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NON-CLINICAL REVIEW(S)

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

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Applicant's letter date:	September 12, 2015
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Product:	XULTOPHY (Insulin degludec and liraglutide [rDNA origin] injection)
Indication:	Improve glycemic control in adults with type 2 diabetes mellitus
Applicant:	Novo Nordisk
Review Division:	Division of Metabolism and Endocrinology Products
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1 Executive Summary

1.1 Introduction

XULTOPHY (Insulin degludec/liraglutide, abbreviated IDegLira) is a fixed-ratio combination of insulin degludec (IDeg, an insulin analog) and liraglutide (a GLP-1 receptor agonist). IDegLira is intended for improvement of glycemic control in adults with type 2 diabetes mellitus (T2DM) via once-daily subcutaneous injection administered at any time of the day. The combination product is provided in a pre-filled pen containing IDeg and liraglutide at a ratio of 100 units:3.6 mg per mL. The pre-filled pen allows for dose adjustments in increments of 1 unit IDeg and 0.036 mg liraglutide. (b) (4) The recommended daily starting dose of IDegLira is (b) (4) units of IDeg and (b) (4) mg liraglutide. Patients transferring from basal insulin treatment or converting form GLP-1 receptor agonists are to be started at a maximum of 16 units of IDeg and 0.6 mg of liraglutide. The maximum starting dose of 16 units/0.6 mg dose is consistent with the recommended start dose of liraglutide (Victoza®).

The IDegLira clinical development program includes five completed phase III trials designed to evaluate the efficacy and safety of IDegLira in subjects with T2DM. In their IDegLira nonclinical development program, the Applicant evaluated two IDegLira combination strengths (600/ (b) (4) nmol and 600/960 nmol) (b) (4) **The IDegLira nonclinical development program includes one primary pharmacology study in rats, one single dose pharmacokinetic study in pigs, a series of repeat dose toxicity studies in rats, and local tolerance studies in pigs and rabbits.** For nonclinical studies, doses are listed in nmol/kg, thereby using the same unit for both the IDeg and liraglutide components. **When comparing doses, 60 nmol of IDeg = 10 U and 100 nmol liraglutide = 0.375 mg liraglutide and maximum recommended human dose of 50 U/1.8 mg = 300 nmol IDeg/480 nmol liraglutide. The nonclinical formulation of 600/960 nmol IDegLira represents the clinical formulation.** (b) (4)

1.2 Brief Discussion of Nonclinical Findings

Pharmacodynamic profile of IDegLira

The primary pharmacodynamic (PD) effects of IDegLira at a ratio of 600nmol/mL IDeg to (b) (4) nmol/mL liraglutide, representing 2.9-fold (for IDeg) and (b) (4)-fold (for liraglutide) the maximum clinical dose of 50 IU/1.8 mg IDegLira, were evaluated in in male Wistar rats (Study No cfle080703). The effects of the combination were compared to the effects of IDeg and liraglutide alone. Three doses of IDegLira, as well as IDeg and liraglutide as individual components, were tested. Twenty-four hour blood glucose profiles, 24-hour food and water consumption and body weight changes were assessed after a single subcutaneous dose. Dose-dependent effects of IDegLira were observed for all parameters measured, including decreases in blood glucose, food and water consumption and body weight that were consistent with those expected based on the established effects of IDeg and liraglutide tested as individual components.

Pharmacokinetic profile of IDegLira

In single dose pharmacokinetic (PK) studies in pigs (Study Nos anp090201, anp090302 and anp090401), the PK of IDeg was shown to be similar when dosed as part of IDegLira or as IDeg alone. The PK of liraglutide when dosed as part of IDegLira showed a tendency towards lower overall C_{max} compared to when dosed as liraglutide alone. However, the total dose-normalized exposure (AUC/Dose) for liraglutide as part of IDegLira was similar to that of liraglutide alone. (b) (4)

In repeat dose toxicity studies in rats, increases in exposures of both IDeg and liraglutide were predictable, dose-proportional and sex independent. Limited systemic accumulation (<2-fold) was observed for the individual components upon repeated administration of IDegLira, which is consistent with the expected steady state concentrations considering the established half-lives of elimination and the dosing interval for the individual components.

Antidrug antibodies to individual components of IDegLira
For IDeg, a limited number of rats (18 of 153) developed anti-IDeg antibodies after repeated dosing with IDegLira. No difference in the plasma exposure observed between antibody positive and negative animals and PD activity was maintained. Thus, the formation of anti-IDeg antibodies was unlikely to have affected the evaluation of PD, PK, and safety of the combination. No anti-liraglutide antibodies were detected in any animal.

Toxicologic profile of IDegLira
Four and 13 week rat toxicity studies (with local tolerance assessments) were conducted with IDegLira administered via the SC route. It is notable that the ratio of IDeg : liraglutide was altered between the 4- and 13-week toxicity studies.

In 13 week study (Study No 209212), with the 600/960 IDeg/Lira nmol/mL formulation (the same IDeg/liraglutide ratio as the formulation for marketing), no adverse effects were observed at 20/32 nmol/kg/day, which represents 2.1-fold (for IDeg) and 3.3-fold (for liraglutide) the maximum clinical dose of 50 IU/1.8 mg IDegLira], lowered blood glucose levels were observed only at the highest dose. Dose-dependent reductions in body weight gain were observed across the dose range. No adverse drug-related histopathology findings were observed. Findings observed in the 13 week toxicity study were consistent with the known pharmacological effects of insulin and/or GLP-1 analogues.

In the 4 week study (Study No 208142) with the 600/ (b) (4) IDeg/Lira nmol/mL formulation, no adverse effects were observed at a dose of 20/54 nmol/kg/day, which represents 2.5-fold (for IDeg) and 4.2-fold (for liraglutide) the maximum clinical dose of 50 IU/1.8 mg IDegLira]. The safety margins are different for 20 nmol/kg/day IDeg in the 13 week (2.1-fold) versus the 4 week (2.5-fold) studies, which was attributed to the TK profile was slightly different between the two studies. At higher doses (60/160 and 30/80 nmol/kg/day), hypoglycemia-related mortalities were observed. Drug-related reductions in food consumption and body weight gain were observed across the dose range. Histopathological findings were noted in adrenal gland (minimal cortical cell vacuolation, consistent with stress), liver (min/slight hepatocellular rarefaction, attributed to excess glycogen deposition) and testes (minimal seminiferous tubular degeneration/apoptosis if spermatocytes, observed previously with insulin degludec and related to hypoglycemia) and were observed primarily at doses ≥30/80 nmol/mL. The maximum tolerated dose was exceeded, leading to premature sacrifices and dose reduction due to hypoglycemia. The fixed-ratio of IDeg/Lira was changed from 600/ (b) (4) nmol/mL to 600/960 nmol/mL and lower doses were administered in the 13 week toxicity study.

The potential for local tissue reactions after single unintended intramuscular or intravenous administration of IDegLira was evaluated in rabbits (Study No 210203). Local reactions were mild and comparable to that of vehicle and not considered to pose any concerns for the clinical use of the product.

Impurities/leachables of IDegLira
All IDegLira impurities were within the approved limits established for liraglutide 6.0 mg/mL (Victoza®) and IDeg 100 U/mL (Tresiba®). Five leachables (b) (4) were identified and quantified in a long-term leachable study with IDegLira. The container closure system is considered suitable for the 100 U/mL insulin degludec and 3.6 mg/mL liraglutide drug product (IDegLira 100 U/3.6 mg per mL) with no safety concerns related to the levels of leachables observed, based on Permissible Daily Exposure levels established in ICHQ3C or the qualification threshold established by the Product Quality Research Institute.

In summary, the nonclinical program revealed findings attributable to the known pharmacological or exaggerated pharmacological effects of IDeg and/or liraglutide components (e.g., hypoglycemia,

decreased body weight gain and decreased food consumption, etc.). No unexpected or novel safety concerns were identified.

1.3 Recommendations

1.3.1 Approvability

Pharmacology/Toxicology recommends approval of IDegLira to improve glycemic control in adults with type 2 diabetes mellitus.

1.3.2 Additional Nonclinical Recommendations

None.

1.3.3 Labeling

The Applicant's proposed labeling is consistent with those approved for insulin degludec and liraglutide prior to PLLR conversion. Pharm/tox finds the proposed label for IDegLira is acceptable, pending any recommendation from Division of Pediatric and Maternal Health (DPMH).

SPONOR'S PROPOSED LABELING
8 USE IN SPECIFIC POPULATIONS
8.1 Pregnancy
Risk Summary

(b) (4)

Clinical Considerations
Disease-associated maternal and/or embryo/fetal risk

Poorly controlled diabetes in pregnancy increases the maternal risk for diabetic ketoacidosis, pre-eclampsia (b) (4)

Data

Animal Data

Insulin degludec

(b) (4)

Liraglutide

Female rats given subcutaneous doses of 0.1, 0.25 and 1.0 mg/kg/day liraglutide, (b) (4) beginning 2 weeks before mating through gestation day 17 had estimated systemic exposures 0.8-, 3-, and 11-times the human exposure at the MRHD based on plasma AUC comparison. The number of early embryonic deaths in the 1 mg/kg/day group increased slightly. Fetal abnormalities and variations in kidneys and blood vessels, irregular ossification of the skull, and a more complete state of ossification occurred at all doses. Mottled liver and minimally kinked ribs occurred at the highest dose. The incidence of fetal malformations in liraglutide-treated groups exceeding concurrent and historical controls were misshapen oropharynx and/or narrowed opening into larynx at 0.1 mg/kg/day and umbilical hernia at 0.1 and 0.25 mg/kg/day.

Pregnant rabbits given subcutaneous doses of 0.01, 0.025 and 0.05 mg/kg/day liraglutide from gestation day 6 through day 18 inclusive, had estimated systemic exposures less than the human exposure at the MRHD of 1.8 mg/day at all doses, based on plasma AUC. Liraglutide decreased fetal weight and dose dependently increased the incidence of total major fetal abnormalities at all doses. The incidence of malformations exceeded concurrent and historical controls at 0.01 mg/kg/day (kidneys, scapula), ≥ 0.01 mg/kg/day (eyes, forelimb), 0.025 mg/kg/day (brain, tail and sacral vertebrae, major blood vessels and heart, umbilicus), ≥ 0.025 mg/kg/day (sternum) and at 0.05 mg/kg/day (parietal bones, major blood vessels). Irregular ossification and/or skeletal abnormalities occurred in the skull and jaw, vertebrae and ribs, sternum, pelvis, tail, and scapula; and dose-dependent minor skeletal variations were observed. Visceral abnormalities occurred in blood vessels, lung, liver, and esophagus. Bilobed or bifurcated gallbladder was seen in all treatment groups, but not in the control group.

In pregnant female rats given subcutaneous doses of 0.1, 0.25 and 1.0 mg/kg/day liraglutide from gestation day 6 through weaning or termination of nursing on lactation day 24, estimated systemic exposures were 0.8-, 3-, and 11-times human exposure at the MRHD of 1.8 mg/day, based on plasma AUC. A slight delay in parturition was observed in the majority of treated rats. Group mean body weight of neonatal rats from liraglutide-treated dams was lower than neonatal rats from control group dams. Bloody scabs and agitated behavior occurred in male rats descended from dams treated with 1 mg/kg/day liraglutide. Group mean body weight from birth to postpartum day 14 trended lower in F2 generation rats descended from liraglutide-treated rats compared to F2 generation rats descended from controls, but differences did not reach statistical significance for any group.

8.2 Lactation

Risk Summary

(b) (4)

(b) (4)

Data

(b) (4)

Insulin degludec

In lactating rats, insulin degludec was (b) (4)

Liraglutide

In lactating rats, liraglutide was (b) (4) approximately 50% of maternal plasma concentrations.

(b) (4)

8.4 Pediatric Use

Safety and effectiveness of XULTOPHY have not been established in pediatric patients.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

XULTOPHY

Insulin degludec
Standard 2-year carcinogenicity studies in animals have not been performed to evaluate the carcinogenic potential of insulin degludec.

In a 52-week study including human insulin (NPH insulin) as comparator, Sprague-Dawley rats were dosed subcutaneously with insulin degludec at 3.3, 6.7, and 10 U/kg/day, resulting in 5 times the human exposure (AUC) when compared to a human subcutaneous dose of 0.75 U/kg/day. Human insulin was dosed at 6.7 U/kg/day. No treatment-related increases in incidences of hyperplasia, benign or malignant tumors were recorded in female mammary glands from rats dosed with insulin degludec and no treatment related changes in the female mammary gland cell proliferation were found using BrdU incorporation. Further no treatment related changes in the occurrence of hyperplastic or neoplastic lesions were seen in any animals dosed with insulin degludec when compared to vehicle or human insulin.

In a combined fertility and embryo-fetal study in male and female rats, treatment with insulin degludec up to 21 U/kg/day prior to mating and in female rats during gestation had no effect on mating performance and fertility.

Liraglutide
A 104-week carcinogenicity study was conducted in male and female CD-1 mice at doses of 0.03, 0.2, 1.0, and 3.0 mg/kg/day liraglutide administered by bolus subcutaneous injection yielding systemic exposures 0.2-, 2-, 10- and 45-times the human exposure, respectively, at the MRHD of 1.8 mg/day based on plasma AUC comparison. A dose-related increase in benign thyroid C-cell adenomas was seen in the 1.0 and the 3.0 mg/kg/day groups with incidences of 13% and 19% in males and 6% and 20% in females, respectively. C-cell adenomas did not occur in control groups or 0.03 and 0.2 mg/kg/day groups. Treatment-related malignant C-cell carcinomas occurred in 3% of females in the 3.0 mg/kg/day group. Thyroid C-cell tumors are rare findings during carcinogenicity testing in mice. A treatment-related increase in fibrosarcomas was seen on the dorsal skin and subcutis, the body surface used for drug injection, in males in the 3 mg/kg/day group. These fibrosarcomas were attributed to the high local concentration of drug near the injection site. The liraglutide concentration in the clinical formulation (6 mg/mL) is 10-times higher than the concentration in the formulation used to administer 3 mg/kg/day liraglutide to mice in the carcinogenicity study (0.6 mg/mL).

A 104-week carcinogenicity study was conducted in male and female Sprague Dawley rats at doses of 0.075, 0.25 and 0.75 mg/kg/day liraglutide administered by bolus subcutaneous injection with exposures 0.5-, 2- and 8-times the human exposure, respectively, resulting from the MRHD based on plasma AUC comparison. A treatment-related increase in benign thyroid C-cell adenomas was seen in males in 0.25 and 0.75 mg/kg/day liraglutide groups with incidences of 12%, 16%, 42%, and 46% and in all female liraglutide-treated groups with incidences of 10%, 27%, 33%, and 56% in 0 (control), 0.075, 0.25, and 0.75 mg/kg/day groups, respectively. A treatment-related increase in malignant thyroid C-cell carcinomas was observed in all male liraglutide-treated groups with incidences of 2%, 8%, 6%, and 14% and in females at 0.25 and 0.75 mg/kg/day with incidences of 0%, 0%, 4%, and 6% in 0 (control), 0.075, 0.25, and 0.75 mg/kg/day groups, respectively. Thyroid C-cell carcinomas are rare findings during carcinogenicity testing in rats.

Studies in mice demonstrated that liraglutide-induced C-cell proliferation was dependent on the GLP-1 receptor and that liraglutide did not cause activation of the REarranged during Transfection (RET) proto-oncogene in thyroid C-cells.

Human relevance of thyroid C-cell tumors in mice and rats is unknown and has not been determined by clinical studies or nonclinical studies *[see Boxed Warning and Warnings and Precautions (5.2)]*.

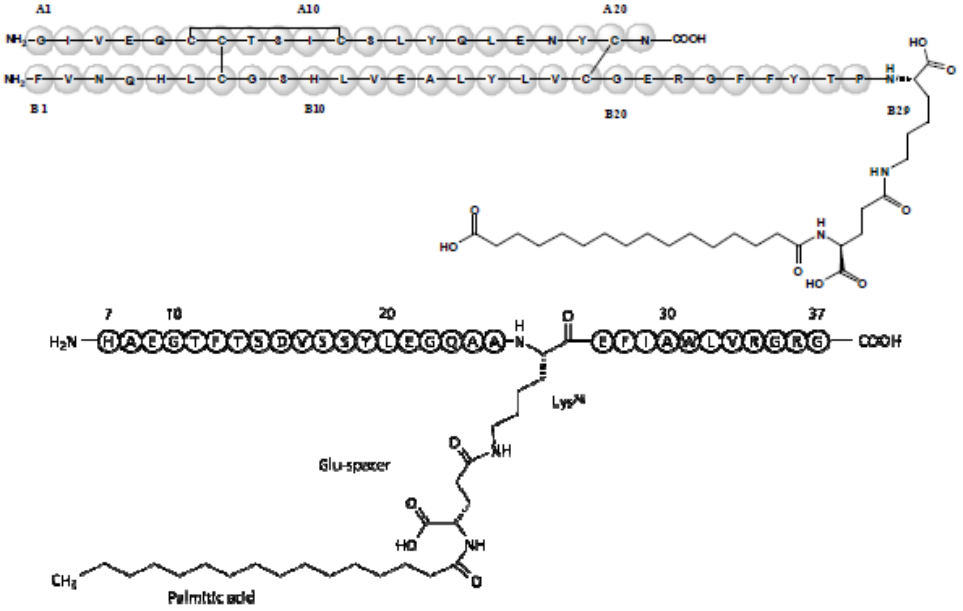
Liraglutide was negative with and without metabolic activation in the Ames test for mutagenicity and in a human peripheral blood lymphocyte chromosome aberration test for clastogenicity. Liraglutide was negative in repeat-dose *in vivo* micronucleus tests in rats.

In rat fertility studies using subcutaneous doses of 0.1, 0.25 and 1.0 mg/kg/day liraglutide, males were treated for 4 weeks prior to and throughout mating and females were treated 2 weeks prior to and throughout mating until gestation day 17. No direct adverse effects on male fertility was observed at doses up to 1.0 mg/kg/day, a high dose yielding an estimated systemic exposure 11- times the human exposure at the MRHD, based on plasma AUC. In female rats, an increase in early embryonic deaths occurred at 1.0 mg/kg/day. Reduced body weight gain and food consumption were observed in females at the 1.0 mg/kg/day dose.

2 Drug Information

2.1 Drug

- CAS Registry Number: 11061-69-0 (human insulin) and 87805-34-3 (glucagon-like peptide 1)
- Generic Name: Insulin degludec and liraglutide
- Code Name: IDegLira: GIC; IDeg: insulin 454
- Chemical Name: LysB29(Nε-hexadecandioyl-γ-Glu) des(B30) human insulin (IDeg) and $Arg^{34}Lys^{26}$-(N-ε-(γ-Glu-(N-α-hexadecanoyl)))-GLP-1[7-37] (liraglutide)
- Molecular Formula: $C_{274}H_{411}N_{65}O_{81}S_6$ (IDeg) and $C_{172}H_{265}N_{43}O_{51}$ (liraglutide)
- Structure or Biochemical Description:



- Pharmacologic Class: Insulin analog (insulin degludec) and GLP-1 receptor agonist (liraglutide)

2.2 Relevant INDs, NDAs, BLAs and DMFs

NDA 203314 for insulin degludec and NDA 022341 for liraglutide

2.3 Drug Formulation

Insulin degludec/liraglutide (100 U/3.6 mg/mL) is a clear and colorless solution filled in a 3 mL cartridge and assembled into a pre-filled disposable pen-injector. The IDegLira product to be marketed is formulated in a vehicle containing glycerol (19.7 mg/mL) (b) (4), zinc (55 µg/mL) (b) (4) and phenol (5.7 mg/mL) (b) (4)

Glycerol and zinc are present in similar or higher concentrations in currently marketed insulin products, e.g., insulin detemir (Levemir®), and insulin glargine (Lantus®) compared to IDegLira. (b) (4) A phenol concentration of (b) (4) mg/mL in IDegLira was tested in the rabbit local toxicity study with only mild local injection site reactions thereby qualifying the concentration of 5.7 mg/mL phenol in the product to be marketed with respect to local toxicity. The amount of phenol administered at the maximum recommended human dose of IDegLira (50 U/1.8 mg) is (b) (4) mg. This is (b) (4) fold below the Reference Dose for Chronic Oral Exposure (RfD) of 0.3 mg/kg/day established by US Environmental Protection Agency (EPA) - Integrated Risk Information System (IRIS). The level of phenol in the IDegLira formulation is therefore considered to be acceptable for use.

Table 1: Drug Formulation of IDegLira

Composition of insulin degludec/liraglutide (100 U/3.6 mg/ml)

Component	Quantity per ml	Function	Reference to standards
Active substance			
Insulin degludec	600 nmol (100 U)	Active substance	Novo Nordisk A/S
Liraglutide	3.6 mg (960 nmol)	Active substance	Novo Nordisk A/S
Excipient			
Phenol	5.70 mg[A]	(b) (4)	Ph Eur, USP, JP
Glycerol	19.7 mg		Ph Eur, USP, JP
Zinc (b) (4)	55.0 µg		Ph Eur, USP, JPE
Hydrochloric acid	q.s.[B]		Ph Eur, USP, JP
Sodium hydroxide	q.s.[B]		Ph Eur, USP, JP
Water for injections	To make 1.00 ml		Ph Eur, USP, JP

[A] An overage (b) (4) is added to compensate for loss during manufacturing

[B] To reach pH 8.15

Insulin degludec/liraglutide formulations tested in clinical trials
Note: Highlighted IDegLira A3 and B3 were tested in nonclinical studies.

Commodity name	IDegLira (A3)	IDegLira (B3)	IDegLira (B5)	IDegLira (100 U/3.6 mg/ml)[A]
Clinical development	Phase 1	Phase 1	Phase 3	Bioequivalence
Quantity per ml				
Active substances				
Liraglutide	(b) (4)	3.60 mg	3.60 mg	3.60 mg
Insulin degludec	600 nmol	600 nmol	600 nmol	600 nmol
Excipients				
Phenol[B]	(b) (4)			5.70 mg
Glycerol				19.7 mg
Zinc				55.0 µg
Hydrochloric acid	q.s.	q.s.	q.s.	q.s.
Sodium hydroxide[C]	q.s.	q.s.	q.s.	q.s.
WFI	To make 1.00 ml	To make 1.00 ml	To make 1.00 ml	To make 1.00 ml
Characteristics of drug product				
pH	(b) (4)			8.15

[A] Composition corresponding to the formulation of insulin degludec/liraglutide (100 U/3.6 mg/ml) intended for the market. This formulation is also referred to as formulation V2 or IDegLira V2
[B] An overage (b) (4) is added to compensate for potential loss during manufacturing
[C] For pH adjustment to reach target pH

2.4 Comments on Novel Excipients

None of the excipients are classified as novel excipients.

2.5 Comments on Impurities/Degradants of Concern

Impurities in drug product

All liraglutide impurity groups have shelf life specification limits and in-use acceptance criteria consistent with the approved limits for liraglutide 6.0 mg/mL (Victoza®). All IDeg impurity groups (b) (4) have shelf life specification limits and in-use acceptance criteria consistent with the proposed limits for insulin degludec 100 U/mL (Tresiba®). The level of (b) (4) impurities has however been qualified in a 4-week toxicology study in rats conducted as part of the IDeg nonclinical program. Consequently, the impurities are qualified and the proposed shelf life specification limits and in-use acceptance criteria for IDegLira are considered acceptable.

Leachables in drug product

IDegLira is supplied in containers (b) (4) This container closure system is identical to the one used for both insulin degludec (Tresiba®) and liraglutide (Victoza®) drug products. Five leachables (b) (4) have been identified and quantified in a long-term leachable study for IDegLira. Based on a maximum recommended daily clinical dose of 50 U/1.8mg IDegLira, the maximum daily exposure of the five substances was calculated.

Fo (b) (4) maximum clinical exposure (b) (4) would be at least (b) (4) fold below the Permissible Daily Exposure ((b) (4) mg/person/day) established in the ICH Q3C guideline for residual solvents. For (b) (4) since there are no PDE values established in ICH Q3C and they are not genotoxic, maximum clinical exposure to any of these (b) (4) would at least (b) (4) fold below the qualification threshold established by the Product Quality Research Institute (Development of Safety Qualification Threshold and Their Use in Orally Inhaled and Nasal Drug Product Evaluation), considering all non-carcinogenic toxic effects. Therefore, these levels are not considered to be associated with any safety risks to the patients.

Maximum clinical exposure to leachables

Compound	CAS number	Maximal concentration (ug/mL)	Max. clinical s.c. exposure (ug/person/day)[a]
(b) (4)			

[a] Based on a the maximum recommended daily clinical dose of 50 U IDeg and 1.8 mg liraglutide (i.e. volume of 0.5 ml/person/day)
[b] (b) (4)

2.6 Proposed Clinical Population and Dosing Regimen

Insulin degludec/liraglutide (IDegLira) is a combination of the basal insulin analog *insulin degludec* (IDeg) and the GLP-1 analog liraglutide. IDegLira is intended for improvement of glycemic control in adults with type 2 diabetes mellitus (T2DM) via once-daily subcutaneous injection administered at any time of the day. The combination product will be provided in a pre-filled pen containing an IDeg/liraglutide ratio of 100 units/3.6 mg per mL. The recommended daily starting dose of IDegLira is (b) (4) units of IDeg and (b) (4) mg liraglutide). A maximum starting dose of 16 units insulin degludec and 0.6 mg liraglutide) can be used for patients transferring from basal insulin treatment or patients converting form GLP-1 receptor agonists. The maximum starting dose of 16 units insulin degludec/0.6 mg liraglutide is consistent with the recommended start dose of liraglutide (Victoza®).

2.7 Regulatory Background

- Oct 2010: pIND/EOP2 memo in DARRTS (1 nonclinical question). Pharm/Tox agreed there was sufficient nonclinical data for IDegLira to support the clinical development of IDegLira.
- Jan 2011: IND 109121 submitted and nonclinical review (PD, PK, and TOX studies) in DARRTS.
- June 2011: Type C Written Response (no nonclinical questions). FDA agreed on the design of 2 proposed pivotal clinical trials.
- August 2013: Type C meeting request to discuss the content and appropriate timing for filing the NDA.
- Sept 2013: Meeting denied; interim data needed from the CV outcome trial under NDA 203314 for IDeg (insulin degludec). In April 2015, FDA has accepted the resubmission of NDA 203314 for review and cross reference could be made for IDegLira.
- April 2015: PSP memo (full waiver request – no nonclinical study planned) and Type B pre-NDA memo in DARRTS (1 nonclinical question – sufficient for NDA filing).
- June 2015: pNDA industry meeting
- Sept 2015: NDA 208583 submission for IDegLira

3 Studies Submitted

3.1 Studies Reviewed

All studies are reviewed.

3.2 Studies Not Reviewed

n/a

3.3 Previous Reviews Referenced

NDA 203314 (IDeg) and NDA 22341 (liraglutide)

4 Pharmacology

4.1 Primary Pharmacology

Study No cfle080703: acute effect of GIC in rats

The effects of GIC (insulin 454+liraglutide combination; e.g., ↓ glucose level, ↓ body weight, and ↓ food consumption) were observed, as seen with insulin 454 or liraglutide alone. However, GIC, at all three doses, resulted in a slightly earlier reduction in blood glucose than insulin 454, while the maximal reduction in blood glucose was slightly smaller for GIC than for insulin 454.

Study title: Acute effects of GIC on blood glucose levels, bodyweight and food intake in rats (GLP)

Reviewer's note: GIC is the code name used for the combined insulin 454 and liraglutide. Insulin 454 was later renamed insulin degludec (IDeg).

Study description:
This study was conducted to explore the pharmacodynamic profile of GIC (insulin 454 and liraglutide) versus insulin 454 or liraglutide alone.

This study evaluated the effects of GIC (liraglutide + insulin 454) on 24-hour glucose profiles, body weight and food intake, compared to the effect of liraglutide alone or insulin 454 alone. Wistar rats (n=6 per group) was divided into 3 substudy groups: 6/16, 15/40, 30/80 nmol/kg (IDeg/Lira). Each substudy group had 4 different dosing groups: placebo, liraglutide, insulin 454, and GIC (insulin 454+liraglutide). A baseline blood sample was collected and bodyweights recorded right before dosing with vehicle (placebo), liraglutide, insulin 454, or GIC. The rats were dosed subcutaneously in the morning and blood was drawn 0.5, 1, 2, 3, 6, and 24 hrs post dosing to determine blood glucose levels. A blood sample was also collected for plasma exposure to determine liraglutide and insulin 454 in the liraglutide/GIC and insulin 454/GIC, respectively. After dosing, cage observation was done during the first 6 hrs and again the following morning at the 24-hr sampling time. The rats had free access to food and water during the study. Food and water weights were measured at baseline and 24 hrs after dosing. The animals were sacrificed at the end of the study.

> Liraglutide: batch TG50297 (Product batch 5199025), 6.0 mg/ml, vehicle for dilution batch TG50655 (Product batch 5199026)
>
> Insulin 454: batch LP454KCV01(Product batch 412_N08429) 600 nmol/ml, vehicle for dilu-tion batch N.A. (Product batch 412_N08429)
>
> GIC: Liraglutide: G1K4S005 (Product batch 412_N08435), 1600 nmol/ml (6.0 mg/ml), Insu-lin 454: LP454KCV01, Vehicle for dilution and placebo group, batch N.A. (Poduct batch 412_N08467)

Study findings:
- IDegLira resulted in a dose-dependent reduction in blood glucose. The effect was gone 24 hrs after dosing.
- IDegLira resulted in dose-dependent decreases in body weight 24 hr after dosing.
- IDegLira resulted in dose-dependent decreases in 24 hr food and water consumption.
- All doses of GIC resulted in a slightly earlier reduction in blood glucose than insulin 454.
- The maximal reduction in blood glucose was slightly smaller for GIC than for insulin 454.

Figure 1 CFIe080703 pharmacology study in rats: blood glucose level

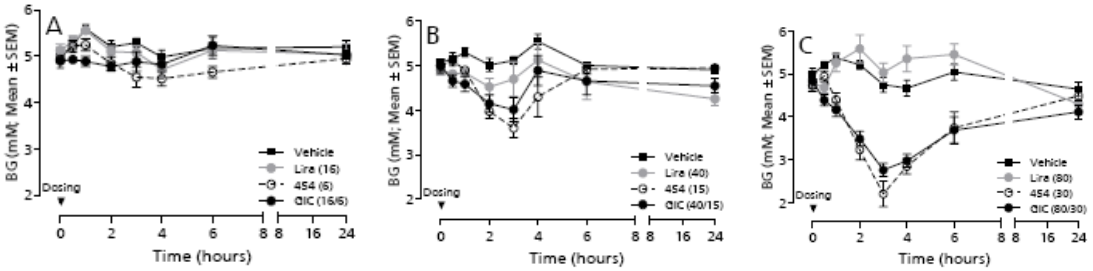


Figure 2 CFIe080703 pharmacology study in rats: 24 hr changes in body weight

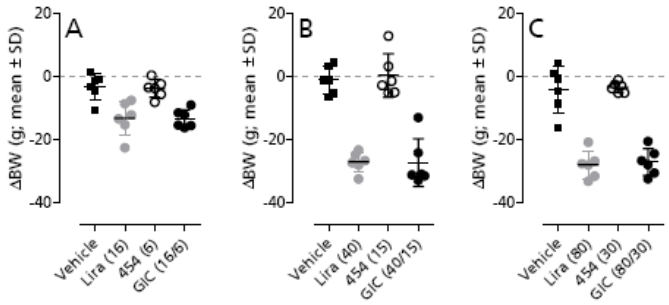


Figure 3 CFIe080703 pharmacology study in rats: food intake

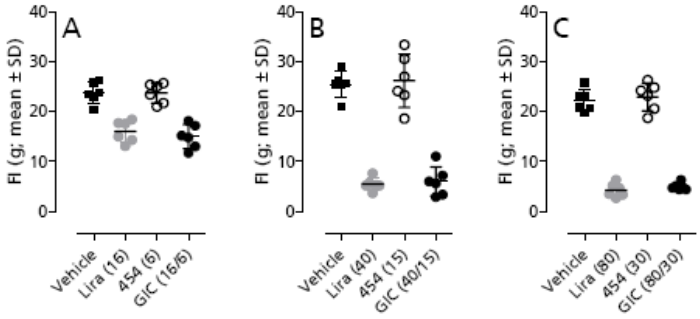


Figure 4 CFIe080703 pharmacology study in rats: water intake

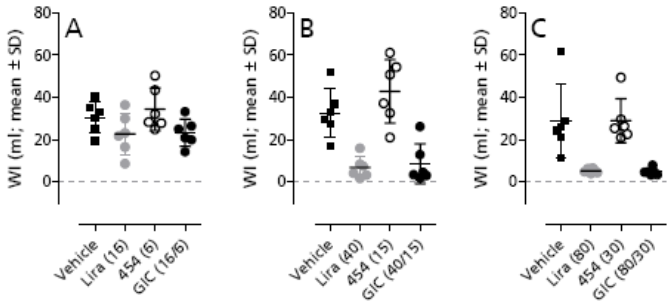


Figure 5 CFIe080703 pharmacology study in rats: 24 hours liraglutide plasma levels

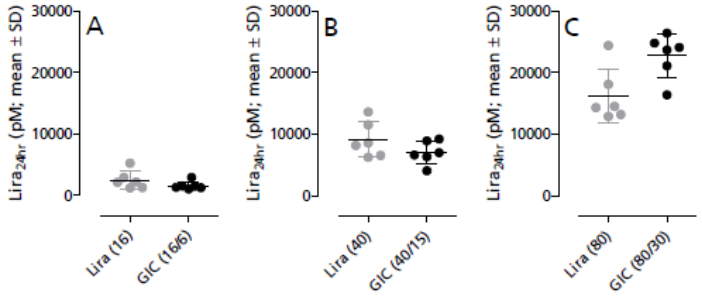
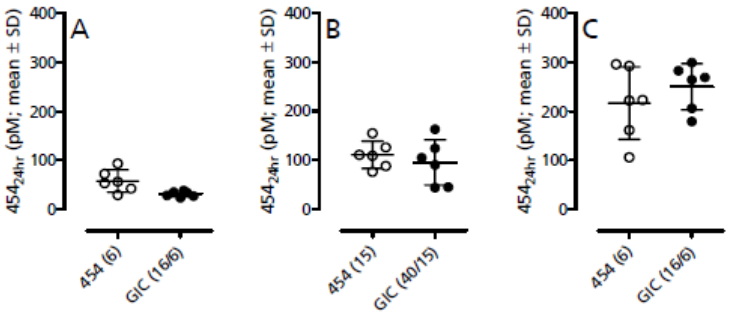


Figure 6 CFIe080703 pharmacology study in rats: 24 hours insulin454 plasma levels



4.2 Secondary Pharmacology

No studies were conducted for IDegLira.

4.3 Safety Pharmacology

In accordance with ICH M3 (R2), safety pharmacology studies were not conducted for IDegLira since each individual component has been tested (under IDeg NDA 203314 and liraglutide NDA 22341). The pharmacological properties of IDeg and liraglutide are mediated via distinct cellular receptors and signaling pathways with no indication of molecular pharmacological interaction.

5 Pharmacokinetics/ADME/Toxicokinetics

The PK profile of IDegLira (600/ (b) (4) and 600/960 formulations) was based on PK/TK parameters obtained in Wistar rats (4 week and 13 week repeat dose toxicity studies) and in female LYD pigs (AnP090201, AnP090302, AnP090401). *Note: For TK rat data, see repeat dose toxicity study section*.

5.1 PK/ADME

Study No anp090201: GIC (B)

Study title: Pharmacokinetics after SC administration to LYD pig of three versions of IGM combination GIC (B) and one version kept stressed in comparison to references of insulin 454 and liraglutide (GLP)

Reviewer's Note: IGM is related to the study project number (Project No IGM0132), and generally refers to IDeg/liraglutide combinations. GIC (B) refers to the 0.6/0.96 mM combination ratio (b) (4) Insulin 454 is the code name for IDeg.

Study description:
The study was conducted to determine the pharmacokinetics in LYD pigs of three formulation versions of IGM mixtures, corresponding to GIC (B) with insulin 454 (0.6 mM) and liraglutide (0.96 mM).

Eight female LYD pigs were dosed with insulin 454 (0.9 nmol/kg BW) and liraglutide (1.44 nmol/kg BW) according to the dosing sequence and dose code formulation listed below. Following subcutaneous administration blood samples were collected as follows: 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 12, 24, 30, 48, and 72 hrs after dosing. Based on the tables below, these three GIC(B) formulations had (b) (4) In addition, GIC(B1) under stressed condition (1 week at 37°C) was also tested perhaps to see if altering drug storage condition would affect its pharmacokinetic profile. The LYD pig was selected by as the test model because of its proven suitability as model for subcutaneous administration in man.

Treatment and days	Pig 1	Pig 2	Pig 3	Pig 4	Pig 5	Pig 6	Pig 7	Pig 8
02 Feb. 2009	A+B	C	D	E	A+B	C	D	E
06 Feb. 2009	E	A+B	C	D	E	A	B	C
10 Feb. 2009	D	E	A+B	C	D	E	A+B	C
16 Feb. 2009	C	D	E	A+B	C	D	E	A+B
20 Feb. 2009	F	F	F	F	F	F	F	F

Code of formulation	Composition (b) (4)	pH (b) (4)	Dose nmol/kg	
			454	liraglutide
A SIBA (M) 0132-0024-0001-2A			0.9	-
B Victoza® 0132-0028-0001-1A				1.44
C, GIC (B1) 0132-0025-0001-2A			0.9	1.44
D, GIC (B2) 0132-0027-0001-1A			0.9	1.44
E, GIC (B3) 0132-0026-0001-1A			0.9	1.44
F is a stressed version of formulation C GIC (B1) 0132-0025-0001-1A	C, kept 1 week at 37°C		0.9	1.44

Study findings:

- The pharmacokinetics of insulin 454 from the three GIC (B) formulations did not show statistical significant differences when compared with the pharmacokinetics from the SIBA (M) formulation, (b) (4)
- For liraglutide, the relative bioavailability (expressed as dose normalized AUC and the values for maximal liraglutide plasma concentration, C_{max}) showed two third of expected values, which such difference was highly significant. In order to elucidate this observation, an explanatory study was later initiated (AnP090401).
- For the two versions of formulation GIC (B1), fresh and stressed after one week at 37°C, there were no statistically significant differences for any of the pharmacokinetic parameters investigated neither for insulin 454 nor for liraglutide.

Summary of pharmacokinetics for insulin 454 from the three GIC (B) formulations and SIBA (M) reference and the stressed GIC (B1) formulation.

Formulation		T_{max} (h)	C_{max} (pM)	λz (h-1)	T½ (h)	AUC (h·pM	AUC_%Extr. (%)	AUC/D (n·pM/nmol/kg)	$AUC_{0-3.5}$ (h·pM)	MRT (h)
A = SIBA										
(M)	Mean	4.2	584	0.1731	4.6	5299	14	5888	1414	8.2
	SD	2.6	215	0.0683	1.7	1050	8	1166	638	2.7
C = GIC										
(B1)9	Mean	3.4	551	0.1602	4.9	5722	21	6358	1396	8.9
	SD	3.0	189	0.0611	1.9	1700	11	1888	579	1.9
D = GIC										
(B2)	Mean	3.1	605	0.1536	5.6	6139	25	6822	1391	9.6
	SD	1.3	94	0.0702	3.0	918	14	1020	231	3.5
E = GIC										
(B3)	Mean	5.3	567	0.1549	4.8	6584	20	7315	1188	9.3
	SD	1.6	116	0.0397	1.3	945	11	1050	311	2.1
F = stressed										
GIC (B1)	Mean	2.5	643	0.1566	4.7	6426	16	7140	1467	8.5
	SD	0.8	160	0.0428	1.2	1372	8	1525	362	1.7
A vs C	P	0.61	0.76	0.71	0.69	0.57	0.21	0.57	0.95	0.60
A vs D	P	0.27	0.75	0.47	0.34	0.15	0.03	0.15	0.91	0.41
A vs E	P	0.35	0.85	0.55	0.81	0.03	0.21	0.03	0.41	0.42
A vs F	P	0.14	0.57	0.59	0.83	0.10	0.73	0.10	0.85	0.82
C vs F	P	0.47	0.35	0.90	0.81	0.41	0.35	0.41	0.79	0.70

Summary of pharmacokinetics for liraglutide from the three GIC (B) formulations and Victoza® reference and the stressed GIC (B1) formulation.

Formulation	Subject	T_{max} (h)	C_{max} (pM)	λz (h-1)	$t_{½}$ (h)	AUC (h·pM)	AUCExt. (%	AUC/D (h·pM/nmol/kg)	MRT (h)
B =									
Victoza®	Mean	6.6	4753	0.0389	18.7	175555	8	121913	29.0
	SD	3.3	2098	0.0099	4.1	91632	3	63633	5.6
C = GIC									
(B1)	Mean	9.9	7359	0.0365	19.4	283113	6	196606	30.6
	SD	7.0	2257	0.0055	2.9	71903	3	49933	4.3
D = GIC									
(B2)	Mean	7.1	6938	0.0387	18.2	257886	5	179087	29.4
	SD	2.5	960	0.0051	2.3	46277	3	32137	3.7
E GIC (B3)	Mean	10.6	7187	0.0395	18.1	285561	6	198306	30.1
	SD	1.5	1550	0.0072	3.4	85481	5	59362	5.0
F = stressed									
GIC (B1)	Mean	10.1	7917	0.0365	19.8	327462	9	227404	32.3
	SD	6.4	1738	0.0074	4.7	107930	5	74951	6.9
B vs C	P	0.66	0.01	0.71	0.88	0.01	0.08	0.01	0.70
B vs D	P	0.75	0.02	0.95	0.75	0.04	0.04	0.04	0.88
B vs E	P	0.01	0.03	0.91	0.77	0.04	0.34	0.04	0.71
B vs F	P	0.22	0.01	0.62	0.65	0.01	0.53	0.01	0.35
C vs F	P	0.94	0.61	1.00	0.83	0.38	0.15	0.38	0.59

Study No anp090302: GIC (A)

Title: Pharmacokinetics after SC administration to LYD Pig of three versions of IGM combination GIC (A) in comparison to references of insulin 454 and liraglutide administered SC and IV (GLP)

Reviewer's Note: IGM is related to the study project number (Project No IGM0132), and generally refers to IDeg/liraglutide combinations. GIC (A) refers to the 0.6/(b) (4) mM combination ratio (b) (4) Insulin 454 is the code name for IDeg.

Study description:
The study was conducted to determine the pharmacokinetics in LYD pigs of three formulation versions of IGM mixtures, corresponding to GIC (A) with insulin 454 (0.6 mM) and liraglutide ((b) (4) mM).

Eight female LYD pigs were dosed with insulin 454 (0.9 nmol/kg BW) and liraglutide (2.4 nmol/kg BW) according to the dosing sequence and dose code formulation listed below. Blood samples were collected prior to *subcutaneous* dosing. Following subcutaneous administration, blood samples were collected as follows: 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 12, 24, 30, 48, and 72 hrs after dosing. Following

intravenous dosing samples were taken at 5, 10, 15, 20, 25, 30, 40, 50 minutes, 1, 1.25, 1.5, 2, 2.5, 3, 4, 5, 6, 24, 48, 72 and 96 hrs after dosing. Based on the tables below, these three GIC(A) formulations had (b) (4) The LYD pig was selected by as the test model because of its proven suitability as model for subcutaneous administration in man.

Treatment and days	Pig 1	Pig 2	Pig 3	Pig 4	Pig 5	Pig 6	Pig 7	Pig 8
06 Mar. 2009	A+B	C	D	E	A+B	C	D	E
10 Mar. 2009	E	A+B	C	D	E	A+B	C	D
16 Mar 2009	D	E	A+B	C	D	E	A+B	C
20 Mar 2009	C	D	E	A+B	C	D	E	A+B
26 Mar 2009 Intravenous administration	A+B	A+B	A+B	A+B	A+B	A+B	A+B	A+B

Code of formulation	Composition (b) (4)	pH (b) (4)	Dose nmol/kg	
			454	liraglutide
A SIBA M 0132-0024-0001-3A			0.9	-
B Victoza® 0132-0028-0001-2A				2.4
C, GIC(A1) 0132-0032-0001-1A			0.9	2.4
D, GIC (A2) 0132-0031-0001-1A			0.9	2.4
E, GIC (A3) 0132-0030-0001-1A			0.9	2.4

Study findings:

- There were no statistically significant difference between the parameters for insulin 454 from any of the three GIC (A) formulations and SIBA (M) formulation (b) (4)
- There were for Victoza® observed a higher dose normalized AUC than obtained from the GIC (A) formulations and the difference was statistically significant at a level of p = 0.01 for the GIC (A1) formulation. All other pharmacokinetic parameters remained unchanged.
- Based on single IV PK data, the plasma elimination half-life was 1.07 h for insulin degludec and 11.4 h for liraglutide. For insulin degludec, the clearance was 0.138 L/hr/kg and the volume of distribution was 0.212 kg. For liraglutide clearance was 0.00263 L/hr/kg and the volume of distribution was 0.0424 L/kg.

Summary of pharmacokinetics for insulin 454 from the three GIC (A) formulations and SIBA (M) as reference.

454 Formulation		T_{max} (h)	C_{max} (pM)	λ_z (h-1)	$t_{½}$ (h)	AUC (pM*h)	AUCExtr (%)	AUC/D (pM*h/ nmol/kg)	AUC0_3.5 (pM*h)	AUC0_12 (pM*h)	BA (%)
A SIBA (M)	Mean	3.00	369	0.1047	7.0	4350	22	4834	885	2860	60
	SD	2.63	160	0.0265	1.7	1522	16	1691	416	1198	21
C GIC (A1)	Mean	3.33	400	0.0970	7.6	4951	31	5502	917	3019	68
	SD	3.03	149	0.0298	1.8	1240	20	1377	299	818	17
D GIC(A2)	Mean	3.59	422	0.1650	4.8	4510	15	5011	915	3242	62
	SD	3.18	96	0.0685	1.7	1333	9	1481	249	808	18
E GIC(A3)	Mean	4.17	401	0.1235	6.8	5456	25	6062	959	3506	75
	SD	2.32	90	0.0630	3.1	1246	17	1385	183	800	17
A vs C	t test p	0.98	0.90	0.84	0.64	0.97	0.60	0.97	0.87	0.92	0.97
A vs D	t test p	0.99	0.38	0.10	0.10	0.94	0.20	0.94	0.63	0.52	0.94
A vs E	t test p	0.53	0.80	0.93	0.69	0.33	0.60	0.33	0.61	0.47	0.33

Summary of pharmacokinetics for liraglutide from the three GIC (A) formulations and Victoza® as reference.

Liraglutide formulation		T_{max} (h)	C_{max} (pM)	Λz (h^{-1})	$t_{½}$ (h)	AUC (pM*h)	AUCExtr (%)	AUC/D (pM*h/ nmol/kg)	BA (%)
B, Victoza	Mean	5.86	16614	0.0433	16.2	539664	6	224860	48
	SD	1.77	3059	0.0051	1.9	101634	2	42347	9
C GIB A1	Mean	5.93	13457	0.0480	14.7	370431	4	154346	33
	SD	2.49	4540	0.0069	2.2	114659	2	47775	10
D GIC A2	Mean	5.50	13788	0.0456	15.9	474656	6	197774	42
	SD	2.73	1913	0.0091	3.9	79481	4	33117	7
E GIC A3	Mean	5.92	12637	0.0457	15.4	447430	5	186429	40
	SD	3.14	2899	0.0069	2.1	97906	2	40794	9
	b vs c	0.95	0.15	0.17	0.21	0.01	0.29	0.01	0.01
	b vs d	0.77	0.05	0.56	0.84	0.19	0.72	0.19	0.19
	b vs e	0.97	0.04	0.47	0.50	0.13	0.83	0.13	0.13

Summary of pharmacokinetic results for insulin 454 from SIBA (M) and liraglutide from Victoza® following intravenous administration

Compound and dose (nmol/kg)	C_0 (pM)	T½ (h)	λz (h^{-1})	AUCINF (pM*h)	AUC/D (pM*h/nmol/kg)	Vz (L/kg)	Cl (l/kg/h)
SIBA (M) 0.9	8	8	8	8	8	8	8
Mean	7303	1.07	0.6502	7280	8089	0.21177	0.13770
SD	1765	0.09	0.0515	1986	2207	0.09062	0.06203
Victoza® 2.4	8	8	8	8	8	8	8
Mean	102032	11.4	0.0618	1124739	468641	0.04240	0.00263
SD	27356	1.6	0.0084	373765	155735	0.02873	0.00183

Study No anp090401: GIC (A) and GIC (B)

Study title: Pharmacokinetics after SC administration to LYD pig of IGM combinations GIC (A) and GIC (B) in comparison to reference of liraglutide (GLP)

Reviewer's note: IGM is related to the study project number (Project No IGM0132), and generally refers to IDeg/liraglutide combinations. GIC (A) refers to the 0.6/ (b) (4) mM combination ratio and GIC (B) refers to the 0.6/0.96 mM combination ratio (b) (4) Insulin 454 is the code name for IDeg.

Study description:
The study was conducted to repeat two formulation versions of IGM mixtures (i.e. GIC (A3) and GIC (B3)) and compare the pharmacokinetic profile between these two formulations.

The 1st part of the study was a repeat of study AnP090201 with dosing 1.44 nmol/kg of liraglutide from both GIC (B3) (0.6 mM insulin 454 and 0.96 mM liraglutide) and the Victoza® reference with (b) (4) mM liraglutide. The 2nd part of the study was a repeat of study AnP090302 with dosing 2.4 nmol/kg of liraglutide from both GIC (A3) (0.6 mM insulin 454 and (b) (4) mM liraglutide) and the Victoza® reference with (b) (4) mM liraglutide. Eight female LYD pigs were dosed with insulin 454 and liraglutide according to the dosing sequence and dose code formulation listed below. The subcutaneous doses were given on the side of the neck in depth of 4 mm, using Novo Pen 4®. Before dosing, a catheter was placed in the jugular vein. A volume of about 5 mL blood was drawn with a syringe from the catheter. Following subcutaneous administration, blood samples were collected at 0, 0.25, 0.5, 0.75., 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 12, 24, 30, 48 and 72 hrs after dosing. Based on the tables below, these two formulations had a (b) (4) different liraglutide concentrations. The LYD pig was selected by as the test model because of its proven suitability as model for subcutaneous administration in man.

Treatment and days	316	317	318	319	320	321	322	323
Week 16	A	B	A	B	A	B	A	B
Week 17	B	A	B	A	B	A	B	A
Week 18	C	D	C	D	C	D	C	D
Week 19	D	C	D	C	D	C	D	C

Code of formulation	Composition (b) (4)	pH (b) (4)	Dose nmol/kg	
			454	Liraglutide
B equals Victoza® 0132-0028-0001-3A			-	1.44
D equals Victoza® 0132-0028-0001-4A			-	2.4
A GIC (B3) 412_N08709 0132-0026-0001-2A			0.9	1.44
C GIC (A3) 412_N08718 0132-0030-0001-2A			0.9	2.4

Study findings:
- Bioavailability of insulin 454 from GIC (B) was 86% and from GIC (A) 92%.
- Bioavailability for liraglutide from the GIC (B) formulation was 46% and from GIC (A) formulations was 52%.
- For the GIC (A) formulation, a slightly later t_{max} (from 5.4 to 8.3 hrs, with p=0.02) and lower C_{max} (from 18786 to 14679 pM, with p=0.01) was observed for liraglutide when compared to the reference formulation. These observations, together with unchanged dose normalized AUC and unchanged plasma elimination, were not be of any significant effect under steady state circumstances.
- For the GIC (B) formulation, no statistical significant difference between the pharmacokinetics parameters was obtained for liraglutide when compared to the Victoza® reference formulation.

Summary of pharmacokinetics for insulin 454 from the GIC (A) and GIC (B) formulations

	Tmax (h)	Cmax (pM)	Lambda_z (h-1)	t½ (h)	AUCINF (h·pM)	AUCExtrap (%)	AUC/D (h·pM/nmol/kg)	F (%)
GIC (B)								
NObs	7	7	7	7	7	7	7	
Mean	3.2	534	0.1188	6.1	6267	13	6963	86.1
SD	2.7	141	0.0262	1.2	598	9	664	8.2
GIC (A)								
NObs	7	7	7	7	7	7	7	
Mean	3.6	467	0.0901	7.9	6722	10	7469	92.3
SD	3.5	228	0.0168	1.5	932	4	1036	12.8
GIC(A) vs GIC(B)	0.80	0.57	0.05	0.03	0.29	0.43	0.29	0.29

Summary of pharmacokinetics for liraglutide from the GIC (A) and GIC (B) formulations and Victoza 1.44 nmol/kg and 2.4 nmol/kg

	Tmax (h)	Cmax (pM)	Lambda_z (h-1)	t½ (h)	AUCINF (h·pM)	AUCExtrap (%)	AUC/D (h·pM/nmol/kg)	F (%)
GIC (B)								
NObs	7	7	7	7	7	7	7	
Mean	8.4	7571	0.0339	21.0	313190	11	217493	46.4
SD	3.4	1623	0.0063	3.8	28978	4	20123	4.3
Victoza® 1.44 nmol/kg								
NObs	7	7	7	7	7	7	7	
Mean	6.3	9080	0.0348	20.1	314230	9	218215	46.6
SD	2.4	2635	0.0033	2.1	62185	3	43184	9.2
GIC (A)								
NObs	7	7	7	7	7	7	7	
Mean	8.3	14679	0.0320	22.1	584286	14	243452	51.9
SD	2.6	3330	0.0046	3.7	90494	7	37706	8.0
Victoza® 2.4 nmol/kg)								
NObs	7	7	7	7	7	7	7	
Mean	5.4	18786	0.0326	21.3	619679	10	258199	55.1
SD	2.6	1448	0.0023	1.4	68352	1	28480	6.1
GIC (B) vs Victoza® 1.44 nmol/kg p	0.16	0.37	0.76	0.59	0.97	0.47	0.97	0.97
GIC (A) vs Victoza® 2.4 nmol/kg p	0.02	0.01	0.76	0.60	0.09	0.16	0.09	0.09

Study No anp080203: Zn

Study title: Liraglutide: Pharmacokinetics after SC administration in LYD pigs of liraglutide (b) (4)

Study description:
This study determined the subcutaneous pharmacokinetics in LYD pigs of liraglutide in the formulation used in phase I clinical trials in comparison (b) (4) The LYD pig was selected by as the test model because of its proven suitability as model for subcutaneous administration in man.

Formulation	Content	Delivery
A	(b) (4)	3 ml Penfill® batch 0132-0010-0001-2A
B		3 ml Penfill® batch 0132-0015-0001-1A
C		3 ml Penfill® batch 0132-0014-0001-1A

Study findings:

- (b) (4)
- The time for maximal plasma concentration occurred (b) (4)
- The relative bioavailability of the formulations in terms of dose normalized area under the curve was (b) (4)

Tabulated summary of pharmacokinetics for liraglutide (b) (4) (b) (4)

Formulation	Subject	Tmax (h)	Cmax (pmol/L)	AUC (h*pmol/L)	AUC/D (h*kg/L)	Lambda_z (1/h)
A	Mean	5.2	20143	581189	242	0.043
	SD	1.9	2094	122573	51	0
	Harmonic mean	4.5	19970	556868	232	0.042
B	Mean	8.4	13214	479796	200	0.037
	SD	2.1	1577	71351	30	0
	Harmonic mean	7.9	13065	470317	196	0.036
C	Mean	14.5	6608	308851	129	0.037
	SD	5.9	1447	85963	36	0
	Harmonic mean	13	6334	289539	121	0.036

*The mean in the table is arithmetic mean

5.2 Toxicokinetics

The TK data was included in 4 week and 13 week repeat dose toxicity studies. See General Toxicology section for details.

6 General Toxicology

6.1 Single-Dose Toxicity

No single-dose studies were conducted with the IDegLira combination product.

6.2 Repeat-Dose Toxicity

Study No 209212: 13 week rat with IDegLira 600/960 (B3)

Study title: GIC B3: 3-month toxicity study in rats with recovery period and satellite groups for Toxicokinetics

Study no.:	209212
Study report location:	SDN2, SN0000
Conducting laboratory and location:	(b) (4)
Date of study initiation:	April 09th 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	NNC 0100-0000-0454 (insulin 454)/NNC 0090-0000-1170 (liraglutide): batch no. XLDP015

Key Study Findings

- NN209212 is the 13 week toxicity study was conducted with the IDegLira 600/960 (B3) formulation with three fixed doses of insulin degludec and liraglutide: 2/3.2, 7/11.2 and 20/32 nmol/kg, respectively. This study showed that the **IDegLira 600/960 (B3)** formulation for 13 weeks was better tolerated than IDegLira 600/ (b) (4) (A3) formulation for 4 weeks in rats. IDegLira (600/960) is the clinical ratio.
- No treatment-related clinical signs were seen in the animals throughout the study and no effects were observed in the food consumption.
- No changes were observed at the ophthalmoscopic examination or in at the hematology evaluation, urinalysis and urine microscopy.
- No changes were seen in organ weights and the changes reported in the subcutaneous injection sites were of equal severity in the vehicle control group and in all groups treated with GIC B3.
- An expected lower body weight and body weight gain was recorded in Group 4 compared to Group 1 due to the treatment with liraglutide. The treatment also resulted in a low level of total protein and globulin (and thereby an increase in albumin/globulin ratio) as well as the high level of carbamide (UREA). These findings are considered related to the expected pharmacological effect of liraglutide (weight reduction). The higher level of chloride observed in Group 4 (both sexes) in Week 13 appeared related to treatment.
- Toxicokinetic data:
    - The C_{max} and $AUC_{0\text{-}24h}$ increased with dose for both male and female animals for both insulin degludec and liraglutide. The increase in exposure was proportional or slightly more than proportional with increasing doses.
    - Limited accumulation was observed for both males and females for both compounds which was consistent with the $t_{½}$ and the dosing interval for both compounds. No clear sex-related differences were observed for any of the compounds.
    - The observed t_{max} ranged from 1 to 4 hrs for IDeg and from 4 to 8 hrs for liraglutide. The estimated t½ ranged from 1.7 to 4.4 for insulin degludec and from 3.1 to 5.8 hours for liraglutide.
    - Assessment of antibody formation showed that antibodies were found for insulin degludec in 1/20, 3/20 and 4/30 animals in the low, medium and high dose groups respectively, and that no antibodies were found for liraglutide. No change in exposure was observed in the insulin degludec antibody positive animals, hence the validity of the toxicity study was unaffected by this finding.

- Since there was no adverse findings in the high dose group, the NOAEL for GIC B3 (IDegLira 600/960) was established at 20/32 nmol/kg/day.

Methods

Doses:	0/0, 2/2.3, 7/11.2, 20/32 (insulin 454/liraglutide nmol/kd/day) *A fixed ratio: 600 nmol insulin 454 and 960 nmol liraglutide*
Frequency of dosing:	Once daily for 13 weeks
Route of administration:	Subcutaneous
Dose volume:	0.5 mg/kg
Formulation/Vehicle:	The vehicle was an aqueous, isotonic solution of glycerol 19.7 mg/mL and phenol (b) (4) mg/mL with a pH of (b) (4): batch no 412_N09545
Species/Strain:	Wistar rats
Number/Sex/Group:	10/sex/group, 4 groups
Age:	5 weeks
Weight:	108-138 g (males), 102-123 g (females)
Satellite groups:	12/sex/group (TK), 5/sex/group (Recovery)
Unique study design:	TK group: Groups 1,2,3 and 4 Recovery Groups 1 and 4 (recovery time: 4 weeks)
Deviation from study protocol:	Some deviations were recorded but had no effect on the study outcome.

Table 2: Study No 209212 13 Week Rat Study - Study Design

Group	Dose* (nmol/kg/day)	Dose concen-tration (nmol/ml)*	Animal Nos (Main study)		Animal Nos (Kinetic)		Animal Nos (Recovery)		Colour code
			Male	Female	Male	Female	Male	Female	
1	0/0	0/0	1-10	11-20	81-94	95-108	193-197	198-202	White
2	3.2/2	6.4/4	21-30	31-40	109-122	123-136			Blue
3	11.2/7	22.4/14	41-50	51-60	137-150	151-164			Green
4	32/20	64/40	61-70	71-80	165-178	179-192	203-207	208-212	Red

*Liraglutide (NNC 0090-0000-1170)/insulin 454 (NNC 0100-0000-0454)

Observations and Results
Mortality
No mortality was observed.

Clinical Signs (daily)
No treatment-related clinical signs and no signs of hypoglycemia were seen in the animals throughout the study. Some animals were seen with thin-hair or having hairless spots and superficial wounds.

Body Weights (once weekly)
Overall, the animals gained weight during the study. However, in Group 4 the weight gain was lower when compared to Group 1. The effect was considered related to the expected pharmacological effect of liraglutide.

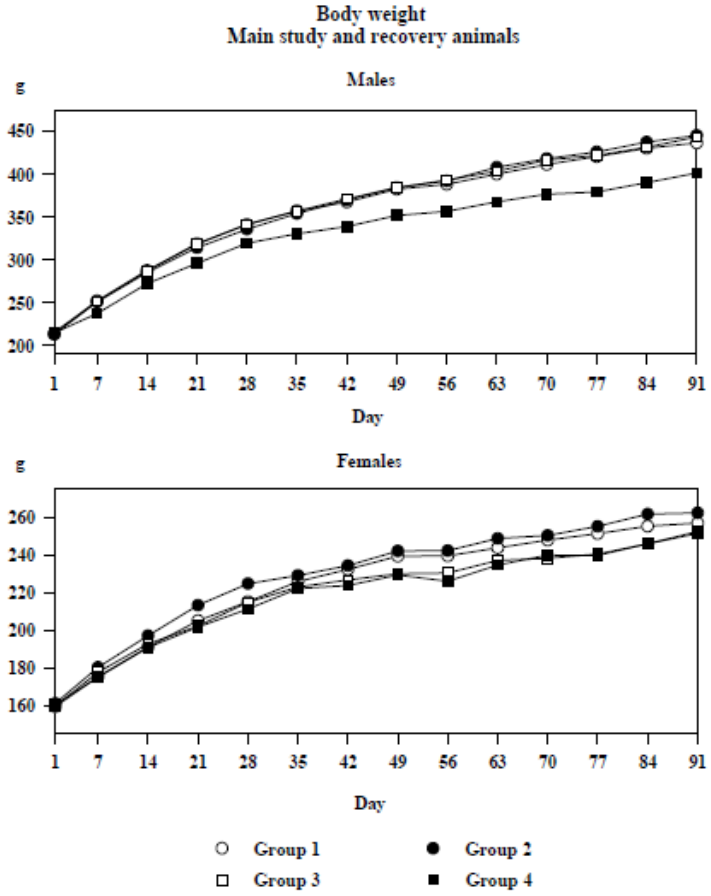
From Day 7 to Day 91, all the males in Group 4 had lower body weights compared to Group 1 and on several occasions, a statistically significant difference was observed.

The main study animals in Groups 2 and 3 gained weight to a similar or slightly higher extent than the animals in Group 1, whereas the TK animals in Groups 2 and 3 had a lower body weight compared to the TK animals in Group 1. In addition, from Day 14 (TK animals) or 21 (main study and recovery animals) to Day 91, the females in Group 4 had slightly lower body weights compared to Group 1. A statistically significant difference was only observed on Day 56 (main study, recovery animals). Moreover, half way through the treatment period, a slightly lower body weight was also observed in Group 3 (main study, recovery as well as TK). However, at the end of the treatment period, the body weights of the TK animals

in Groups 1, 3 and 4 were comparable. Overall, the results were consistent with slight drug-related decreases in body weight gain compared to vehicle controls at the high-dose in males, and the mid and high-dose in females (see figure below).

During the recovery period, the males and the females in Group 4 gained less weight compared to the animals in Group 1.

Figure 7 Study No 209212 13-week toxicity study in rats: body weight



Feed Consumption (once weekly)
The overall food intake during the treatment period was comparable in the main and recovery animals for both sexes. For the TK animals, a tendency towards an overall lower food intake was observed in the males, whereas the opposite was observed for the females. During the recovery period, the food intake was comparable for Groups 1 and 4 (both sexes).

Ophthalmoscopy (before start of dosing and termination)
No treatment-related findings were found.

ECG
This was not done in animals.

Hematology (Weeks 6, 13, and 17)
No clear treatment related changes were observed.

The activated partial thromboplastin time was statistically significantly shorter in Group 4 females on all sampling timepoints (Weeks 6, 13 and 17). However, the values of Group 1 females were higher than Group 1 males. Therefore, the finding was considered related to Group 1 instead of Group 4. In addition, the finding was not linked to other toxicological findings. Therefore, this finding was considered incidental.

In Week 6, an effect on the white blood cells mainly in Group 4 animals was observed. For the males, an increase was observed in the white blood cell count resulting in a significant increase in the number of lymphocytes, whereas a shift was observed in the females. This resulted in a, significant increase in percentage of lymphocytes and thereby a significant decrease in the percentage of neutrophils. Since such finding was only observed in Week 6, it was considered to be of no toxicological importance.

All other findings were considered incidental with no toxicological relevance.

Table 3: Study No 209212 13 Week Rat Study – Hematology Parameters

The group mean values as a percentage of the control (Group 1) for parameter, where a significant difference was observed, are summarised below:

Parameter	Week	Sex	Group 2	Group 3	Group 4
Haemoglobin (Hb)	13	F	-	**↓	-
	17	F			*↓
Haematocrit (HT)	17	F			*↓
Mean cell haemoglobin concentration (MCHC)	6	M	-	**↓	**↓
Lymphocytes (LYMPHO)	6	M	-	-	**↑
% Lymphocytes (LYMPHO)	6	F	-	-	**↑
White blood cell count (WBC)	6	M	-	-	**↑
Neutrophiles (NEUTRO)	6	F	-	*↓	*↓
% Neutrophils	6	F	-	*↓	***↓
Monocytes (logarithmic Transformed)	13	M	-	-	*↑
Activated partial thromboplastin time (APTT)	6	F	-	-	**↓
	13	F	-	-	*↓
	17	F			*↓

M: male F: female
- No statistical significant difference observed
Statistically significant results were reported as P<0.05(*), P<0.01(**) or P<0.001(***)
↑ : increase ↓ : decrease

Clinical Chemistry (Weeks 6, 13, and 17)
As expected, a decrease in the glucose levels was recorded. In Group 4, on Day 1, the decrease was observed at 1, 2, and 12 hours after dosing for males and at 2, 4 and 12 hours after dosing for females. On Day 91 a decrease was observed at 1, 2 and 4 hours after dosing (both sexes) and at 6 and 8 hours after dosing (females) of Group 4. At the end of treatment, a decrease was also observed in Group 2 at 2 hours and in Group 3 at 2 and 4 hours after treatment. On a few occasions, increases in the glucose levels were observed. This was considered a result of the animals reacting to the insulin treatment (rebound effect).

In Week 6, a statistically significantly higher level of ALT and AST was observed in Group 4 males (Group 4 ALT and AST: 0.99±0.26 and 1.53±0.32 vs. Group 1 ALT and AST: 0.72±0.12 and 1.23±0.18). However, the increase was marginal and no changes were observed during the remaining part of the study. The changes in liver enzymes appear transient since the 13 week timepoints showed no increases. Increased ALT and AST were also observed in the 4 week toxicity study in rats.

In Week 6, a tendency towards a lower level of total protein and globulin was observed in Group 4 (both sexes). In Week 13, the low levels in Group 4 were statistically significantly different from Group 1 (both sexes). At the end of the recovery period, the levels were still statistically significantly lower for the males,

whereas only a tendency toward a lower level was observed in the females of Group 4. The albumin/globulin ratio was statistically significantly higher in Group 4 males on all three sampling occasions and a tendency towards the same was observed in Group 4 females.

The level of carbamide (UREA) had statistically significantly increased in males of Groups 2 and 3 in Week 6 and in Group 4 in Weeks 13 and 17. However, on all three occasions, a tendency towards an increase was observed for the remaining males as well as for the females. In Week 13, a significantly higher level of chloride was observed in both sexes of Group 4 as well as in Group 3 females.

The low level of total protein and globulin (and thereby the increase in albumin/globulin ratio) as well as the high level of carbamide were considered related to the expected pharmacological effect of liraglutide (weight reduction). Moreover, the higher level of chloride observed in Group 4 (both sexes) in Week 13 was related to treatment.

Table 4: Study No 209212 13 Week Rat Study – Clinical Chemistry Parameters

Groups significant from Group 1

Parameter	Week	Sex	Group 2	Group 3	Group 4
Alanine aminotransferase (ALAT)	6	M	-	-	***↑
	13	F	*↑	-	-
	17	F			*↓
Aspartate aminotransferase (ASAT)	6	M	-	-	**↑
	13	F	*↑	-	-
	17	F			**↓
Triglycerides (TRIG)	6	M	-	-	**↓
Carbamide (UREA)	6	M	*↑	**↑	-
	13	M	-	-	**↑
	17	M			*↑
Glucose (GLUC)	13	F	-	-	*↑
	17	M			**↑
Magnesium (Mg)	6	F	-	**↓	*↓
Chloride (Cl)	13	M	-	-	**↑
	13	F	-	**↑	*↑
Protein (total) (PROTEIN)	13	M	-	-	*↓
	13	F	-	-	***↓
	17	M			**↓
Globulin	13	M	-	-	**↓
	13	F	-	-	*↓
	17	M			***↓
Albumin/Globulin (ALB/G) ratio	6	M	-	-	*↑
	13	M	-	-	*↑
	17	M			**↑

M: male F: female
- No statistical significant difference observed
Statistically significant results were reported as P<0.05(*), P<0.01(**) or P<0.001(***)
↑ : increase ↓ : decrease

Urinalysis (Weeks 6, 13, and 17)
There were no treatment related findings.

Gross Pathology (termination)
Red discoloration primarily to a slight degree was reported at some of the subcutaneous injection sites. No difference between the dose groups with respect to incidence and severity of this red discoloration was observed. The remaining findings were minor and unremarkable.

Organ Weights (termination)
At the end of the treatment period, the relative weight (% of body weight) was statistically significantly higher for the kidneys in Group 4 males and for the ovaries in Group 4 females. However, these were not correlated with any microscopic findings. In addition, the absolute and relative (% of brain weight) weight

of the adrenals in Group 3 females was statistically significantly low. However, there were no changes observed in Group 4, therefore, this was considered incidental.

At the end of the recovery period, the relative weight (% of body weight) was statistically significantly higher for the liver and spleen and statistically significantly lower for the thymus in Group 4 males. For the females of Group 4, the relative weight (% of body weight) for the ovaries was statistically significantly higher, whereas both the absolute and relative (% of body weight) weight of the heart and the absolute and relative (% of brain weight) weight of the liver were statistically significantly low.

Taking into consideration that no findings were observed in any organs at the end of the treatment period, it was unlikely that changes should have occurred at the end of the recovery period and therefore, the findings were considered incidental. However, as histopathology was not performed for the recovery animals, this could not be confirmed.

Histopathology
Adequate Battery
Yes
Peer Review
Yes
Histological Findings
The changes reported at the subcutaneous injection sites were considered to be of a similar nature and severity in the control group and in Group 4 (high dose group, 20/32 nmol/kg/day IDegLira). The changes were considered related to the vehicle and/or the procedure of subcutaneous injection rather than to treatment with GIC B3.

All other findings reported were considered to be within the background incidence of findings reported in this age and strain of laboratory maintained rats and as such of no toxicological significance. *Note: N.A.D. is denoted as "No Abnormality Detected" in the histopathology tables.*

Table 5: Study No 209212 13 Week Rat Study – Histopath Findings

```
PATHOLOGY REPORT                                    PAGE       :    2/ 107
SUMMARY TABLES                                  Sponsor ref No :    209212
-------------------------------------------------------------------------
TEST ARTICLE  : GIC B3                          PATHOL. NO.: 70255 GN
TEST SYSTEM   : RAT, 3 MONTHS/4 WEEKS REC., SC  DATE       : 26-SEP-10
SPONSOR       : Novo Nordisk A/S                PathData©System V6.2a2
-------------------------------------------------------------------------
NUMBER OF ANIMALS WITH NECROPSY FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0
-------------------------------------------------------------------------
                     DOSE GROUP:      01        02        03        04
                            SEX:     M   F     M   F     M   F     M   F
ORGAN/FINDING        ANIM.EXAM.:    10  10    10  10    10  10    10  10
-------------------------------------------------------------------------
KIDNEYS                         :
- reduced in size.              :     -   -     -   -     -   -     -   1
.........................................................................
URETERS                         :
- dilated.                      :     -   -     -   -     -   -     -   1
.........................................................................
TESTES                          :
- diminished in size.           :     -   -     -   -     1   -     -   -
.........................................................................
THYROID GLAND                   :
- enlarged.                     :     -   -     -   -     1   -     -   -
.........................................................................
THYMUS                          :
- discoloration: red.           :     -   -     -   -     1   -     -   -
.........................................................................
LYMPH NODES                     :
- discoloration: red.           :     1   -     2   -     2   -     1   -
.........................................................................
INJECTION SITE                  :
- discoloration: red.           :     -   1     3   1     3   2     2   2
.........................................................................
```

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PATHOLOGY REPORT                                        PAGE       :    3/ 107
SUMMARY TABLES                                    Sponsor ref No :    209212
-------------------------------------------------------------------------------
TEST ARTICLE  : GIC B3                                  PATHOL. NO.: 70255 GN
TEST SYSTEM   : RAT, 3 MONTHS/4 WEEKS REC., SC          DATE       : 26-SEP-10
SPONSOR       : Novo Nordisk A/S                        PathData@System V6.2a2
-------------------------------------------------------------------------------
NUMBER OF ANIMALS WITH NECROPSY FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: R1
-------------------------------------------------------------------------------
                     DOSE GROUP:      01        02        03        04
                            SEX:     M   F     M   F     M   F     M   F
ORGAN/FINDING        ANIM.EXAM.:     5   5     -   -     -   -     5   5
-------------------------------------------------------------------------------

Necropsy observations recorded, but no table to print with parameter
options selected


PATHOLOGY REPORT                                        PAGE       :    4/ 107
SUMMARY TABLES                                    Sponsor ref No :    209212
-------------------------------------------------------------------------------
TEST ARTICLE  : GIC B3                                  PATHOL. NO.: 70255 GN
TEST SYSTEM   : RAT, 3 MONTHS/4 WEEKS REC., SC          DATE       : 26-SEP-10
SPONSOR       : Novo Nordisk A/S                        PathData@System V6.2a2
-------------------------------------------------------------------------------
NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0
-------------------------------------------------------------------------------
           DOSE GROUP:      01        02        03        04
           SEX       :     M   F     M   F     M   F     M   F
           NO.ANIMALS:    10  10    10  10    10  10    10  10
-------------------------------------------------------------------------------
HEART                :    10  10     -   -     -   -    10  10
             N.A.D.  :     5   9     -   -     -   -     6  10
...............................................................................
- Mononucl cells focal:     5   -     -   -     -   -     4   -
             Grade 1:      3   -     -   -     -   -     4   -
             Grade 2:      2   -     -   -     -   -     -   -
- Fibrosis, focal    :     1   -     -   -     -   -     -   -
             Grade 2:      1   -     -   -     -   -     -   -
- Hemorrhage, focal  :     -   1     -   -     -   -     -   -
             Grade 1:      -   1     -   -     -   -     -   -
-------------------------------------------------------------------------------
LUNG                 :    10  10     -   -     -   -    10  10
             N.A.D.  :     3   5     -   -     -   -     2   6
...............................................................................
- Mononucl cells focal:     1   1     -   -     -   -     -   1
             Grade 1:      1   1     -   -     -   -     -   1
- Alveolar macrophages:     1   1     -   -     -   -     3   1
             Grade 1:      1   1     -   -     -   -     3   1
- Alveolar haemorrhage:     6   4     -   -     -   -     6   2
             Grade 1:      6   4     -   -     -   -     6   2
- Osseous metaplasia :      -   -     -   -     -   -     2   1
-------------------------------------------------------------------------------
TONGUE               :     9  10     -   -     -   -    10  10
             N.A.D.  :     -   -     -   -     -   -     1   -
...............................................................................
- Hemorrhage/inflamma.:    9  10     -   -     -   -     9  10
-------------------------------------------------------------------------------
LIVER                :    10  10     -   -     -   -    10  10
             N.A.D.  :     7   6     -   -     -   -     5   8
...............................................................................
- Necrosis, focal    :     -   1     -   -     -   -     -   -
             Grade 1:      -   1     -   -     -   -     -   -
- Vacuolation        :     3   -     -   -     -   -     3   1
             Grade 1:      3   -     -   -     -   -     2   1
             Grade 2:      -   -     -   -     -   -     1   -
- Mononucl cells/EMH :     2   3     -   -     -   -     2   1
             Grade 1:      2   3     -   -     -   -     2   1
-------------------------------------------------------------------------------
```

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SUMMARY TABLES Sponsor ref No : 209212

TEST ARTICLE : GIC B3 PATHOL. NO.: 70255 GN
TEST SYSTEM : RAT, 3 MONTHS/4 WEEKS REC., SC DATE : 26-SEP-10
SPONSOR : Novo Nordisk A/S PathData©System V6.2a2

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0

DOSE GROUP:	01		02		03		04	
SEX :	M	F	M	F	M	F	M	F
NO.ANIMALS:	10	10	10	10	10	10	10	10
PANCREAS :	10	10	-	-	-	-	10	10
N.A.D. :	9	8	-	-	-	-	7	8
- Atrophy, exocrine :	1	2	-	-	-	-	3	2
Grade 1:	1	2	-	-	-	-	2	2
Grade 2:	-	-	-	-	-	-	1	-
KIDNEYS :	10	10	-	-	-	-	10	10
N.A.D. :	4	4	-	-	-	-	7	7
- Atrophy, diffuse :	-	-	-	-	-	-	-	1
Grade 5:	-	-	-	-	-	-	-	1
- Tub baso/dilation :	2	2	-	-	-	-	2	-
Grade 1:	2	2	-	-	-	-	2	-
- Mineralization :	-	2	-	-	-	-	-	-
Grade 1:	-	2	-	-	-	-	-	-
- Mononucl cells focal:	3	1	-	-	-	-	1	-
Grade 1:	3	1	-	-	-	-	1	-
- Tubular hyaline cast:	-	-	-	-	-	-	-	1
Grade 1:	-	-	-	-	-	-	-	1
- Pelvic dilation :	2	2	-	-	-	-	1	2
URETERS :	10	10	-	-	-	-	9	10
N.A.D. :	10	10	-	-	-	-	9	9
- Dilation :	-	-	-	-	-	-	-	1
TESTES :	10	-	-	-	1	-	10	-
N.A.D. :	10	-	-	-	-	-	10	-
- Tubular atrophy :	-	-	-	-	1	-	-	-
Grade 5:	-	-	-	-	1	-	-	-
PROSTATE GLAND :	10	-	-	-	-	-	10	-
N.A.D. :	10	-	-	-	-	-	9	-
- Mononucl cells focal:	-	-	-	-	-	-	1	-
Grade 1:	-	-	-	-	-	-	1	-

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SUMMARY TABLES Sponsor ref No : 209212

TEST ARTICLE : GIC B3 PATHOL. NO.: 70255 GN
TEST SYSTEM : RAT, 3 MONTHS/4 WEEKS REC., SC DATE : 26-SEP-10
SPONSOR : Novo Nordisk A/S PathData@System V6.2a2

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0

DOSE GROUP:	01		02		03		04	
SEX :	M	F	M	F	M	F	M	F
NO.ANIMALS:	10	10	10	10	10	10	10	10
UTERUS :	-	10	-	-	-	-	-	10
- Estrus :	-	2	-	-	-	-	-	4
- Metestrus :	-	2	-	-	-	-	-	3
- Diestrus :	-	3	-	-	-	-	-	1
- Proestrus :	-	3	-	-	-	-	-	2
PITUITARY GLAND :	10	10	-	-	-	-	10	10
N.A.D. :	10	9	-	-	-	-	10	10
- Cyst, focal :	-	1	-	-	-	-	-	-
THYROID GLAND :	10	10	-	-	1	-	10	10
N.A.D. :	10	10	-	-	-	-	10	10
- Colloid retention :	-	-	-	-	1	-	-	-
Grade 3:	-	-	-	-	1	-	-	-
ADRENAL GLANDS :	10	10	-	-	-	-	10	10
N.A.D. :	7	9	-	-	-	-	5	8
- Z fasc vacuolation :	3	1	-	-	-	-	2	-
Grade 1:	2	1	-	-	-	-	2	-
Grade 2:	1	-	-	-	-	-	-	-
- Z ret/fasc vacuolat.:	-	-	-	-	-	-	3	2
Grade 1:	-	-	-	-	-	-	2	2
Grade 2:	-	-	-	-	-	-	1	-
SPLEEN :	10	10	-	-	-	-	10	10
N.A.D. :	10	-	-	-	-	-	10	5
- Increased EMH :	-	10	-	-	-	-	-	5
Grade 1:	-	-	-	-	-	-	-	1
Grade 2:	-	3	-	-	-	-	-	2
Grade 3:	-	7	-	-	-	-	-	2

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SUMMARY TABLES                                    Sponsor ref No :    209212
-------------------------------------------------------------------------------
TEST ARTICLE  : GIC B3                                  PATHOL. NO.: 70255 GN
TEST SYSTEM   : RAT, 3 MONTHS/4 WEEKS REC., SC          DATE       : 26-SEP-10
SPONSOR       : Novo Nordisk A/S                        PathData@System V6.2a2
-------------------------------------------------------------------------------
NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0
-------------------------------------------------------------------------------
            DOSE GROUP:      01        02        03        04
            SEX       :    M    F    M    F    M    F    M    F
            NO.ANIMALS:   10   10   10   10   10   10   10   10
-------------------------------------------------------------------------------
THYMUS                :   10   10    -    -    1    -   10   10
                N.A.D. :    2    5    -    -    -    -    4    7
...............................................................................
- Incr lymphocytolysis:    -    -    -    -    -    -    1    1
                Grade 1:    -    -    -    -    -    -    1    1
- Hemorrhage, focal   :    8    5    -    -    1    -    5    3
                Grade 1:    6    5    -    -    1    -    4    3
                Grade 2:    2    -    -    -    -    -    1    -
-------------------------------------------------------------------------------
MANDIBULAR LN RIGHT   :   10    9    -    -    -    -    9   10
                N.A.D. :    7    8    -    -    -    -    4    4
...............................................................................
- Hemorrhage, sinusoid:    3    1    -    -    -    -    5    6
                Grade 1:    2    1    -    -    -    -    5    5
                Grade 2:    1    -    -    -    -    -    -    1
-------------------------------------------------------------------------------
PAROTID GLAND, RIGHT  :   10   10    -    -    -    -   10   10
                N.A.D. :    9    9    -    -    -    -    9   10
...............................................................................
- Atrophy, acinar     :    1    -    -    -    -    -    -    -
                Grade 1:    1    -    -    -    -    -    -    -
- Basophilia, acinar  :    -    -    -    -    -    -    1    -
                Grade 1:    -    -    -    -    -    -    1    -
- Mononucl cells focal:    -    1    -    -    -    -    -    -
                Grade 1:    -    1    -    -    -    -    -    -
-------------------------------------------------------------------------------
PATHOLOGY REPORT                                        PAGE       :    8/ 107
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-------------------------------------------------------------------------------
TEST ARTICLE  : GIC B3                                  PATHOL. NO.: 70255 GN
TEST SYSTEM   : RAT, 3 MONTHS/4 WEEKS REC., SC          DATE       : 26-SEP-10
SPONSOR       : Novo Nordisk A/S                        PathData@System V6.2a2
-------------------------------------------------------------------------------
NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0
-------------------------------------------------------------------------------
            DOSE GROUP:      01        02        03        04
            SEX       :    M    F    M    F    M    F    M    F
            NO.ANIMALS:   10   10   10   10   10   10   10   10
-------------------------------------------------------------------------------
INJECTION SITE        :   10   10    3    1    3    2   10   10
                N.A.D. :    1    -    -    -    -    -    -    1
...............................................................................
- Ulceration, epiderm.:    1    -    -    -    -    -    -    -
                Grade 2:    1    -    -    -    -    -    -    -
- Erosion, epidermal  :    -    -    -    -    -    -    1    1
                Grade 1:    -    -    -    -    -    -    1    1
- Necrosis, s.c.      :    3    6    2    -    2    2    6    4
                Grade 1:    3    5    1    -    2    2    4    4
                Grade 2:    -    1    1    -    -    -    2    -
- Inflam cells, s.c.  :    8   10    3    1    3    2   10    9
                Grade 1:    4    7    1    1    1    1    4    6
                Grade 2:    4    3    2    -    2    1    5    3
                Grade 3:    -    -    -    -    -    -    1    -
- Incr fibr/coll s.c. :    9    6    3    1    3    2    9    8
                Grade 1:    3    2    -    -    -    -    3    2
                Grade 2:    4    3    2    1    2    1    4    6
                Grade 3:    2    1    1    -    1    1    2    -
- Hemorrhage, s.c.    :    4    5    2    -    3    2    4    3
                Grade 1:    4    5    -    -    2    1    3    3
                Grade 2:    -    -    1    -    1    1    -    -
                Grade 3:    -    -    1    -    -    -    1    -
- Needle canal        :    -    1    -    -    -    -    -    -
-------------------------------------------------------------------------------
```

Special Evaluation

Blood samples were collected from animals at pre-dose, 1, 2, 4, 6, 8, 12 and 24 hrs post dose on Days 1 and Day 91 for glucose analysis. A decrease in the glucose levels was observed as expected. In Group 4, on Day 1, the decrease was observed on at 1, 2, and 12 hrs after dosing for males and at 2, 4 and 12 hrs after dosing for females. On Day 91, a decrease was observed at 1, 2 and 4 hrs after dosing (both

sexes) and at 6 and 8 hrs after dosing (females). At the end of treatment, a decrease was also observed in Group 2 at 2 hrs and in Group 3 at 2 and 4 hrs after treatment. There were a few occasions that increases in the glucose levels were observed. This was considered a result of the animals reacting to the insulin treatment (rebound effect).

Table 6: Study No 209212 13 Week Rat Study – Blood Glucose

Groups significant from Group 1

Parameter (plasma glucose)	Day	Sex	Group 2	Group 3	Group 4
1 hour	1	M	-	-	**↓
2 hour	1	M	-	-	**↓
	1	F	*↓	-	***↓
	91	F	*↓	***↓	***↓
4 hour	91	F	-	*↓	***↓
6 hour	91	F	-	-	**↓
8 hour	1	M	-	-	*↑
12 hour	1	M	-	-	*↓
24 hour	91	F	-	-	*↑

M: male F: female
Statistically significant results were reported as P<0.05(*), P<0.01(**) or P<0.001(***)
↑ : increase ↓ : decrease
- No statistical significant difference observed

Blood samples were collected for antibody analysis before start of treatment, in Week 13 and in Week 17 (recovery animals). There were 8 out of 70 treated animals were positive for antibodies towards insulin 454, whereas there were no antibodies towards liraglutide. Based on the result of the plasma glucose analysis, there was no indication of a neutralizing effect of these antibodies.

Table 7: Study No 209212 13 Week Rat Study – Anti-Drug Antibodies

Results overview anti-insulin 454 antibodies

Group	Group description	Main Study animal No. Positive/Total	Recovery study animal No. Positive/Total Day 92/93	Recovery study animal No. Positive/Total Day 119
1	Control	0/20 (0%)	0/10 (0%)	0/10 (0%)
2	Low	1/20 (5%)	-	-
3	Medium	3/20 (15%)	-	-
4	High	2/20 (10 %)	2*/10 (20%)	2*/10 (20%)

* Animal No. 204 and No. 209 were positive both at day 92 and day 119

Toxicokinetics

Blood samples were collected from animals at pre dose, 1, 2, 4, 6, 8, 12 and 24 hrs post dose on Days 1 and Day 91. All animals dosed with IDegLira were exposed systemically to both compounds during the study. The observed t_{max} ranged from 1-4 hrs for insulin 454 and from 4-8 hrs for liraglutide. The estimated t½ ranged from 1.7-4.4 hrs for insulin 454 and from 3.1-5.8 hrs for liraglutide. The C_{max} and AUC_{0-24h} increased with dose (both male and female) for both insulin 454 and liraglutide (AUC0-inf was used for insulin 454). The increase in exposure was proportional or more than proportional with dose. Limited systemic accumulation was observed for both males and females for both compounds. This was consistent with the terminal t½ and the dosing interval for both compounds. No sex differences in the TK parameters were observed for any of the compounds.

Table 8: Study No 209212 13 Week Rat Study – TK Parameters

Estimated toxicokinetic parameters for liraglutide

Period	Dose (nmol/kg)	Sex	C_{max} (pM)	t_{max} (h)	AUC_{0-24} (h*pM)	AUC_{0-8h} (h*pM)	AUC_{0-12h} (h*pM)	AUC (h*pM)	$AUC_{\%extra}$ (%)	Rac_{Obs}
Day 1	3.2	Female	6060	6	NA	35500	NA	NC	NC	-
		Male	5250	6	NA	30500	40200	NC	NC	-
	11.2	Female	27200	4	266000	180000	234000	268000	0.913	-
		Male	27300	4	220000	142000	183000	228000	3.13	-
	32	Female	79200	6	816000	479000	666000	832000	1.98	-
		Male	80500	6	834000	499000	688000	848000	1.62	-
Week 13	3.2	Female	7120	4	72900	38300	54800	76300	4.46	1.08[a]
		Male	4560	6	59200	27300	41600	64600	8.42	1.03[b]
	11.2	Female	25800	4	226000	151000	196000	229000	1.26	0.85[c]
		Male	22700	4	265000	122000	191000	277000	4.37	1.20[c]
	32	Female	99300	6	1060000	580000	833000	1100000	3.29	1.30[c]
		Male	83600	8	994000	476000	734000	1040000	4.48	1.19[c]

[a] AUC_{0-8h} used in calculation of Rac_{obs}, [b] AUC_{0-12h} used in calculation of Rac_{obs}, [c] AUC_{0-24h} used in calculation of Rac_{obs}

Estimated toxicokinetic parameters for insulin 454

Period	Dose (nmol/kg)	Sex	C_{max} (pM)	t_{max} (h)	AUC_{0-24h} (h*pM)	AUC_{0-8h} (h*pM)	AUC_{0-12h} (h*pM)	AUC (h*pM)	$AUC_{\%extra}$ (%)	Rac_{obs}
Day 1	2	Female	3610	2	NA	15400	NA	16600	7.41	-
		Male	4250	2	NA	13700	NA	15700	12.9	-
	7	Female	14600	2	NA	55700	59400	60700	2.05	-
		Male	13500	2	NA	49900	NA	53300	6.30	-
	20	Female	43000	2	NA	161000	173000	178000	2.55	-
		Male	35400	2	NA	161000	174000	179000	3.09	-
Week 13	2	Female	5380	1	NA	22700	NA	NR	35.6	1.47[a]
		Male	5170	2	NA	22300	25500	26700	4.38	1.63[a]
	7	Female	21100	2	NA	75900	83200	86300	3.59	1.40[b]
		Male	12100	2	91800	74700	83000	94500	2.80	1.50[a]
	20	Female	46400	2	231000	201000	220000	232000	0.729	1.27[b]
		Male	30200	4	228000	184000	209000	233000	1.84	1.20[b]

[a] AUC_{0-8h} used in calculation of Rac_{obs}, [b] AUC_{0-12h} used in calculation of Rac_{obs}

Dosing Solution Analysis

The analysis of dose formulations revealed a recovery of 88-125 % for insulin 454 and a recovery of 78-92 % for liraglutide.

Group	Recovery % insulin 454	Recovery % liraglutide
1	-	-
2	108-125	84-91
3	107-114	85-89
4	88-103	78-92

Study No 208142: 4 week rat with IDegLira 600/(b) (4) (A3)

Study title: NNC 0090-0000-1170: NNC 0100-0000-0454 ((b) (4):600): 4-weeks toxicity study by subcutaneous administration in Wistar rats

Study no.:	208142
Study report location:	SDN2, SN0000
Conducting laboratory and location:	Novo Nordisk A/S, Denmark
Date of study initiation:	April 09th 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	NNC 0100-0000-0454 (insulin 454) NNC 0090-0000-1170 (liraglutide)

Key Study Findings

- NN208142 is a four week toxicity study with the **IDegLira (A3)** formulation used doses of insulin degludec/liraglutide of 10/27, 30/80, **60/160 nmol/kg**, respectively. IDegLira (600/(b) (4)) was not used as the clinical ratio, which was 600/960.
- However, the dosing regimen was altered during the course of the study. Group 4 was terminated and Group 3 was reduced from 30/80 to 20/54 nmol/kg/day, because these doses exceeded the maximum tolerated dose due to hypoglycemia.
- Expected pharmacological effects:
  - Six mortalities, caused by hypoglycemia, occurred in the high dose group (60/160 nmol/kg/day) on Days 3 and 9. Blood samples from the high dose group (Days 4 and 9) and mid dose group (Day 9) showed severe hypoglycemia (< 2 mmol/L).
  - There was dose-related lower body weight and food consumption in animals treated with IDegLira.
  - Lower blood glucose levels were observed in all groups treated with IDegLira.
- Treatment/stress-related findings (organ weight/histopathological changes):
  - These findings were considered to be related to the combined effects of two compounds (hypoglycemia attributed to insulin degludec and liraglutide and reduced food intake attributed to liraglutide).
  - The prostate weight (absolute and relative) of IDegLira -treated animals were lower compared to that of controls. The finding correlates with macroscopic findings (e.g., small prostate glands and seminal vesicles) in Group 3 males, but not with any microscopic findings.
  - In testes, there was treatment related minimal degeneration of tubular cell and apoptotic spermatocytes. The findings occurred primarily at the higher dose and were reversible.
  - The adrenal gland weight (absolute and relative) of IDegLira-treated Group 2 males were lower compared to that of controls. In the adrenal glands, increases in cortical cell vacuolation were seen in a dose-dependent manner. These findings were considered to reflect an increased glucocorticoid (cortisol) synthesis by the adrenal glands occurring as an adaptive effect to stress due to hypoglycemia. In the liver, increases in hepatocellular rarefaction were seen in a dose-dependent manner. These changes were likely due to increased glycogen deposition due to hyperinsulinemia in healthy animals.
  - The heart weights (absolute) of IDegLira-treated animals (Groups 2 and 3 males and Group 3 females) were lower compared to that of controls. This finding, however, was related to body weight changes rather than an actual change in heart weight. It also did not correlate with any microscopic finding.
- Vehicle related findings:
  - Subcutaneous discoloration at the injection site was seen in all groups, including controls.
  - Minimal to moderate hemorrhage, inflammatory cell infiltrates, necrosis, and fibrosis/granulation tissue formation were seen at the injection site of all animals (Groups 1, 2, and 3).
- TK data:
  - No differences in the toxicokinetics between sexes were found for either insulin degludec or liraglutide. The increase in plasma and serum exposure as expressed by C_{max} and AUC was

proportional to increases in doses for both insulin degludec and liraglutide. An increase in exposure of less than 2-fold was seen at Day 28 compared to Day 1 which was consistent with the half-life and dosing interval of both compounds.

- The observed t_{max} ranged from 1 to 4 hrs for IDeg and from 4 to 12 hrs for liraglutide. The estimated $t_{½}$ ranged from 2.1 to 4.9 hrs for IDeg and from 2.7 to 5.3 hrs for liraglutide.
- Assessment of antibody formation showed that antibodies were detected for insulin degludec in 3/42 and 7/41 animals in the low (IDegLira 10/27) and medium (IDegLira 30/80 then 20/54) dose groups respectively, and that no antibodies were found for liraglutide. No change in exposure was observed in the insulin degludec antibody positive animals, hence the validity of the toxicity study was unaffected by this finding.

- The NOAEL for insulin454/liraglutide was established at 20/54 nmol/kg/day, based on adverse clinical signs, decreased body weight gain and other hypoglycemia-related and pathologies, and severe injection site reactions, at higher doses.

Methods

Study design:

Doses:	0/0, 10/27, 30/80 (reduced to 20/54 on Day 12), 60/160 (reduced to 45/120on Day 9 before being terminated on Day 10) (IDeg/Lira nmol/kg/day)
Frequency of dosing:	Once daily for 4 weeks
Route of administration:	Subcutaneous
Dose volume:	1 mg/kg/day
Formulation/Vehicle:	(b) (4) mg/mL phenol and (b) (4) mg/mL glycerol
Species/Strain:	Wistar rats
Number/Sex/Group:	10/sex/group, 4 groups
Age:	8 weeks old
Weight:	240-252g for males and 183-194g for females
Satellite groups:	TK: 12/sex/group
Unique study design:	TK group
Deviation from study protocol:	Some deviations were recorded but had no effect on the study outcome.

Table 9: Study No 208142 4 Week Rat Study - Study Design

Group	Day	Dose Level Liraglutide/Insulin 454 (nmol/kg/day)	Main Study Animals		Satellite Animals	
			Males	Females	Males	Females
1	1-28/29	0/0 (vehicle)	1-10	102-110, 193	41-52	141-152
2	1-28/29	27/10	11-20	111-120	53-64	153-164
3	1-9	80/30	21-30	121-130	65-76	165-176
	10-11	No treatment				
	12-28/29	54/20				
4	1-7	160/60	31-34, 36-40, 89	131-140	77-88	177-181, 183-185, 187, 189-190, 192
	8	No treatment				
	9	120/45				
	10	No treatment				

Main study animals from Group 1, 2 and 3 were terminated on Days 28-29 whereas all Group 4 animals were animals were terminated on Day 11.

Details	GIC (Amount pr ml)	Vehicle (Amount pr ml)
Insulin 454	600 nmol (0.6 mM)	-
Liraglutide	(b) (4) nmol ((b) (4) mM)	-
Phenol	(b) (4) mg ((b) (4) mM)	(b) (4) mg (b) (4) mM)
Glycerol	(b) (4) mg (b) (4) mM)	(b) (4) mg (b) (4) mM)
Zinc (b) (4)	(b) (4) µg (b) (4)	-
Sodium hydroxide	q.s.	q.s.
Hydrochloride acid	q.s.	q.s.
Water for injection	1 ml	1 ml
pH	8.2	8.2
Batch no.	412_N08203	412_N08204
Date of manufacture	14 April 2008	14 April 2008
Expiry date	21 May 2008	21 May 2008
Storage	at 2-8°C protected from direct sunlight	

Observations and Results
Mortality
Eleven deaths occurred.

- 5 females and 1 male from Group 4 were found dead on Days 3-9 (3 females were satellite animals and not subjected to necropsy)
- 2 males from Group 3 were terminated due to large ulcers at the injection site on Day 14 and Day 23 respectively (both animals were satellite animals and not subjected to necropsy)
- 2 females from Group 2 (Day 2) and 1 female from Group 3 (Day 29) were terminated due to soft tissue injuries occurring after blood sampling (all animals were satellite animals and not subjected to necropsy).

Clinical Signs (twice daily)
Animals in the two high dose groups (Groups 3 and 4) appeared subdued with piloerection and in some instances hunched back, due to hypoglycemia, confirmed by blood glucose measurements on Days 4 and 9. As a consequence of these findings, dosing of Group 4 animals was stopped on Day 8, the dose level was reduced to 120/45 nmol/kg/day on Day 9. Similarly, dosing of Group 3 animals were stopped on Days 10 and 11 and the dose level was reduced to 54/20 nmol/kg/day from Day 12.

Table 10: Study No 208142 4 Week Rat Study - Clinical Signs

Number of affected main study animals with observation (total number of observations)

Clinical observation	Male				Female			
	1	2	3	4 *	1	2	3	4 *
Subdued			10 (21)	10 (38)			10 (20)	10 (37)
Piloerection			10 (12)	10 (38)			10 (10)	10 (37)
Hunched back				4 (4)				
Isotonic glucose injected s.c.				10 (19)				10 (18)
Drinking water with glucose				10 (10)				12 (12)
Moribund (isolated)								2 (2)
Found dead				1 (1)				2 (2)
Terminated pre-schedule				9 (9)				8 (8)

The reactions were more frequent and more pronounced in animals treated with IDegLira and the effect appeared to be dose-related.

Body Weights (twice weekly)
The body weight of main study Group 3 males were statistically significantly lower compared to control animals (Group 1) on Days 14, 17, 21 and 24. Similarly Group 3 females were statistically significantly lower compared to control animals (Group 1) on Days 14 and 17.

The overall body weight gain (Days 1 – 28) for Group 3 males and females was also statistically significantly lower compared to the body weight gain of the control animals (Group 1). The body weight and body weight gain of Group 2 was also lower compared to the controls, but not statistically significant.

A similar pattern was seen for the satellite animals and statistical significance were attained at several occasions during the dosing period for both Group 2 and 3. On Day 35, the body weight of Group 2 and 3 remained lower, but not statistically significantly lower, indicating partial recovery from the body weight reduction.

Feed Consumption (twice weekly)
Lower food consumption was seen in animals treated with IDegLira during the entire dosing period. The effect was most pronounced in the beginning of the dosing period (Days 1 – 7).

Ophthalmoscopy
No treatment related findings were found.

Hematology (termination)
Some hematology parameters were minor but statistically significantly higher or lower in IDegLira-treated animals compared to controls. None of the changes in red blood cell related parameters and platelet related parameters indicated any toxicological effects of the IDegLira treatment. On the other hand, the increase in white blood cells and changes in leukocyte counts could be treatment related although no clear dose response was observed. In addition, the coagulation parameters were comparable among all groups.

Table 11: Study No 208142 4 Week Rat Study - Hematology Parameters

Haematology Parameters Attaining Statistical Significance

Parameter (Abbreviation)	Males	Females
Red blood cell count (RBC)	↓ Group 2*, 3*	
Haematocrit (HCT) calculated value	↓ Group 2*	
Red blood cell dist. width (RDW) calculated value		↑ Group 3**
Haemoglobin dist. width (HDW) calculated value		↑ Group 3*
Mean platelet volume (MPV)	↓ Group 2*, 3*	
White blood cell count (WBC)	↑ Group 2*, 3*	↑ Group 3**
Differential leucocyte count:		
NEU NEUP	↓ Group 2*	↓ Group 2**, 3*
LYM LYMP	↑ Group 2*, 3* ↑ Group 2*	↑ Group 3** ↑ Group 2*, 3*
EOS EOSP		↓Group 2*, 3**
LUC LUCP	↑ Group 2***, 3***	

↑ Increase, ↓ Decrease, * = P<0.05, ** = P<0.01, *** = P<0.001

Clinical Chemistry (termination)
Some parameters were statistically significantly higher or lower in IDegLira-treated animals compared to controls. The changes in ASAT (AST), UREA and CK seemed to be treatment-related. These findings correlated with liver rarefaction (excess glycogen deposition), and body weight decreases. No correlative kidney histopathology was noted for increased BUN, which could be due to dehydration (water consumption was not measured). Oher changes in enzymes, proteins or ions did not indicate any toxicological effects of the IDegLira- treatment.

Table 12: Study No 208142 4 Week Rat Study - Clinical Chemistry Parameters

Clinical Chemistry Parameters Attaining Statistical Significance

Parameter (Abbreviation)	Males	Females
Aspartate aminotransferase (ASAT)	↑ Group 2**, 3***	
Alanine aminotransferase (ALAT)	↑ Group 3*	↑ Group 3*
Lactate dehydrogenase (LDH)	↑ Group 2*, 3**	↑ Group 3**
Carbamide (UREA)	↑ Group 2**, 3***	
Calcium (CA)	↑ Group 2***, 3**	
Albumin (ALB)		↓ Group 2**, 3**
Total Protein (TP)		↓ Group 2*, 3*
Creatin kinase (CK)	↑ Group 2***, 3***	↑ Group 3***
Inorganic Phosphate (PHOS)	↓ Group 3	
Sodium (NA)	↑ Group 2**	
Potassium (K)	↑ Group 3*	

↑ Increase, ↓ Decrease, * = P<0.05, ** = P<0.01, *** = P<0.001

Urinalysis (during the last week of dosing)
Wet cages were observed on Day 4 for Group 3 and 4 females. There was a higher (but not statistically significant) urine volume in IDegLira–treated males and females in the last week of dosing when compared to controls. There was a lower (but statistically significant) level of ketones in Group 3 males compared to controls. However, no similar trend was seen in females.

Gross Pathology (termination)
In the main study animals, subcutaneous discoloration at the injection site was observed in Groups 1, 2 and 3 (8/10, 8/10 and 10/10 for male animals and 9/10, 2/10 and 3/10 for female animals respectively) on Days 29-30. However the range of changes (minimal-moderate) was comparable among groups. Similar finding was observed in Group 4 (9/9 for male animals and 6/6 for female animals) on Day 11. In addition, in Group 3, two male animals showed small prostate glands and seminal vesicle, which were correlated with the decreased prostate weight seen in Group 3.

Organ Weights
Organs were collected from animals and weighted at termination: adrenals, brain, heart, kidneys, liver, lungs, pituitary, spleen, thymus, ovaries, uterus, epididymides, prostate, and testes. A dose dependent reduction in prostate weight was seen. The absolute and relative prostate weight of Group 2 and 3 males was statistically significantly lower compared with controls. The absolute (but not the relative) heart weight of Groups 2 and 3 males and Group 3 females was statistically significantly lower compared with controls. The absolute and relative adrenal weight of Group 2 males was statistically significantly lower compared with the control group.

Histopathology
Adequate Battery
Yes.
Peer Review
No.

Histological Findings
In the testes, there was a dose-dependent (minimal) increase in the incidences of tubular cell degeneration and apoptotic spermatocytes in Groups 1, 2 and 3 animals (1/10, 1/10 and 4/10 for tubular cell degeneration and 0/10, 1/10 and 5/10 for apoptotic spermatocytes respectively). The Sertoli cells appeared unaffected. No signs of reduced amount of sperm in testes and epididymides were seen.

In the adrenal glands, a dose dependent (minimal) increase in cortical cell vacuolation was observed in Groups 1, 2 and 3 animals (0/10, 2/10 and 7/10 in males and 0/10, 2/10 and 5/10 in females respectively). This is likely reflective of an increased stress caused by hypoglycemia and reduced food intake.

In the liver, a dose dependent (minimal) increase in hepatocellular rarefaction was observed in Groups 1, 2 and 3 animals (3/10, 6/10 and 9/10 in males and 0/10, 3/10 and 8/10 in females respectively).

At the injection site, hemorrhage, inflammatory cell infiltration, necrosis and fibrosis/granulation tissue formation were observed in the subcutis across all groups and ranged from minimal to moderate. These changes correlated with macroscopic findings.

Table 13: Study No 208142 4 Week Rat Study - Histopath Findings

Pathology - Intergroup Comparison of Histopathology Observations

208142 - NNC 0090-0000-1170:NNC 0100-0000-0454 (b) (4) 600)

4-week toxicity study by subcutaneous administration in Wistar Rats

Observations: Non Neo-Plastic	MALES			FEMALES		
	0 nmol/kg	27/10 nmol/kg	80/30 nmol/kg	0 nmol/kg	27/10 nmol/kg	80/30 nmol/kg
Number of Animals on Study :	10	10	10	10	10	10
Number of Animals Completed:	(10)	(10)	(10)	(10)	(10)	(10)
ADRENAL GLANDS;						
Examined	(10)	(10)	(10)	(10)	(10)	(10)
Within Normal Limits	5	4	1	1	3	1
Hemorrhage; cortex	(0)	(0)	(1)	(0)	(0)	(0)
minimal	0	0	1	0	0	0
Vacuolation; increased; cortex	(0)	(2)	(7)	(0)	(2)	(5)
minimal	0	2	7	0	2	5
Vacuolation; increased; cortex; focal	1	1	0	0	0	0
Accessory Nodule	2	4	3	5	4	5
Angiectasis	1	0	1	2	2	1
Inflammatory Cell Infiltration; cortex	(0)	(0)	(0)	(1)	(0)	(0)
minimal	0	0	0	1	0	0
Inflammatory Cell Infiltration; mononuclear cells; cortex	(1)	(0)	(2)	(1)	(0)	(0)
minimal	1	0	2	1	0	0
Inflammatory Cell Infiltration; mononuclear cells; cortex; focal	0	0	0	0	3	1
Fibrosis; subcapsular; focal	0	0	0	0	0	1
AORTA;						
Examined	(10)	(0)	(10)	(10)	(0)	(10)
Within Normal Limits	9	0	10	10	0	10
Hemorrhage; tunica media; focal	1	0	0	0	0	0
BONE MARROW, FEMUR;						
Examined	(10)	(0)	(10)	(10)	(0)	(10)
Within Normal Limits	10	0	6	10	0	6
Fatty Infiltration; increased	(0)	(0)	(4)	(0)	(0)	(4)
minimal	0	0	3	0	0	4
slight	0	0	1	0	0	0
BONE MARROW, STERNUM;						
Examined	(10)	(0)	(10)	(10)	(0)	(10)
Within Normal Limits	10	0	6	9	0	6
Fatty Infiltration; increased	(0)	(0)	(4)	(0)	(0)	(4)
minimal	0	0	4	0	0	4
Myeloid Cells; increased	(0)	(0)	(0)	(1)	(0)	(0)
minimal	0	0	0	1	0	0

Pathology - Intergroup Comparison of Histopathology Observations

208142 - NNC 0090-0000-1170:NNC 0100-0000-0454 ((b) (4) 600)
4-week toxicity study by subcutaneous administration in Wistar Rats

Observations: Non Neo-Plastic	MALES			FEMALES		
	0 nmol/kg	27/10 nmol/kg	80/30 nmol/kg	0 nmol/kg	27/10 nmol/kg	80/30 nmol/kg
Number of Animals on Study :	10	10	10	10	10	10
Number of Animals Completed:	(10)	(10)	(10)	(10)	(10)	(10)
BRAIN;						
Examined	(10)	(0)	(10)	(10)	(0)	(10)
Within Normal Limits	6	0	10	10	0	10
Vacuolation; increased; white matter	(4)	(0)	(0)	(0)	(0)	(0)
minimal	2	0	0	0	0	0
slight	2	0	0	0	0	0
COAGULATING GLANDS;						
Examined	(10)	(0)	(10)	(-)	(-)	(-)
Within Normal Limits	10	0	10	-	-	-
EPIDIDYMIDES;						
Examined	(10)	(0)	(10)	(-)	(-)	(-)
Within Normal Limits	3	0	4	-	-	-
Debris; lumen	(1)	(0)	(3)	(-)	(-)	(-)
minimal	1	0	3	-	-	-
Inflammatory Cell Infiltration; mononuclear cells; interstitium	(7)	(0)	(6)	(-)	(-)	(-)
minimal	7	0	6	-	-	-
ESOPHAGUS;						
Examined	(10)	(0)	(10)	(10)	(0)	(10)
Within Normal Limits	10	0	10	10	0	10
HARDERIAN GLANDS;						
Examined	(10)	(0)	(10)	(10)	(0)	(10)
Within Normal Limits	10	0	10	8	0	9
Dilation; acinus	(0)	(0)	(0)	(2)	(0)	(1)
minimal	0	0	0	2	0	1
HEART;						
Examined	(10)	(0)	(10)	(10)	(0)	(10)
Within Normal Limits	8	0	7	8	0	9
Inflammatory Cell Infiltration; mononuclear cells; interstitial	(2)	(0)	(2)	(2)	(0)	(1)
minimal	2	0	2	2	0	1
Congestion	0	0	1	0	0	0

Pathology - Intergroup Comparison of Histopathology Observations

208142 - NNC 0090-0000-1170:NNC 0100-0000-0454 ((b) (4) 600)
4-week toxicity study by subcutaneous administration in Wistar Rats

Observations: Non Neo-Plastic	MALES			FEMALES		
	0 nmol/kg	27/10 nmol/kg	80/30 nmol/kg	0 nmol/kg	27/10 nmol/kg	80/30 nmol/kg
Number of Animals on Study :	10	10	10	10	10	10
Number of Animals Completed:	(10)	(10)	(10)	(10)	(10)	(10)
INJECTION SITE;						
Examined	(10)	(10)	(10)	(10)	(10)	(10)
Within Normal Limits	0	1	1	0	2	2
Hemorrhage; subcutaneous	(7)	(9)	(8)	(10)	(4)	(5)
minimal	4	3	3	7	4	5
slight	3	5	2	2	0	0
moderate	0	1	3	1	0	0
Inflammation; mononuclear cells; subcutaneous	(9)	(7)	(9)	(10)	(7)	(7)
minimal	6	5	5	10	7	6
slight	3	1	4	0	0	1
moderate	0	1	0	0	0	0
Inflammation; neutrophils; subcutaneous	(0)	(0)	(0)	(0)	(1)	(0)
minimal	0	0	0	0	1	0
Necrosis; subcutaneous	(7)	(7)	(3)	(7)	(1)	(1)
minimal	3	6	2	5	1	1
slight	3	1	1	2	0	0
moderate	1	0	0	0	0	0
Ulceration; epidermis; focal	0	1	0	0	0	0
Cavity; subcutaneous	4	6	3	3	1	0
Granuloma; subcutaneous	0	0	0	0	2	0
Granuloma; subcutaneous; focal	1	0	1	4	0	2
Folliculitis; subcutaneous	(0)	(0)	(0)	(1)	(0)	(0)
minimal	0	0	0	1	0	0
Pigmentation; macrophage; subcutaneous	(1)	(0)	(1)	(1)	(0)	(2)
minimal	1	0	1	1	0	2
Encrustation; epidermal; focal	0	0	0	2	0	0
Fibrosis/Granulation Tissue; subcutaneous	(10)	(9)	(8)	(9)	(5)	(5)
minimal	8	5	7	5	5	4
slight	2	4	1	4	0	1
INTESTINE, CECUM;						
Examined	(10)	(0)	(10)	(10)	(0)	(10)
Within Normal Limits	9	0	10	9	0	10
Congestion	1	0	0	1	0	0

Pathology - Intergroup Comparison of Histopathology Observations

208142 - NNC 0090-0000-1170:NNC 0100-0000-0454 ((b) (4) 600)
4-week toxicity study by subcutaneous administration in Wistar Rats

Observations: Non Neo-Plastic	MALES			FEMALES		
	0 nmol/kg	27/10 nmol/kg	80/30 nmol/kg	0 nmol/kg	27/10 nmol/kg	80/30 nmol/kg
Number of Animals on Study :	10	10	10	10	10	10
Number of Animals Completed:	(10)	(10)	(10)	(10)	(10)	(10)
INTESTINE, COLON;						
Examined	(10)	(0)	(10)	(10)	(0)	(10)
Within Normal Limits	10	0	10	10	0	10
INTESTINE, DUODENUM;						
Examined	(10)	(0)	(10)	(10)	(0)	(10)
Within Normal Limits	10	0	10	10	0	9
Fibrosis; submucosa; focal	0	0	0	0	0	1
Degeneration; brunners gland; epithelium; focal	0	0	0	0	0	1
INTESTINE, ILEUM;						
Examined	(10)	(0)	(10)	(10)	(0)	(10)
Within Normal Limits	10	0	10	10	0	10
INTESTINE, JEJUNUM;						
Examined	(10)	(0)	(10)	(10)	(0)	(10)
Within Normal Limits	10	0	10	10	0	10
INTESTINE, RECTUM;						
Examined	(10)	(0)	(10)	(10)	(0)	(10)
Within Normal Limits	10	0	9	9	0	10
Inflammatory Cell Foci; mononuclear cells; submucosa	(0)	(0)	(1)	(1)	(0)	(0)
minimal	0	0	1	1	0	0
KIDNEYS;						
Examined	(10)	(0)	(10)	(10)	(1)	(10)
Within Normal Limits	4	0	3	0	0	0
Cast(S)	(0)	(0)	(1)	(0)	(0)	(0)
minimal	0	0	1	0	0	0
Fibrosis; peritubular	(0)	(0)	(1)	(0)	(0)	(1)
minimal	0	0	1	0	0	1
Hydronephrosis	(0)	(0)	(0)	(0)	(1)	(0)
severe	0	0	0	0	1	0
Mineralization; corticomedullary junction	(0)	(0)	(1)	(9)	(0)	(10)
minimal	0	0	1	5	0	7
slight	0	0	0	4	0	3

Pathology - Intergroup Comparison of Histopathology Observations

208142 - NNC 0090-0000-1170:NNC 0100-0000-0454 ((b) (4) 600)
4-week toxicity study by subcutaneous administration in Wistar Rats

Observations: Non Neo-Plastic	MALES			FEMALES		
	0 nmol/kg	27/10 nmol/kg	80/30 nmol/kg	0 nmol/kg	27/10 nmol/kg	80/30 nmol/kg
Number of Animals on Study :	10	10	10	10	10	10
Number of Animals Completed:	(10)	(10)	(10)	(10)	(10)	(10)
KIDNEYS; (continued)						
Inflammatory Cell Infiltration; mononuclear cells; interstitial	(3)	(0)	(3)	(6)	(0)	(2)
minimal	3	0	3	6	0	2
Tubular Lysis	(2)	(0)	(0)	(1)	(0)	(0)
minimal	2	0	0	1	0	0
Congestion	0	0	0	3	0	1
Pigmentation; tubular	(0)	(0)	(2)	(4)	(0)	(7)
minimal	0	0	2	3	0	6
slight	0	0	0	1	0	1
Dilation; tubular	(0)	(0)	(0)	(3)	(0)	(1)
minimal	0	0	0	3	0	1
Basophilia; tubular	(4)	(0)	(4)	(4)	(0)	(7)
minimal	4	0	4	4	0	7
LIVER;						
Examined	(10)	(10)	(10)	(10)	(10)	(10)
Within Normal Limits	0	3	1	0	2	1
Congestion	1	0	0	1	1	0
Inflammatory Cell Infiltration; mononuclear cells; periportal	(3)	(0)	(2)	(7)	(2)	(3)
minimal	3	0	2	7	2	3
Inflammatory Cell Foci; parenchymal	(9)	(7)	(8)	(9)	(6)	(6)
minimal	9	7	8	9	6	6
Apoptosis; single cell	(1)	(0)	(1)	(0)	(0)	(0)
minimal	1	0	1	0	0	0
Vacuolation; hepatocellular	(0)	(0)	(0)	(2)	(0)	(0)
minimal	0	0	0	2	0	0
Rarefaction; increased; hepatocellular	(3)	(6)	(9)	(0)	(3)	(8)
minimal	3	4	5	0	3	8
slight	0	2	3	0	0	0
LUNGS WITH BRONCHI;						
Examined	(10)	(1)	(10)	(10)	(1)	(10)
Within Normal Limits	3	0	3	2	0	1
Macrophage Aggregation; foamy; alveolar	(3)	(0)	(4)	(3)	(0)	(8)
minimal	2	0	4	3	0	8
slight	1	0	0	0	0	0

Pathology - Intergroup Comparison of Histopathology Observations

208142 - NNC 0090-0000-1170:NNC 0100-0000-0454 ((b) (4) 600)

4-week toxicity study by subcutaneous administration in Wistar Rats

Observations: Non Neo-Plastic	MALES			FEMALES		
	0 nmol/kg	27/10 nmol/kg	80/30 nmol/kg	0 nmol/kg	27/10 nmol/kg	80/30 nmol/kg
Number of Animals on Study :	10	10	10	10	10	10
Number of Animals Completed:	(10)	(10)	(10)	(10)	(10)	(10)
LUNGS WITH BRONCHI; (continued)						
Inflammatory Cell Infiltration; mononuclear cells; perivascular	(1)	(0)	(2)	(3)	(0)	(0)
minimal	1	0	2	3	0	0
Inflammatory Cell Infiltration; mononuclear cells; interstitial	(3)	(1)	(2)	(1)	(0)	(3)
minimal	3	1	2	1	0	3
Inflammatory Cell Infiltration; eosinophils; perivascular	(2)	(0)	(2)	(2)	(0)	(7)
minimal	1	0	2	2	0	7
slight	1	0	0	0	0	0
Inflammatory Cell Infiltration; neutrophils; alveolar	(0)	(0)	(1)	(0)	(0)	(0)
minimal	0	0	1	0	0	0
Inflammatory Cell Infiltration; neutrophils; perivascular	(0)	(0)	(1)	(0)	(0)	(0)
minimal	0	0	1	0	0	0
Hemorrhage; agonal; alveolar	(2)	(0)	(1)	(2)	(0)	(3)
minimal	1	0	0	2	0	3
slight	1	0	1	0	0	0
Fibrosis; interstitial	(0)	(0)	(1)	(0)	(0)	(0)
minimal	0	0	1	0	0	0
Congestion; agonal	1	1	0	2	1	0
Edema; agonal; alveolar	(1)	(0)	(0)	(0)	(0)	(0)
slight	1	0	0	0	0	0
Metaplasia; osseous; alveolar	0	1	0	0	0	0
LYMPH NODE, AXILLARY;						
Examined	(10)	(0)	(9)	(10)	(1)	(9)
Within Normal Limits	10	0	6	9	0	7
Not Examined: NOT PRESENT	0	0	1	0	0	1
Germinal Center Development Increased	0	0	1	1	0	0
Macrophage Aggregation; focal	0	0	2	0	1	2
Hemorrhage	(0)	(0)	(0)	(0)	(1)	(0)
minimal	0	0	0	0	1	0
LYMPH NODE, MESENTERIC;						
Examined	(10)	(0)	(10)	(10)	(0)	(10)
Within Normal Limits	7	0	10	7	0	7
Hemorrhage; agonal; sinusoid	(0)	(0)	(0)	(2)	(0)	(0)
minimal	0	0	0	1	0	0

Pathology - Intergroup Comparison of Histopathology Observations

208142 - NNC 0090-0000-1170:NNC 0100-0000-0454 ((b) (4) 600)

4-week toxicity study by subcutaneous administration in Wistar Rats

Observations: Non Neo-Plastic	MALES			FEMALES		
	0 nmol/kg	27/10 nmol/kg	80/30 nmol/kg	0 nmol/kg	27/10 nmol/kg	80/30 nmol/kg
Number of Animals on Study :	10	10	10	10	10	10
Number of Animals Completed:	(10)	(10)	(10)	(10)	(10)	(10)
LYMPH NODE, MESENTERIC; (continued)						
slight	0	0	0	1	0	0
Histocytosis; sinusoid	(2)	(0)	(0)	(0)	(0)	(1)
minimal	2	0	0	0	0	1
Macrophage Aggregation; focal	1	0	0	2	0	2
MAMMARY GLANDS;						
Examined	(7)	(0)	(9)	(10)	(0)	(10)
Within Normal Limits	7	0	6	9	0	8
Not Examined: NOT PRESENT	3	0	1	0	0	0
Hemorrhage; alveolar	(0)	(0)	(1)	(0)	(0)	(0)
minimal	0	0	1	0	0	0
Pigmentation; epithelial	(0)	(0)	(2)	(1)	(0)	(2)
minimal	0	0	2	1	0	1
slight	0	0	0	0	0	1
SKELETAL MUSCLE;						
Examined	(10)	(0)	(10)	(10)	(0)	(10)
Within Normal Limits	7	0	2	5	0	6
Degeneration; hyaline; myofiber	(3)	(0)	(8)	(5)	(0)	(3)
minimal	3	0	6	5	0	3
slight	0	0	2	0	0	0
Inflammatory Cell Foci; mononuclear cells; interstitium	(0)	(0)	(0)	(0)	(0)	(1)
minimal	0	0	0	0	0	1
NERVE, OPTIC;						
Examined	(8)	(0)	(10)	(10)	(0)	(9)
Within Normal Limits	8	0	10	10	0	9
Not Examined: NOT PRESENT	2	0	0	0	0	1
NERVE, SCIATIC;						
Examined	(10)	(0)	(10)	(10)	(0)	(10)
Within Normal Limits	10	0	10	10	0	10
OVARIES;						
Examined	(-)	(-)	(-)	(10)	(0)	(10)

Pathology - Intergroup Comparison of Histopathology Observations

208142 - NNC 0090-0000-1170:NNC 0100-0000-0464 ((b) (4) 600)

4-week toxicity study by subcutaneous administration in Wistar Rats

Observations: Non Neo-Plastic	MALES			FEMALES		
	0 nmol/kg	27/10 nmol/kg	80/30 nmol/kg	0 nmol/kg	27/10 nmol/kg	80/30 nmol/kg
Number of Animals on Study :	10	10	10	10	10	10
Number of Animals Completed:	(10)	(10)	(10)	(10)	(10)	(10)
OVARIES; (continued)						
Within Normal Limits	-	-	-	10	0	10
PANCREAS;						
Examined	(10)	(0)	(10)	(10)	(0)	(10)
Within Normal Limits	4	0	7	5	0	6
Vacuolation; acinar cell	(5)	(0)	(2)	(4)	(0)	(3)
minimal	5	0	2	4	0	3
Inflammatory Cell Infiltration; mononuclear cells; perivascular	(2)	(0)	(2)	(1)	(0)	(1)
minimal	2	0	2	1	0	1
Degeneration; acinar cell; focal	2	0	1	2	0	0
Dilation; duct; focal	0	0	1	0	0	0
PARATHYROID GLANDS;						
Examined	(8)	(0)	(9)	(8)	(0)	(10)
Within Normal Limits	6	0	9	5	0	10
Not Examined: NOT PRESENT	2	0	1	2	0	0
PITUITARY GLAND;						
Examined	(9)	(0)	(10)	(10)	(0)	(10)
Within Normal Limits	8	0	10	8	0	10
Not Examined: NOT PRESENT	1	0	0	0	0	0
Cyst; adenohypophysis	1	0	0	2	0	0
PROSTATE GLAND;						
Examined	(10)	(0)	(10)	(-)	(-)	(-)
Within Normal Limits	7	0	6	-	-	-
Atrophy; epithelium	(1)	(0)	(0)	(-)	(-)	(-)
minimal	1	0	0	-	-	-
Inflammatory Cell Infiltration; mononuclear cells; interstitium	(3)	(0)	(3)	(-)	(-)	(-)
minimal	3	0	3	-	-	-
Degeneration; epithelium	(0)	(0)	(1)	(-)	(-)	(-)
minimal	0	0	1	-	-	-
Secretion; decreased	0	0	1	-	-	-

Pathology - Intergroup Comparison of Histopathology Observations

208142 - NNC 0090-0000-1170:NNC 0100-0000-0464 ((b) (4) 600)

4-week toxicity study by subcutaneous administration in Wistar Rats

Observations: Non Neo-Plastic	MALES			FEMALES		
	0 nmol/kg	27/10 nmol/kg	80/30 nmol/kg	0 nmol/kg	27/10 nmol/kg	80/30 nmol/kg
Number of Animals on Study :	10	10	10	10	10	10
Number of Animals Completed:	(10)	(10)	(10)	(10)	(10)	(10)
SALIVARY GLAND, MANDIBULAR;						
Examined	(10)	(0)	(10)	(10)	(0)	(10)
Within Normal Limits	9	0	10	9	0	10
Atrophy; granular duct	1	0	0	0	0	0
Vacuolation; acinar cell	(0)	(0)	(0)	(1)	(0)	(0)
minimal	0	0	0	1	0	0
SEMINAL VESICLES;						
Examined	(10)	(0)	(10)	(-)	(-)	(-)
Within Normal Limits	7	0	5	-	-	-
Degeneration; epithelium	(3)	(0)	(2)	(-)	(-)	(-)
minimal	2	0	0	-	-	-
slight	1	0	2	-	-	-
Secretion; decreased	0	0	2	-	-	-
SKIN;						
Examined	(10)	(0)	(10)	(10)	(0)	(10)
Within Normal Limits	10	0	9	10	0	9
Hyperplasia; epithelial; focal	0	0	1	0	0	0
Encrustation; focal	0	0	0	0	0	1
SPINAL CORD, CERVICAL;						
Examined	(9)	(0)	(10)	(10)	(0)	(10)
Within Normal Limits	8	0	10	10	0	10
Not Examined: NOT PRESENT	1	0	0	0	0	0
Vacuolation; increased; white matter	(1)	(0)	(0)	(0)	(0)	(0)
minimal	1	0	0	0	0	0
SPINAL CORD, THORACIC;						
Examined	(10)	(0)	(10)	(10)	(0)	(10)
Within Normal Limits	9	0	10	10	0	10
Vacuolation; increased; white matter	(1)	(0)	(0)	(0)	(0)	(0)
minimal	1	0	0	0	0	0
SPINAL CORD, LUMBAR;						
Examined	(10)	(0)	(9)	(10)	(0)	(10)

Pathology - Intergroup Comparison of Histopathology Observations

208142 - NNC 0090-0000-1170:NNC 0100-0000-0454 ((b) (4) 600)
4-week toxicity study by subcutaneous administration in Wistar Rats

Observations: Non Neo-Plastic	MALES			FEMALES		
	0 nmol/kg	27/10 nmol/kg	80/30 nmol/kg	0 nmol/kg	27/10 nmol/kg	80/30 nmol/kg
Number of Animals on Study :	10	10	10	10	10	10
Number of Animals Completed:	(10)	(10)	(10)	(10)	(10)	(10)
SPINAL CORD, LUMBAR; (continued)						
Within Normal Limits	10	0	9	10	0	10
Not Examined: INSUFFICIENT TISSUE TO EVALUATE	0	0	1	0	0	0
SPLEEN;						
Examined	(10)	(0)	(10)	(10)	(0)	(10)
Within Normal Limits	7	0	5	6	0	10
Congestion; subcapsular	0	0	0	1	0	0
Hematopoiesis; increased	(3)	(0)	(2)	(2)	(0)	(0)
minimal	3	0	2	2	0	0
Marginal Zone Increased Size	(0)	(0)	(2)	(1)	(0)	(0)
minimal	0	0	2	1	0	0
Decreased Number Of Lymphocytes; pals	(0)	(0)	(1)	(0)	(0)	(0)
minimal	0	0	1	0	0	0
STOMACH, GLANDULAR;						
Examined	(10)	(0)	(10)	(10)	(0)	(10)
Within Normal Limits	10	0	7	8	0	7
Inflammatory Cell Infiltration; neutrophils; submucosa; focal	0	0	0	0	0	1
Inflammatory Cell Foci; lymphocytic	0	0	1	1	0	1
Fibrosis; submucosa; focal	0	0	1	0	0	0
Dilation; glandular; focal	0	0	1	1	0	1
STOMACH, NONGLANDULAR;						
Examined	(10)	(0)	(10)	(10)	(0)	(10)
Within Normal Limits	9	0	9	10	0	8
Vacuolation; epithelium; focal	1	0	0	0	0	0
Hyperkeratosis; focal	1	0	1	0	0	0
Inflammatory Cell Infiltration; mononuclear cells; submucosa; focal	0	0	0	0	0	1
Inflammatory Cell Infiltration; eosinophils; submucosa; focal	0	0	0	0	0	1
TESTES;						
Examined	(10)	(10)	(10)	(-)	(-)	(-)
Within Normal Limits	8	2	1	-	-	-
Atrophy; seminiferous tubule; focal	0	0	1	-	-	-
Degeneration; seminiferous tubule	(1)	(1)	(4)	(-)	(-)	(-)

208142 - NNC 0090-0000-1170:NNC 0100-0000-0454 ((b) (4) 600)
4-week toxicity study by subcutaneous administration in Wistar Rats

Observations: Non Neo-Plastic	MALES			FEMALES		
	0 nmol/kg	27/10 nmol/kg	80/30 nmol/kg	0 nmol/kg	27/10 nmol/kg	80/30 nmol/kg
Number of Animals on Study :	10	10	10	10	10	10
Number of Animals Completed:	(10)	(10)	(10)	(10)	(10)	(10)
TESTES; (continued)						
minimal	1	1	4	-	-	-
Vacuolation; sertoli cell	(4)	(7)	(6)	(-)	(-)	(-)
minimal	4	7	6	-	-	-
Apoptosis; spermatocyte	(0)	(1)	(5)	(-)	(-)	(-)
minimal	0	1	5	-	-	-
THYMUS;						
Examined	(10)	(1)	(10)	(10)	(0)	(10)
Within Normal Limits	6	0	6	7	0	6
Cyst	2	0	4	3	0	4
Hemorrhage; cortex	(0)	(1)	(0)	(0)	(0)	(0)
minimal	0	1	0	0	0	0
Hemorrhage; medulla	(0)	(1)	(0)	(0)	(0)	(0)
minimal	0	1	0	0	0	0
Hemorrhage; agonal; medulla	(2)	(0)	(0)	(0)	(0)	(0)
minimal	2	0	0	0	0	0
THYROID GLANDS;						
Examined	(10)	(0)	(9)	(10)	(0)	(10)
Within Normal Limits	8	0	7	8	0	7
Not Examined: NOT PRESENT	0	0	1	0	0	0
Cyst; ultimobranchial	1	0	0	0	0	0
Hypertrophy; vacuolar; follicular cell; diffuse	2	0	2	0	0	2
Detachment; follicular cell	(0)	(0)	(1)	(1)	(0)	(0)
minimal	0	0	1	1	0	0
Ectopic Thymus	0	0	0	0	0	1
Inflammatory Cell Infiltration; mononuclear cells; interstitial	(0)	(0)	(0)	(1)	(0)	(0)
minimal	0	0	0	1	0	0
Hypoplasia; c-cell; focal	0	0	0	0	0	1
TONGUE;						
Examined	(10)	(0)	(10)	(10)	(0)	(10)
Within Normal Limits	10	0	9	10	0	10
Inflammatory Cell Infiltration; mononuclear cells; interstitium	(0)	(0)	(1)	(0)	(0)	(0)
minimal	0	0	1	0	0	0

Pathology - Intergroup Comparison of Histopathology Observations

208142 - NNC 0090-0000-1170:NNC 0100-0000-0454 ((b) (4) 600)
4-week toxicity study by subcutaneous administration in Wistar Rats

Observations: Non Neo-Plastic	MALES			FEMALES		
	0 nmol/kg	27/10 nmol/kg	80/30 nmol/kg	0 nmol/kg	27/10 nmol/kg	80/30 nmol/kg
Number of Animals on Study :	10	10	10	10	10	10
Number of Animals Completed:	(10)	(10)	(10)	(10)	(10)	(10)
TRACHEA;						
Examined	(10)	(0)	(10)	(10)	(0)	(10)
Within Normal Limits	9	0	10	9	0	10
Dilation; glandular; focal	1	0	0	1	0	0
URINARY BLADDER;						
Examined	(10)	(0)	(10)	(10)	(0)	(10)
Within Normal Limits	9	0	8	7	0	9
Inflammatory Cell Foci; mononuclear cells; submucosa	(1)	(0)	(1)	(3)	(0)	(1)
minimal	1	0	1	3	0	1
Eosinophilic Inclusions; transitional cell	(0)	(0)	(1)	(0)	(0)	(0)
minimal	0	0	1	0	0	0
UTERUS;						
Examined	(-)	(-)	(-)	(10)	(0)	(10)
Within Normal Limits	-	-	-	10	0	10
VAGINA;						
Examined	(-)	(-)	(-)	(10)	(0)	(10)
Within Normal Limits	-	-	-	10	0	8
Hemorrhage; submucosa	(-)	(-)	(-)	(0)	(0)	(1)
minimal	-	-	-	0	0	1
Inflammation; neutrophilic; epithelium; lumen	(-)	(-)	(-)	(0)	(0)	(1)
minimal	-	-	-	0	0	1
SALIVARY GLAND, PAROTIS;						
Examined	(10)	(0)	(10)	(10)	(0)	(10)
Within Normal Limits	7	0	7	6	0	4
Vacuolation; increased; acinar cell	(2)	(0)	(1)	(3)	(0)	(4)
minimal	2	0	1	3	0	3
slight	0	0	0	0	0	1
Inflammatory Cell Infiltration; mononuclear cells; periductal	(1)	(0)	(1)	(1)	(0)	(0)
minimal	1	0	1	1	0	0
Hypertrophy; basophilic; acinar cell	(0)	(0)	(1)	(1)	(0)	(2)
minimal	0	0	1	1	0	2
Hypertrophy; basophilic; acinar cell; focal	0	0	0	0	0	1

Pathology - Intergroup Comparison of Histopathology Observations

208142 - NNC 0090-0000-1170:NNC 0100-0000-0454 ((b) (4) 600)
4-week toxicity study by subcutaneous administration in Wistar Rats

Observations: Non Neo-Plastic	MALES			FEMALES		
	0 nmol/kg	27/10 nmol/kg	80/30 nmol/kg	0 nmol/kg	27/10 nmol/kg	80/30 nmol/kg
Number of Animals on Study :	10	10	10	10	10	10
Number of Animals Completed:	(10)	(10)	(10)	(10)	(10)	(10)
EYES;						
Examined	(10)	(0)	(10)	(10)	(0)	(10)
Within Normal Limits	9	0	9	10	0	10
Fold; retina	0	0	1	0	0	0
Mineralization; lens	1	0	0	0	0	0
LYMPH NODE, MANDIBULARIS;						
Examined	(0)	(0)	(0)	(1)	(0)	(0)
Within Normal Limits	0	0	0	0	0	0
Hemorrhage; agonal; sinusoid	(0)	(0)	(0)	(1)	(0)	(0)
minimal	0	0	0	1	0	0
JOINT, KNEE;						
Examined	(10)	(0)	(10)	(10)	(0)	(10)
Within Normal Limits	10	0	10	10	0	10
BONE, STERNUM;						
Examined	(10)	(0)	(10)	(10)	(0)	(10)
Within Normal Limits	10	0	10	10	0	10
BONE, FEMUR;						
Examined	(10)	(0)	(10)	(10)	(0)	(10)
Within Normal Limits	10	0	10	10	0	10

Special Evaluation (Days 1 and 28 for glucose analysis and antibody analysis, Day 35 for TK) blood glucose values were lower in Groups 3 and 4 on Day 1 compared to controls. Similar findings were seen in Groups 2 and 3 on Day 28 from 1-8 hrs after dosing.

Eleven samples (one incidental from pre-dose Group 2, three from Group 2, and seven from Group 3) were found positive for antibodies towards insulin 454, whereas no samples were found positive for liraglutide antibodies. However, as it was estimated that the concentration of insulin 454 and liraglutide in the antibody samples was below the level of interference, this finding was considered treatment-related.

The group mean blood glucose level of main study animals positive for insulin 454 antibodies were slightly lower than corresponding group mean blood glucose level of main study animals negative for insulin 454 antibodies. However, the blood glucose levels in the antibody positive animals were within the range found in the antibody negative animals, indicating that the level of insulin 454 antibodies did not change the effect of IDeg/Lira.

Toxicokinetics
Blood samples were collected on Days 1 and 28 (before dosing, 1, 2, 4, 6, 8, 12 and 24 hrs after dosing).

All animals dosed with IDegLira were exposed systemically to both drug components during the study.

Based on the available data, there were no sex differences for either insulin 454 or liraglutide. However, there appeared to be an approximate dose proportional increase in C_{max} and AUC for both insulin 454 and liraglutide. There appeared to be an indication of slightly higher exposure to insulin 454 on Day 28 compared to Day 1, however the increase was < 2-fold.

The limited accumulation observed from Day 1 to Day 28 was consistent with the half-life and dosing interval of both compounds. The observed t_{max} ranged from 1-4 hrs (for insulin 454) and from 4-12 hrs (liraglutide) post dosing, whereas the estimated t½ ranged from 2.1-4.85 hrs (for insulin 454) and from 2.65-5.25 hrs (liraglutide).

Table 14: Study No 208142 4 Week Rat Study - TK Profile

Estimated toxicokinetic parameters for insulin 454.

Period	Dose (nM/kg)	Gender	Cmax (pM)	Tmax (h)	AUC (h*pM)	AUCtau (h*pM)	%AUCextra	AUClast (h*pM)	CL/f (L/h/kg)	Vz/f (L/kg)
1	10	Female	15400	2.00	86300	82600	4.30	82600	0.116	0.399
		Male	17600	2.00	106000	102000	3.82	102000	0.0945	0.286
	30	Female	42800	2.00	306000	303000	0.807	303000	0.0981	0.448
		Male	48800	2.00	354000	349000	1.27	349000	0.0848	0.431
	60	Female	69300	4.00	475000	470000	0.946	470000	0.126	0.624
		Male	74100	4.00	629000	614000	2.47	614000	0.0953	0.584
28	10	Female	24500	1.00	109000	106000	2.02	106000	0.0940	0.657
		Male	25800	2.00	136000	134000	1.85	134000	0.0748	0.463
	20	Female	61300	2.00	275000	273000	0.630	273000	0.0733	0.334
		Male	56700	2.00	301000	298000	1.12	298000	0.0672	0.354

Estimated toxicokinetic parameters for liraglutide.

Period	Dose (nmol/kg)	Gender	Cmax (pM)	Tmax (h)	AUC (h*pM)	AUCtau (h*pM)	%AUCextra	AUClast (h*pM)	CL/f (L/h/kg)	Vz/f (L/kg)
1	27	Female	47700	8.00	540000	536000	0.605	536000	0.0500	0.192
		Male	46600	6.00	515000	503000	2.35	503000	0.0524	0.286
	80	Female	138000	6.00	1540000	1520000	1.72	1520000	0.0518	0.256
		Male	216000	12.0	3100000	2920000	5.70	2920000	0.0258	0.155
	160	Female	198000	4.00	2620000	2560000	2.30	2560000	0.0611	0.304
		Male	241000	8.00	3150000	2940000	6.88	2940000	0.0507	0.385
28	27	Female	52400	8.00	550000	544000	1.08	544000	0.0497	0.216
		Male	54300	8.00	647000	633000	2.16	633000	0.0427	0.224
	54	Female	114000	6.00	1330000	1320000	1.07	1320000	0.0410	0.181
		Male	114000	6.00	1430000	1390000	3.23	1390000	0.0389	0.233

Dosing Solution Analysis
The drug product was analyzed by HPLC. The results were within limits.

ID no. in dept. 202	Sample description*	Component	Labelled content [nmol/ml]	Determined content [nmol/ml]
GIC pr. 205659638	GIC pr 67, 0/0, 22/4-08, -20°C, GLP 208142	Insulin 454	(b) (4	(b) (4)
GIC pr. 205659654	GIC 68, 27/10, 22/4-08, -20°C, GLP 208142			
GIC pr. 205659655	GIC 69, 80/30, 22/4-08, -20°C, GLP 208142			
GIC pr. 205659656	GIC 67, 160/60, 22/4-08, -20°C, GLP 208142			
GIC pr. 205659657	GIC St, 1600/ 600, 22/4-08, -20°C, GLP 208142		600	
GIC pr. 205659658	GIC pr 67, 0/0, 19/5-08, -20°C, GLP 208142		(b) (4	
GIC pr. 205659659	GIC 68, 27/10, 19/5-08, -20°C, GLP 208142			
GIC pr. 205659660	GIC 81, 54/20, 19/5-08, -20°C, GLP 208142			
GIC pr. 205659661	GIC St, 1600/ 600, 19/5-08, -20°C, GLP 208142		600	
GIC pr. 205659638	GIC pr 67, 0/0, 22/4-08, -20°C, GLP 208142	Liraglutide	(b) (4	
GIC pr. 205659654	GIC 68, 27/10, 22/4-08, -20°C, GLP 208142			
GIC pr. 205659655	GIC 69, 80/30, 22/4-08, -20°C, GLP 208142			
GIC pr. 205659656	GIC 67, 160/60, 22/4-08, -20°C, GLP 208142			
GIC pr. 205659657	GIC St, 1600/ 600, 22/4-08, -20°C, GLP 208142			
GIC pr. 205659658	GIC pr 67, 0/0, 19/5-08, -20°C, GLP 208142			
GIC pr. 205659659	GIC 68, 27/10, 19/5-08, -20°C, GLP 208142			
GIC pr. 205659660	GIC 81, 54/20, 19/5-08, -20°C, GLP 208142			
GIC pr. 205659661	GIC St, 1600/ 600, 19/5-08, -20°C, GLP 208142			

* ID no., concentration (liraglutide/insulin 454), sampling date, storage, study no.

Study No 208068: 2 week rat with IDegLira (A3)

Study title: GIC: 2-weeks Dose Range Finding Study by Subcutaneous Administration in Wistar Rats

Study no.:	208068
Study report location:	SDN2, SN0000
Conducting laboratory and location:	Novo Nordisk A/S
Date of study initiation:	Feb 07th 2008
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	GIC INJ ((b) (4) /0.6) mM (b) (4) Vehicle GIC INJ ((b) (4) /0.6) mM. (b) (4) Batch No: 412_N08081 and 412_N08084

Key Study Findings

- NN 208068 is the 2 week DRF study with the IDegLira (A3) formulation to investigate the toxicity of a fixed combination of insulin degludec and liraglutide, following once daily subcutaneous administration to rats for two weeks. The doses of insulin degludec/liraglutide were 30/80, 60/160 and 90/240 nmol/kg, respectively. IDegLira (600/ (b) (4)) was not used as the clinical ratio, which was 600/960.
  - Expected pharmacological effects:
    - Three mortalities, most likely caused by low blood glucose levels, occurred on Day 14: one Group 3 female (60/160 nmol/kg/day), one Group 4 female and one Group 4 male (90/240 nmol/kg/day).

- There were lower body weight and lower overall body weight gain from 30/80 nmol/kg/day.
- There was also lower overall group mean food consumption from 30/80 nmol/kg/day.
- Lower blood glucose levels were observed from 30/80 nmol/kg/day, which were more pronounced after 14 days of dosing.

- Treatment-related findings (examined microscopically from Groups 1 and 4 only) :
  - Small superficial ulcers at the injection site from 30/80 nmol/kg/day
  - A reduced number of reticulocytes in males from 30/80 nmol/kg/day
  - Lower (absolute and relative) liver weight in males from 30/80 nmol/kg/day
  - Liver congestion and mononuclear infiltration were seen in Group 4 males (1/5 and 2/5 vs. 0/5 and 0/5 in Group 1 males, respectively) whereas liver congestion and inflammatory cell foci were seen in Group 4 females (1/5 and 3/5 vs. 0.5 and 0/5 in Group 1 females, respectively).
- Vehicle related findings:
  - There was redness of subcutis at the injection site in some animals from all groups, including controls.
  - Minimal-slight inflammatory reaction (sometimes with hemorrhages) was observed at the injection site in animals from control and high dose group and in animals from the other groups showing macroscopic changes at the injection site.
- TK data:
  - The exposure of insulin degludec and liraglutide increased with increasing doses. Due to the limited amount of data (n=1; 1 male and 1 female) and the variability in the plasma and serum concentration data, no conclusions could be drawn regarding sex differences and time dependency. The t_{max} was observed from 1.0 to 3.0 hrs for insulin degludec and from 1.0 to 9.0 hrs for liraglutide in non dose-dependent manner.

Methods

Study design:

Doses:	0/0, 30/80, 60/160, 90/240 (insulin 454/liraglutide nmol/kd/day)
Frequency of dosing:	Once daily for 14 days
Route of administration:	Subcutaneous
Dose volume:	It was according to animal most recent body weight.
Formulation/Vehicle:	(b) (4) mg/mL phenol + (b) (4) mg/mL glycerol
Species/Strain:	Wistar rats
Number/Sex/Group:	5/sex/group, 4 groups
Age:	6 weeks old
Weight:	119-173 g for males and 101-120 g for females
Satellite groups:	There was no TK group.
Unique study design:	TK parameters were measured.
Deviation from study protocol:	Some deviations were recorded but had no effect on the study outcome.

Table 15: Study No 208068 2 Week Rat Study - Study Design

Group	Test Substance	Concentration (nmol/ml)	Dose Level (nmol/kg/day)	Dose Volume (ml/kg)	Animals	
					Males	Females
1	Vehicle	0/0	0/0	0.15	1-3, 4/24[A], 5	101-105
2	insulin 454 / liraglutide	600 (b) (4)	30/80	0.05	6-10	106-110
3		600	60/160	0.1	11-15	111-115
4		600	90/240	0.15	16-20	116-120

[A] Animal no. 4 was replaced by no. 24 on Day 2

Body Weight (g)	Administered Dose Volume			
	Group 1	Group 2	Group 3	Group 4
100-149	0.02	0.01	0.01	0.02
150-166	0.02	0.01	0.02	0.02
167-233	0.03	0.01	0.02	0.03
234-250	0.04	0.01	0.02	0.04

Details	GIC (amount pr ml)	Vehicle (amount pr ml)
Insulin 454	600 nmol (0.6 mM)	-
Liraglutide	(b) (4)	(b) (4)
Phenol		
Glycerol		
Zinc (b) (4)		
Sodium hydroxide	q.s.	q.s.
Hydrochoride acid	q.s.	q.s.
Water for injection(WFI)	1 ml	1 ml
Batch no.	412-N08081 Insulin 454: LP454K4T01 Liraglutide: GIK4S005	412-N08084
Date of manufacture	13 February 2008	13 February 2008
Expiry date	15 March 2008	15 March 2008
Storage:	At 2-8°C protected from direct sunlight	Room temperature

Observations and Results
Mortality
Three deaths were found on Day 14 (1 Group 3 female, 1 Group 4 female, 1 Group 4 male) with suspected hypoglycemia.

Clinical Signs (twice daily)
No clinical signs preceded the three deaths found on Day 14. Small wounds were observed at the injection site for some animals (more apparent in GIC treated animals but not dose relationship).

Incidence of Small Wounds at the Injection Site

Sex	Males				Females			
Group	1	2	3	4	1	2	3	4
No. of animals affected	1	2	3	3	0	1	2	0
No. of observations (days)	1	2	4	7	0	1	1	0

Body Weights (twice weekly)
GIC treated animals had lower body weight and body weight gain from Day 3 and throughout the study, when compared to the vehicle treated animals.

Table 6 Group Mean Overall Body Weight Gain (Day -1 – 15)

Sex	Males				Females			
Group	1	2	3	4	1	2	3	4
Body weight gain (g)	57.7	29.9	22.8	11.7	22.1	21.4	14.2	17.2
Body weight gain (% of group 1)	100%	52%	40%	20%	100%	97%	64%	78%

Feed Consumption (twice weekly)
GIC treated animals had lower food consumption when compared to the vehicle treated animals.

Sex	Males				Females			
Group	1	2	3	4	1	2	3	4
Food consumption (g/rat)	288.4	227.3	192.4	195.8	197.3	186.1	155.2	176.5
Food consumption (% of group 1)	100%	79%	67%	68%	100%	94%	79%	89%

Ophthalmoscopy
Not performed.

ECG
Not performed.

Hematology (at termination)
A reduced number of reticulocytes were seen in males of all GIC treated groups compared to the controls (57%, 64%, 50% vs 100% for Groups 2, 3, 4, vs. Group 1 respectively).

Clinical Chemistry (Days 1 and 14)
The glucose levels were altered by the GIC treatment. On Day 1, 1 hr after dosing, the glucose levels were higher in GIC-treated animals, whereas 3 hr after dosing, the glucose levels were lower in GIC-treated animals compared to the vehicle treated animals. The glucose levels were about the same between pre-dosing and 9-24 hr after dosing, except the high dose group (Group 4) by which blood glucose levels were still lower after 9 hrs after dosing. On Day 14, lower glucose levels were observed in all GIC-treated groups (1-3 hrs after dosing) and in Group 3 (9 hrs after dosing). However, no dose-dependent pattern was seen.

Group Mean Blood Glucose Level (Day 14)

Sex	Males			Females		
Group	1	3	4	1	3	4
Blood glucose (mmol/L)	5.6	2.5	1.7	4.7	2.3	3.5
Blood glucose ((% of group 1)	100%	45%	29%	100%	49%	74%

Urinalysis
Not performed.

Gross Pathology
Three deaths did not correlate with any specific pathological changes (some changes were congestion in lungs and liver). At necropsy, redness of subcutis at the injection site was seen in some animals (including controls).

Organ Weights
Organs (e.g., kidneys, liver, thyroid gland, and pancreas) were collected and weighted at termination. The absolute and relative liver weight of GIC treated males were lower compared to the liver weight found in the vehicle treated males. No treatment-related changes were seen in absolute or relative kidney weight.

Absolute organ weight	Males				Females			
	1	2	3	4	1	2	3	4
Kidney	100%	95%	87%	92%	100%	100%	99%	104%
Liver	100%	75%	73%	69%	100%	98%	91%	96%

Relative organ weight	Males				Females			
	1	2	3	4	1	2	3	4
Kidney	100%	105%	100%	106%	100%	100%	106%	108%
Liver	100%	83%	84%	78%	100%	98%	97%	98%

Histopathology
Tissues (e.g., kidneys, liver, thyroid gland, pancreas, injection site) were examined from Groups 1 and 4
Adequate Battery
Yes, only limited tissues were collected for microscopic examination.
Peer Review
No.
Histological Findings
A minimal-slight inflammatory reaction was seen (sometimes with hemorrhages) at the injection site in all animals (including controls). There were minimal inflammatory cell foci in Group 4 females (3/5 vs 0/5 in Group 1 females) and minimal mononuclear infiltration in Group 4 males (2/5 vs. 0/5 Group 1 males).

Table 16: Study No 208068 2 Week Rat Study - Histopath Findings

Pathology - Intergroup Comparison of Histopathology Observations

208068 - GIC
2-wk dose range finding study by subcutaneous administration in Wistar rats

Observations: Non Neo-Plastic	MALES				FEMALES			
Removal Reasons: All of those SELECTED	0 nmol/kg	30/80 nmol/kg	60/160 nmol/kg	90/240 nmol/kg	0 nmol/kg	30/80 nmol/kg	60/160 nmol/kg	90/240 nmol/kg
Number of Animals on Study :	5	5	5	5	5	5	5	5
Number of Animals Completed:	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)
INJECTION SITE;								
Examined	(5)	(1)	(1)	(5)	(5)	(3)	(2)	(5)
Within Normal Limits	0	0	0	0	1	0	0	1
Not Examined: CANNABILIZED	0	0	0	0	0	0	1	0
Fibrosis; dermal	(1)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
minimal	1	0	0	0	0	0	0	0
Fibrosis; subcutaneous	(5)	(0)	(0)	(5)	(4)	(1)	(2)	(4)
minimal	4	0	0	3	2	1	2	3
slight	1	0	0	2	2	0	0	1
Hemorrhage; subcutaneous	(3)	(0)	(0)	(2)	(2)	(1)	(0)	(0)
minimal	3	0	0	2	2	1	0	0
Infiltration, Mononuclear; subcutaneous	(4)	(0)	(0)	(1)	(1)	(0)	(1)	(3)
minimal	4	0	0	1	1	0	1	3
Degeneration; vein	(0)	(0)	(0)	(0)	(0)	(0)	(1)	(0)
minimal	0	0	0	0	0	0	1	0
Degeneration; myofiber	(0)	(0)	(1)	(2)	(2)	(1)	(1)	(1)
minimal	0	0	0	2	2	1	1	1
slight	0	0	1	0	0	0	0	0
Infiltration, Mixed Inflammatory Cell; subcutaneous	(0)	(0)	(1)	(2)	(2)	(2)	(1)	(0)
minimal	0	0	0	2	2	0	0	0
slight	0	0	1	0	0	2	1	0
Injection Canal	0	0	1	0	0	2	0	0
Injection Canal; muscular	0	0	0	0	0	0	0	1
Granulation Tissue	(0)	(1)	(0)	(0)	(0)	(0)	(0)	(0)
minimal	0	1	0	0	0	0	0	0

Pathology - Intergroup Comparison of Histopathology Observations

208068 - GIC
2-wk dose range finding study by subcutaneous administration in Wistar rats

Observations: Non Neo-Plastic	MALES				FEMALES			
Removal Reasons: All of those SELECTED	0 nmol/kg	30/80 nmol/kg	60/160 nmol/kg	90/240 nmol/kg	0 nmol/kg	30/80 nmol/kg	60/160 nmol/kg	90/240 nmol/kg
Number of Animals on Study :	5	5	5	5	5	5	5	5
Number of Animals Completed:	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)
KIDNEYS;								
Examined	(5)	(1)	(1)	(5)	(5)	(0)	(0)	(5)
Within Normal Limits	4	1	1	3	0	0	0	1
Not Examined: CANNABILIZED	0	0	0	0	0	0	1	0
Basophilia; tubule	(1)	(0)	(0)	(1)	(2)	(0)	(0)	(3)
minimal	1	0	0	1	2	0	0	3
Calcification; corticomedullary junction	(0)	(0)	(0)	(0)	(4)	(0)	(0)	(2)
minimal	0	0	0	0	4	0	0	2
Autolysis	0	0	0	1	0	0	0	1
LIVER;								
Examined	(5)	(1)	(0)	(5)	(5)	(0)	(0)	(5)
Within Normal Limits	2	1	0	1	3	0	0	1
Not Examined: CANNABILIZED	0	0	0	0	0	0	1	0
Congestion	0	0	0	1	0	0	0	1
Inflammatory Cell Foci; parenchyma	(3)	(0)	(0)	(3)	(0)	(0)	(0)	(3)
minimal	3	0	0	3	0	0	0	3
Infiltration, Mononuclear; periportal	(0)	(0)	(0)	(2)	(2)	(0)	(0)	(1)
minimal	0	0	0	2	2	0	0	1
PANCREAS;								
Examined	(5)	(1)	(0)	(5)	(5)	(0)	(0)	(5)
Within Normal Limits	4	1	0	2	5	0	0	4
Not Examined: CANNABILIZED	0	0	0	0	0	0	1	0
Infiltration, Mononuclear; interstitium	(1)	(0)	(0)	(3)	(0)	(0)	(0)	(0)
minimal	1	0	0	3	0	0	0	0
Autolysis	0	0	0	0	0	0	0	1
THYROID GLANDS;								
Examined	(5)	(1)	(0)	(5)	(5)	(0)	(0)	(5)
Within Normal Limits	4	1	0	4	5	0	0	4
Not Examined: CANNABILIZED	0	0	0	0	0	0	1	0
Cyst	1	0	0	1	0	0	0	0
Autolysis	0	0	0	0	0	0	0	1

Pathology - Intergroup Comparison of Histopathology Observations

208068 - GIC
2-wk dose range finding study by subcutaneous administration in Wistar rats

Observations: Non Neo-Plastic	MALES				FEMALES			
Removal Reasons: All of those SELECTED	0 nmol/kg	30/80 nmol/kg	60/160 nmol/kg	90/240 nmol/kg	0 nmol/kg	30/80 nmol/kg	60/160 nmol/kg	90/240 nmol/kg
Number of Animals on Study :	5	5	5	5	5	5	5	5
Number of Animals Completed:	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)
UTERUS;								
Examined	(0)	(1)	(0)	(0)	(0)	(1)	(0)	(0)
Within Normal Limits	0	1	0	0	0	0	0	0
Not Examined: CANNABILIZED	0	0	0	0	0	0	1	0
Dilation	0	0	0	0	0	1	0	0
LUNGS;								
Examined	(0)	(0)	(1)	(1)	(0)	(0)	(0)	(1)
Within Normal Limits	0	0	1	0	0	0	0	0
Not Examined: CANNABILIZED	0	0	0	0	0	0	1	0
Congestion	(0)	(0)	(0)	(1)	(0)	(0)	(0)	(1)
moderate	0	0	0	1	0	0	0	1
Edema; alveolus	(0)	(0)	(0)	(1)	(0)	(0)	(0)	(0)
slight	0	0	0	1	0	0	0	0
Infiltration, Mixed Inflammatory Cell; perivascular	(0)	(0)	(0)	(1)	(0)	(0)	(0)	(0)
minimal	0	0	0	1	0	0	0	0
Compression	0	0	0	0	0	0	0	1

Toxicokinetics

Days 1 and 14 (before dosing, 1, 3, 9, and 24 hrs after dosing)

Due to limited data, no conclusions could be drawn with regards to sex difference and dose-response relationship. The T_{max} was approx. 1-3 hrs for insulin 454 and approx. 1-9 hrs for liraglutide with no does-response relationship.

Table 17: Study No 208068 2 Week Rat Study - TK Profile

Estimated toxicokinetic parameters for insulin 454 following subcutaneous administration of NN9068

Day	Dose (nmol/kg)	Gender	AUC_{last} (h*pM)	C_{max} (pM)	T_{max} (h)
1	30	Female	162000[b]	29900	1.00
		Male	95500[a]	7040	3.00
	60	Female	172000[b]	34300	3.00
		Male	271000[a]	20200	3.00
	90	Female	269000[a]	35600	3.00
		Male	548000[a]	60400	3.00
14	30	Female	260000[a]	43500	1.00
		Male	347000[a]	59800	3.00
	60	Female	385000[b]	74500	1.00
		Male	430000[a]	52700	3.00
	90	Female	573000[a]	79600	3.00
		Male	1760000[a]	237000	3.00

[a]AUC_{0-24} [b]AUC_{0-9}

Estimated toxicokinetic parameters for liraglutide following subcutaneous administration of NN9068

Day	Dose (nmol/kg)	Gender	AUC_{last} (h*pM)	C_{max} (pM)	T_{max} (h)
1	80	Female	716000[b]	135000	3.0
		Male	676000[a]	68000	9.0
	160	Female	1340000[b]	211000	9.0
		Male	1600000[a]	164000	9.0
	240	Female	1160000[a]	96300	9.0
		Male	1950000[a]	219000	3.0
14	80	Female	1340000[a]	251000	3.0
		Male	2000000[a]	244000	3.0
	160	Female	2340000[b]	373000	9.0
		Male	3340000[a]	336000	3.0
	240	Female	2730000[a]	423000	3.0
		Male	2660000[a]	440000	1.0

[a]AUC_{0-24} [b]AUC_{0-9}

Dosing Solution Analysis

The drug product was analyzed by HPLC. The results were within limits.

Analytical results :
Period of analysis : Feb. 2008

Product batch No.	Analyzed in Dept. no.	Analyzed	Test Method	Limits	Result
412_N08081	202	Liraglutide content	RP-HPLC	(b) (4)	
		454 content			
		ID Liraglutide		Complies	Complies
		ID 454		Complies	Complies

7 Genetic Toxicology

In accordance with ICH M3 (R2) no genotoxicity studies were performed with IDegLira.

The genotoxicity assessments of IDeg and liraglutide were performed under IDeg NDA 203314 and liraglutide NDA 22341. In a standard genotoxicity battery of *in vitro* and *in vivo* studies, liraglutide demonstrated no genotoxic potential. Genotoxicity studies were not performed with IDeg as it is considered a biotechnology-derived product without mutagenic potential.

8 Carcinogenicity

No carcinogenicity studies were conducted for the IDegLira fixed ratio combination.

For IDeg, the nonclinical in vitro and in vivo studies on cell proliferation and neoplasm formation showed no adverse findings and the carcinogenic potential of IDeg was not greater than that of human insulin.

For liraglutide, the carcinogenic potential was assessed in 104-week studies in mice and rats. Liraglutide caused local, injection site related fibrosarcomas in male mice at the highest dose and thyroid C-cell tumors in rodents. Studies in mice also demonstrated that liraglutide-induced C-cell proliferation was dependent on the GLP-1 receptor and that liraglutide did not cause activation of the REarranged during Transfection (RET) proto-oncogene in thyroid C-cells.

9 Reproductive and Developmental Toxicology

No development and reproductive toxicity studies have been conducted for IDegLira.

With IDeg, minor fetal and infant effects were observed which were secondary to the lowered maternal blood glucose levels. The animal reproduction studies did not reveal any difference between IDeg and human insulin regarding embryotoxicity and teratogenicity.

Liraglutide has been shown to be teratogenic in rats and has been shown to cause reduced growth and increased total major abnormalities in rabbits.

10 Special Toxicology Studies

Local tolerance

Study No 207451: SC pig with IDeg/Lira (600/ (b) (4))

The histopathological changes induced by GIC and the vehicle at the site of injection were considered to be mild, only marginally above the minimal reactions induced by saline, and therefore considered acceptable for subcutaneous administration.

Study title: GLP-1-insulin combination GIC: local toxicity in pigs 2 and 5 days after subcutaneous injection (GLP)

Study description:
This study evaluated the local tissue reaction in the subcutaneous tissue at the injection site 2 and 5 days after subcutaneous injection of the formulation that will be used in the clinical trials and to compare it with the reaction after injection of the vehicle and sterile isotonic saline. Four female SPF pigs were anaesthetized, shaved and washed on the back. On either side of the spine and just behind the shoulders, six injection sites were marked. Three formulations, one GIC formulation (Formulation #3; Batch No 412_N08004), one vehicle formulation (Formulation #1; Batch No 412_N08005) and sterile isotonic saline (Formulation 2; Batch No 7432051), were administered on Day 0 on the left side of the back and on Day 3 on the right side of the back. All injections were given with a NovoPen® 3.0, with a 28G needle mounted with a device which allowed the needle to be introduced approx. 4 mm. For all injections, the dose volume was 200µl.

Contents of the test and control articles

Formulations	pH	Content Liraglutide/O454	Zn content	Other excipients	Other
Formulation 1	pH 8.2	none / none	(b) (4)	Glycerol: (b) (4) mM Phenol: (b) (4) mM	
Formulation 2	NA	none / none		none	(b) (4) mM (b) (4)
Formulation 3	pH 8.2	(b) (4) mM liraglutide/ 0.6 mM O454		Glycerol: (b) (4) mM Phenol: (b) (4) mM	

Study findings:

- On Day 1, all pigs had a reduced appetite. No other clinical signs were seen.
- At necropsy, the injection sites showed that GIC in three of four injection sites induced edematous changes 5 days after injection. No other changes were seen at the injection sites at necropsy. The reaction was somewhat delayed, as no reactions were seen earlier. No macroscopic changes were seen after vehicle or saline. No adherences to the underlying skeletal muscle were seen.
- Two days after injection with GIC and the vehicle, the histopathological changes were characterized by inflammatory reactions in the range of minimal to slight, whereas saline induced only minimal inflammatory reactions.
- Five days after injection, the histopathological changes induced by GIC and the vehicle were characterized by inflammatory reactions in the range of minimal to slight, whereas inflammatory reactions induced by saline were either none or minimal.
- Overall, GIC did not cause local tissue reactions beyond the range recorded in the vehicle group at any time point. No correlation with macroscopic findings at necropsy was evident.

Study No 210203: IV IM SC rabbit with IDegLira (600/960)

A single dose administered either subcutaneously, intramuscularly or intravenously to female rabbits, caused local clinical reactions (erythema) caused by the injection procedure. No drug related macroscopic and microscopic changes were noted.

Study title: GIC B3: local tolerance study in rabbits (GLP)

Reviewer's note: IDegLira (600/960) represents the clinical ratio. However, GICB(3) formulation was highly similar to, but not identical to, the final clinical formulation for marketing.

Study description:
This study, conducted at (b) (4) assessed local tolerance of the GIC B3 at the injection sites after subcutaneous, intramuscular and intravenous injection. Fifteen female albino rabbits (New Zealand White) were given a single injection in the neck, ear or thigh, respectively, with the test item in right side and a single injection with the vehicle in the left side. The injection sites were observed (hemorrhage, bruising, erythema and swelling) and scored prior to treatment and in addition to this 3 hours ±15 minutes after treatment. Thereafter the injection sites were observed daily. The animals were killed on Day 5 and a macroscopic examination was performed. The injection sites were preserved and examined microscopically.

Group	Animal Nos (Female)	Colour code	Route of treatment	Dose volume (ml/injection site)	Dose concen-tration (nmol/ml) NNC 0090-0000-1170 960 / NNC 0100-0000-0454	Dose (nmol/injection site) NNC 0090-0000-1170 960 / NNC 0100-0000-0454
1	1 – 5	White	Subcutaneous (neck, left side)	0.05 ml	0	0
			Subcutaneous (neck, right side)	0.05 ml	960 /600	48/30
2	6 – 10	Blue	Intramuscular (left thigh),	0.05 ml	0	0
			Intramuscular (right thigh)	0.05 ml	960/600	48/30
3	11 - 15	Green	Intravenous (left ear)	0.05 ml	0	0
			Intravenous (right ear)	0.05 ml	960/600	48/30

Study findings:

- Minor local reactions (mostly erythema) were observed after intravenous injection of the test item and the vehicle. The reactions were considered related to the route of administration and not to the test item or vehicle.

- The macroscopic and microscopic changes observed following subcutaneous, intramuscular and intravenous injection of the vehicle or the test item were considered to be related to the procedure of treatment/needle introduction and/or injection of the vehicle rather than a direct treatment-related effect, as the changes were considered to be of a similar severity and incidence following treatment with the vehicle and the test item.

11 Integrated Summary and Safety Evaluation

NDA 208583 was submitted by Novo Nordisk for IDegLira injection to treat type 2 diabetes mellitus. IDegLira will be administered to patients via once-daily subcutaneous injection at any time of the day. This is a fixed-ratio combination product provided in a pre-filled pen containing IDeg and liraglutide at a ratio of 100 units: 3/6 mg per mL. IDeg (insulin analog) was approved under NDA 203314 whereas liraglutide (GLP-1 receptor agonist) was approved under NDA 22341. The nonclinical program included one pharmacology study in rats, single dose pharmacokinetic studies in LYD pigs, repeat dose toxicity studies in rats and local tolerance studies in pigs and rabbits.

Pharmacology profile of IDegLira
In a single subcutaneous dose rat study (**Study No cfle080703**), IDegLira ((600/ (b) (4) nmol/mL) decreased blood glucose levels, decreased body weight and decreased food/water consumption as seen with IDeg or liraglutide alone. IDegLira, however, had a slightly earlier blood glucose reduction but with a smaller maximal blood glucose reduction than IDeg alone.

Pharmacokinetic profile of IDegLira
There were 4 pharmacokinetic studies conducted in LYD pigs. In **Study No anp090201**, three versions of GIC(B) [IDegLira(600/960)] were given to LYD pigs via subcutaneous administration. (b) (4) This study showed that three GIC(B) formulations had similar pharmacokinetics profile when compared to IDeg alone. In **Study No anp090302**, three versions of GIC(A) [IDegLira(600/ (b) (4))] were given to LYD pigs via subcutaneous administration. (b) (4) This study showed that three GIC(A) formulations had similar pharmacokinetics profile when compared to IDeg alone. In both studies, the relative bioavailability for liraglutide was less than expected when compared to liraglutide alone (b) (4) In **Study No anp090401**, two formulations, GIC(B) [IDegLira(600/960)] and GIC(A) [IDegLira (600/ (b) (4))], were administered to LYD pigs via subcutaneous administration. (b) (4) The study showed that IDegLira (600/ (b) (4)) had slightly higher bioavailability of IDeg or liraglutide than IDegLira (600/960) – IDeg: 92% vs. 86% and liraglutide: 52% vs. 46%, respectively. While IDegLira (600/ (b) (4)) had a slightly later T_{max} and lower C_{max} for liraglutide when compared to Victoza®, IDegLira (600/960) had similar pharmacokinetic profile compared to Victoza® reference formulation. In **Study No anp080203**, (b) (4) were given to LYD pigs via subcutaneous administration. (b) (4)

In repeat dose toxicity studies, systemic exposure was confirmed in all animals dosed with IDegLira. The exposure increases were approximately dose-proportional. Systemic accumulation of <2 fold was observed after up to 13 weeks of dosing, which was expected based on the component drugs' half-lives. No antibodies were observed towards liraglutide, whereas antibodies against IDeg were observed at a similar frequency to what was found detected in previous IDeg studies.

Repeated dose toxicity (4 wk and 13 wk) studies
In the 13-week study (**Study No 209212** with IDegLira (600/960); GICB(3)), relative weights (% of body weight) of kidneys and ovaries were increased. These changes were considered related to the reduced body weight gain. There were no macroscopic or microscopic changes of toxicological significance in those organs. Injection site reactions in the IDegLira dosed animals were similar to those receiving the vehicle. Notably there were no treatment-related findings in the adrenal gland, liver, prostate, seminal

vesicles or testes, which were observed at doses that caused severe hypoglycemia in the 4 week study. Consequently the NOAEL was concluded to be 20/32 mmol/kg/day IDegLira, the highest dose tested.

In the 4-week study (**Study No 208142** with IDegLira (600/(b) (4)); GICA(3)), the absolute and relative body weight-relative weight of the prostate and the absolute weight of the heart were decreased in IDegLira treated rats compared to those of the controls. These changes were considered related to the pronounced reduction in food consumption and reduced body weight gain. There were macroscopic and microscopic findings in adrenal gland (increased cortical cell vacuolation; attributable to an increased glucocorticoid synthesis as an adaptive stress response to decreased blood glucose levels exacerbated by the decreased food consumption observed in this study), liver (increased minimal to slight rarefaction due to increased storage of glycogen), prostate and seminal vesicles (smaller size in 2 out of 10 males dosed with 20/54 nmol/kg/day IDegLira and reduced prostate weight in this group with no histopathological correlate), testicular tubules (minimal apoptosis of spermatocytes in stage VII/VIII, likely related to hyperinsulinemia), and at injection site (dose-related increase in ulcerations and mild to moderate inflammatory cell infiltration, necrosis and fibrosis/granulation tissue with no difference between dosed and vehicle treated animals). No drug-related histopathological findings were observed in the heart. The changes in the prostate, seminal vesicles and stage specific spermatocyte apoptosis were considered an effect of the hypoglycemia, reduced food consumption and reduced body weight gain observed in this study (related to the pharmacological effect of liraglutide and/or insulin). These effects were not observed in 13 week toxicity studies where severe hypoglycemia was absent, indicating effects on adrenal, liver, and male reproductive tissues are unlikely to occur in humans with appropriate management of plasma glucose.

Local tolerance
Local tolerance (SC) was assessed in two separate studies in pigs and rabbits, the latter also investigating the effect of other routes of administration (IM or IV). In **Study No 207451**, IDegLira (600/(b) (4)) was given to pigs via subcutaneous injection. The study showed that findings at injection sites (inflammatory) were observed on both Days 2 and 5 after injection and in IDegLira and vehicle treated animals. In **Study No 210203**, IDegLira (600/960) was given to rabbits via various routes of administration (SC, IM and IV). The study showed that findings at injection sites (inflammatory) were observed in IDegLira and vehicle treated animals and regardless the route of administration. Overall, local tissue reactions were mild and comparable to that of vehicle and considered not to pose any concerns regarding tolerability.

Impurities
Both IDeg and liraglutide impurities have shelf life specification limits and in use acceptance criteria consistent with the proposed limits for 100 U/mL Tresiba® (IDeg) and 6.0 mg/mL Victoza® (liraglutide). (b) (4) impurities of IDeg were qualified in a toxicity study in rats under Tresiba® development program. In addition, five leachables (b) (4) from container closure system were identified and quantified in a long-term leachable study for IDegLira. Based on maximum clinical exposure (b) (4) ((b) (4) µg/person/day), this level would be (b) (4) fold below Permissible Daily Exposure established in the ICHQ3C. Based on the maximum clinical exposure (b) (4) ((b) (4) µg/person/day), this level would be (b) (4) below the qualification threshold established by the Product Quality Research Institute, Development of Safety Qualification Threshold and Their Use in Orally Inhaled and Nasal Drug Product Evaluation and below the TTC per ICH M7. Overall, the potential human exposure levels to these impurities and leachables were evaluated and no safety concerns were identified.

Safety margin
The NOAEL of 20/32 nmol/kg/day IDeg/liraglutide, obtained from the 13 week toxicity study in rats was used for calculation of the animal to human safety margins. The exposure at NOAEL in the toxicology study of longest duration (13 weeks) measured as $AUC_{0\text{-}24hr}$ and C_{max} at steady state were compared to the human exposures at the highest dose administered in the therapeutic confirmatory trial (Study No NN9068-3697), identical to the maximum recommended human daily dose of IDegLira (50 IU/1.8mg). The animal/human AUC safety margins were 2.1X for IDeg and 3.3X for liraglutide. These safety margins in non-diabetic animal models, along with the absence of novel risks identified for the IDegLira

combination versus either drug alone, indicate that from a Pharmacology/Toxicology point-of-view the once-daily administration of the fixed-ratio combination of IDegLira at doses up to 50 IU/1.8mg is safe.

Table 18: Exposure Multiples

Exposure (AUC)-based Safety Margin in Pivotal Studies of IDegLira

Study	NOAEL (nmol)	Mean AUC (ng hr/mL)	Safety Margin based on AUC at NOAEL*
13 week rat 600/960 nmol IDegLira	20/32 IDeg/Lira	IDeg: 227.5 Lira: 1070 @ Week 13	**IDeg: 2.1x** **Lira: 3.3x**
4 week rat 600/ (b) (4) IDegLira	20/54 IDeg/Lira	IDeg: 288 Lira: 1380 @ Day 28	**IDeg: 2.5x** **Lira: 4.2x**

* $AUC_{0\text{-}24h}$ at max recommended clinical dose of 50U/1.8 mg IDegLira = 113/327 ng hr/mL (Study No N9068-3697).

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Model-derived estimates of IDeg exposure at steady-state for subjects at the maximum dose (50 units/1.8 mg) of IDegLira

	N	$AUC_{0\text{-}24h,IDeg,SS}$ Geometric Mean (pmol/L*h)	$AUC_{0\text{-}24h,IDeg,SS}$ Between-subject CV (%)	$C_{max,IDeg,SS}$ Geometric Mean (pmol/L)	$C_{max,IDeg,SS}$ Between-subject CV (%)
Maximum dose (50 units)	296	113×10^3	20.1	5196	18.1

Obtained from the subset of individuals, who reached the maximum dose of IDegLira (50 units IDeg and 1.8 mg liraglutide) at Week 26.

Model-derived estimates of liraglutide exposure at steady-state for subjects at the maximum dose of IDegLira (50 units/1.8 mg)

	N	$AUC_{0\text{-}24h,lira,SS}$ Geometric Mean (pmol/L*h)	$AUC_{0\text{-}24h,lira,SS}$ Between-subject CV (%)	$C_{max,lira,SS}$ Geometric Mean (pmol/L)	$C_{max,lira,SS}$ Between-subject CV (%)
Maximum dose (1.8 mg)	290	327×10^3	26.4	14791	24.3

Obtained from the subset of subjects who reached the maximum dose of IDegLira (50 units IDeg and 1.8 mg liraglutide) at Week 26

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

MIYUN M TSAI-TURTON
05/12/2016

CALVIN L ELMORE
05/12/2016
I concur.

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA Number: NDA 208583 **Applicant: Novo Nordisk** **Stamp Date: September 14, 2015**

Drug Name: IDegLira (Xultophy) **NDA/BLA Type: 505(b)(2)**

On **initial** overview of the NDA/BLA application for filing:

Insulin degludec/liraglutide (IDegLira) by Novo Nordisk is the formulated fixed-ratio combination drug product of insulin degludec and liraglutide. Insulin degludec (long acting basal insulin analog) was approved under NDA 203314 as TRESIBA on September 25, 2015. IDegLira was also approved as Xultophy® in the EU in September 2014. Liraglutide (GLP-1 analog) was approved drug under NDA 22341 as Victoza® in 2009.

IDegLira is indicated for once daily SC administration for type two diabetes mellitus (T2DM) treatment. The clinical formulation for IDegLira is 100 U insulin degludec and 3.6 mg/ml liraglutide. PDS290 IDegLira pen-injector intended for market is a prefilled pen-injector containing a 3 ml cartridge, as used for approved products Levemir® and NovoLog ®. The pen-injector could provide up to 50 dose steps which will allow for dose adjustments in increments of 1 unit insulin degludec and 0.036 mg liraglutide.

Composition of insulin degludec/liraglutide (100 U/3.6 mg/ml)

Component	Quantity per ml	Function	Reference to standards
Active substance			
Insulin degludec	600 nmol (100 U)	Active substance	Novo Nordisk A/S
Liraglutide	3.6 mg (960 nmol)	Active substance	Novo Nordisk A/S
Excipient			
Phenol	5.70 mg[A]	(b) (4)	Ph Eur, USP, JP
Glycerol	19.7 mg		Ph Eur, USP, JP
Zinc (b) (4)	55.0 µg		Ph Eur, USP, JPE
Hydrochloric acid	q.s.[B]		Ph Eur, USP, JP
Sodium hydroxide	q.s.[B]		Ph Eur, USP, JP
Water for injections	To make 1.00 ml		Ph Eur, USP, JP

[A] An overage (b) (4) is added to compensate for loss during manufacturing

[B] To reach pH 8.15

The IDegLira clinical development program includes 5 completed phase III trials designed to evaluate the efficacy and safety of IDegLira in T2DM subjects. Two pivotal trials (Trials 3697 and 3912) were designed to evaluate the benefit-risk profile of IDegLira specifically against IDeg and liraglutide. This included an assessment of the contribution of the individual components of the fixed-ratio combination to its primary efficacy effect.

Nonclinical Development

The nonclinical studies include 2-, 4-, and 13- week repeated dose toxicity studies in rats and local tolerance studies in rabbits and pigs. The studies were submitted and reviewed in the original IND submission in 2011. Drug-related effects in the repeated dose studies with IDegLira were attributed to the pharmacological or exaggerated pharmacological effects of insulin and GLP-1 analogues. In local tolerance studies, the local reaction was mild and comparable to that

of vehicle. There were no genotoxicity, carcinogenicity, or reproductive/developmental toxicity studies performed with IDegLira. In addition, the impurities were qualified and the proposed shelf-life specification limits and in use acceptance criteria for IDegLira were met. Leachables in the drug product were evaluated and are included in the NDA. Five leachables (b) (4) have been identified and quantified in the CMC section (Module 3).

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	X		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		
4	Are all required and requested IND studies (in accord with 505 (b)(1) and (b)(2) including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	X		
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	X		Once daily SC injection
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	X		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	X		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m^2 or comparative serum/plasma levels) and in accordance with 201.57?	X		Sections 8 & 13
10	Have any impurity, degradant, extractable/leachable, etc. issues been addressed? (New toxicity studies may not be needed.)	X		Leachables are addressed in under CMC - Module 3.
11	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			N/A
12	If the applicant is entirely or in part supporting the safety of their product by relying on nonclinical information for which they do not have the right to the underlying data (i.e., a 505(b)(2) application referring to a previous finding of the agency and/or literature), have they provided a scientific bridge or rationale to support that reliance? If so, what type of bridge or rationale was provided (e.g., nonclinical, clinical PK, other)?	X		Acute PK at 600 IDeg/ (b) (4) Lira PK in rats (general tox)/guinea pigs (formulation development) TOX - Single, 2 wk, 4,wk, 13 wk, local tolerance

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? YES

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

N/A

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

N/A

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

MIYUN M TSAI-TURTON
10/27/2015

DAVID B CARLSON
10/27/2015
Fileable for nonclinical