24.6 vs 20.9[(1)] 28.7 vs 14.3[(2)] 50 vs 10[(3)]	1.8[(1)] 1.8[(2)] 0[(3)]		
Headache	Incidence	%	11.2 vs 11.6[(1)] 13.6 vs 10.6[(2)] 10 vs 10[(3)]	3.6[(1)] 3.6[(2)] 0[(3)]		

Abbreviations: PGR: plasma glucose recovery (first increase in plasma glucose of ⩾20 mg/dL from baseline without administration of rescue IV glucose)
*Notes: *Broad pool includes following trials: 16137 (1°pivotal), 17145 (2° pivotal), 17084 (bridging), 16136 (immunogenicity), 15126 (dose-finding), 14013 Part 2 (first in man)*
[1)]Placebo-controlled pool (trials 16137, 17145) [(2)]Broad pool[(3)]Pediatric trial (17086).*

3.7. Benefit-risk assessment and discussion

3.7.1. Importance of favourable and unfavourable effects

Dasiglucagon is a ready for use formulation which does not require constitution before administration to the patient; this is important as usually the product is expected to be administered in an emergency context by caregivers that are not healthcare professionals and thus might be uncomfortable to prepare and perform injections. Moreover, there will be no delay in the treatment that could potentially be caused by the reconstitution time.

The efficacy of dasiglucagon for treatment of severe hypoglycemia in adults and children >6 years with diabetes mellitus was established. Dasiglucagon proved superior to placebo in the time required to increase plasma glucose during insulin-induced hypoglycemia in adults and children >7 years (the median time to recovery was about 10 minutes). At several time points analysed, a greater proportion of patients experienced plasma glucose recovery with dasiglucagon compared to placebo. The data presented showed overall similar numerical results between the performances of dasiglucagon and the active comparator GlucaGen, and non-inferiority with the latter was shown in a post-hoc analysis.

Whereas the indication includes all patients with diabetes mellitus, no T2DM patients were enrolled in the clinical trials, but a similar response is expected given the similar pathophysiology of hypoglycemia. When dasiglucagon was stored up to 25°C it was non-inferior compared to when kept refrigerated. Some uncertainties concerned the elder population since very few patients >65 years were enrolled in the studies. No real-life studies were performed.

The safety database for dasiglucagon was sufficient to adequately characterize its safety profile in the adult and peadiatric patients (all >6 years) with diabetes given the intended use of dasiglucagon. However, the number of peadiatric patients is limited, and this hampers the conclusion on the observed higher nausea and vomiting frequency in this population, apparently limited to 12 to 17-year subgroup. The number of elderly patients included is very limited.

Although the incidence of anti-dasiglucagon ADA is low, there are no complete follow up data for positive patients.

For the use of dasiglucagon in pregnancy, it is suggested only if the expected benefit justifies the potential risk to the foetus.

The safety evaluation of dasiglucagon could not detect very rare adverse effects or those caused by prolonged or cumulative exposure. These limitations are less meaningful for the indication as acute potentially life-saving episodic treatment.

Regarding the important potential risk of "*Drug administration error leading to loss of drug benefit'*, in addition to information in the SmPC/PL/IFU, an administration leaflet and an instructional video are implemented. The administration leaflet and the audio-visual training materials are expected to support the appropriate use of Zegalogue.

3.7.2. Balance of benefits and risks

The most important effects about efficacy are related to the time required to increase plasma glucose concentration of ≥20 mg/dL from baseline. This time was lower with dasiglucagon compared to placebo (10 vs 30-40 min), and the difference was statistically and clinically significant. When compared to GlucaGen (an active comparator), a similar time was observed (but no formal comparison was performed). Some uncertainties remain mainly regarding the efficacy/safety in in older subjects.

The safety database for dasiglucagon was sufficient to adequately characterize its safety profile in the adult and pediatric patients (all >6 years) with diabetes given the intended use of dasiglucagon.

3.8. Conclusions

The overall benefit/risk balance of Zegalogue (dasiglucagon) is positive, subject to the conditions stated in section "Recommendations".

4. Recommendations

Outcome

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considers by consensus that the benefit-risk balance of Zegalogue (dasiglucagon) is favourable in the following indication:

Zegalogue is indicated for the treatment of severe hypoglycaemia in adults, adolescents, and children aged 6 years and over with diabetes mellitus.

The CHMP therefore recommends the granting of the marketing authorisation subject to the following conditions:

Conditions or restrictions regarding supply and use

Medicinal product subject to medical prescription.

Other conditions and requirements of the marketing authorisation

- **Periodic Safety Update Reports**

The requirements for submission of periodic safety update reports for this medicinal product are set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and any subsequent updates published on the European medicines web-portal.

The marketing authorisation holder shall submit the first periodic safety update report for this product within 6 months following authorisation.

Conditions or restrictions with regard to the safe and effective use of the medicinal product

- **Risk Management Plan (RMP)**

The marketing authorisation holder (MAH) shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the marketing authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.

- **Additional risk minimisation measures**

Prior to launch of Zegalogue (dasiglucagon), for the treatment of severe hypoglycaemia in adults, adolescents, and children aged 6 years and over with diabetes mellitus, in each EU Member State, the Marketing Authorisation Holder (MAH) must agree on the content and format of the educational materials, including communication media, distribution modalities, and any other aspects of the programme, with National Competent Authority.

The educational materials are aimed at providing guidance on how to minimise the important potential risk in the RMP of drug administration error leading to loss of drug benefit.

The MAH shall ensure that in each Member State where Zegalogue is marketed, all healthcare professionals and patients/caregivers who are expected to prescribe, supply, or use the medicinal product have access to the following:

- Administration leaflet
- Instructional video

The **administration leaflet** should contain the following key elements:

- Patients should receive the administration leaflet from their healthcare professionals upon initial prescription of Zegalogue and after training.
- Patients and family members or caregivers should be informed on how to recognize the signs and symptoms of severe hypoglycaemia and the risks of prolonged hypoglycaemia. Early symptoms of hypoglycaemia should be described.
- The importance not to test the single-dose device in advance, not to remove the single-dose device from the protective case in advance (the single-dose device should be all the time kept in the protective case) and to ensure that the patient understands that each single-dose pre-filled syringe/pen can only be used once.
- The importance to call for emergency medical help or a healthcare provider right away after Zegalogue is injected. Even if the subcutaneous injection of Zegalogue helps the person to wake up, it should be advised to still call for emergency medical help right away.
- If the patient does not respond within 15 minutes, an additional dose of Zegalogue from a new device may be administered while waiting for emergency assistance.
- After the injection is given, the unconscious person should be rolled on to their side to prevent choking.

- The importance of correct storage of the medicinal product should be emphasized.
- The Package Leaflet (PL) and Instruction for use at the end of PL should be referenced for more detailed information regarding administration and handling of Zegalogue.
- Patients can use the administration leaflet to teach those around them how to correctly handle and administer Zegalogue.
- The administration leaflet should contain a URL and QR code to a website where patients can access the instructional video.

The **instructional video** should contain the following key elements:

- To reinforce the correct handling and administration, step-by-step instructions on the appropriate use of Zegalogue should be provided.
- The instructional video should be concise, focused and suitable for the use without delay in emergency situation to immediately help the patient.
- The importance to call for emergency medical help or a healthcare provider right away after Zegalogue is injected. Even if the injection of Zegalogue helps the person to wake up, it should be advised to still call for emergency medical help right away.
- If the patient does not respond within 15 minutes, an additional dose of Zegalogue from a new device may be administered while waiting for emergency assistance.
- After the injection is given, the unconscious person should be rolled on to their side to prevent choking.

New Active Substance Status

Based on the CHMP review of the available data, the CHMP considers that dasiglucagon is to be qualified as a new active substance in itself as it is not a constituent of a medicinal product previously authorised within the European Union. (Refer to Appendix on new active substance (NAS)).