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APPLICATION NUMBER:

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PHARMACOLOGY REVIEW(S)

DEPARTMENT OF HEALTH AND HUMAN SERVICES
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CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

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Product:	Insulin glargine and lixisenatide injection
Indication:	Type 2 diabetes
Applicant:	Sanofi Aventis
Review Division:	Metabolism and Endocrine Products (DMEP)
Reviewer:	Feleke Eshete, Ph.D.
Supervisor/Team Leader:	Todd Bourcier, Ph.D.
Division Director:	Jean-Marc Guettier, M.D.
Project Manager:	Martin White, M.S.

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1. Executive Summary

1.1 Introduction

This is a marketing application by Sanofi Aventis for a fixed dose combination of insulin glargine and lixisenatide for type 2 diabetes mellitus as an adjunct to diet and exercise. The applicant intends to market two fixed-ratio solutions to provide insulin glargine daily doses from 10 to 60 units, while limiting the lixisenatide dose to a maximum of 20 µg. The dose of lixisenatide ranges from 5-20 µg and would be titrated based on the dose of insulin glargine required by the patient, and the ratio in the pen being used. The formulation is to be delivered by prefilled disposable pen-injectors at 2:1 ratio of insulin glargine to lixisenatide for daily doses up to 40 units, and 3:1 ratio for insulin glargine doses up to 60 units.

1.2 Brief Discussion of Nonclinical Findings

Sanofi Aventis cross-referenced the nonclinical information from the approved insulin glargine and lixisenatide (NDA 208,471 under review) to support the marketing application. The sponsor also submitted limited number of studies addressing cross-receptor activation, testing for potential exacerbation of known toxicities by glucagon-like peptide-1 receptor (GLP1-R) agonists or insulins, and for qualification of potential impurities and degradants in the drug substances.

Insulin glargine (HOE901) is a human insulin analogue with modifications to alter the isoelectric point of the molecule towards neutral pH resulting in a prolonged absorption from the injection site. Insulin glargine shares many of the pharmacodynamic properties of human insulin. It is a full agonist at the insulin receptor, but exhibited higher binding affinity to the insulin-like growth factor -1 receptor (IGF-1R). Nonclinical findings have suggested that the effects of human insulin and insulin glargine were additive. The glucose lowering activity of insulin glargine at therapeutic dose and its catecholamine releasing properties at high dose were not different from other marketed insulins. Intravenous clinical testing has shown that the glucose-lowering effect of insulin glargine on a molar basis was similar to that of human insulin. Its metabolic transformations are also similar to that of human insulin.

Lixisenatide (AVE0010) is a DPP-4 resistant GLP-1 receptor (GLP-1R) agonist that is structurally related to exendin-4, a 39 amino acid peptide, marketed as exenatide. Marketing application for lixisenatide as monotherapy (NDA 208,471) is currently under review for type 2 diabetes mellitus (T2DM) indication for subcutaneous daily administration of 10 µg for 14-days as initiation dose and 20 µg as maintenance dose.

Lixisenatide exhibited about 4-times greater *in vitro* receptor binding affinity at the GLP-1R compared to human GLP-1. Lixisenatide induced glucose-dependent insulin secretion in culture, promoting insulin secretion at glucose concentration of > 10 mM, but not 5.6 mM concentration.

Acute OGTTs were performed in dogs with individual drug products and the combination, measuring insulin and C-peptide in serum in addition to blood glucose. Both the monotherapy and the combination treatments reduced the glucose surge and maintained it up to 8 hrs. The effects of the combination on blood glucose were similar to that of lixisenatide alone during the absorptive phase (post prandial) and to that of insulin glargine in the post-absorptive phase[1]. Serum C-peptide was higher than controls in the post-absorptive phase in the groups that received lixisenatide alone, except at low dose (0.15 µg/kg). C-peptide release was however suppressed when lixisenatide was administered in combination with higher dose of insulin glargine.

The suppression of glucose surge and maintenance of glucose level appear to be dependent on lixisenatide during the absorptive phase and at lower doses of insulin glargine. The post-absorptive effect appears to be a reflection of the additive effect of endogenous insulin due to lixisenatide and insulin glargine injection. However, the contribution of lixisenatide wanes as the dose of insulin glargine is increased since the release of endogenous insulin is suppressed as evidenced by the suppression of C-peptide at higher dose of insulin glargine. Corroborative evidence for suppression of endogenous insulin secretion also comes from the reversible degranulation of the β-cells of the islets of Langerhans rats and dogs in the toxicology studies, indicating a decline in insulin production due to exogenous supply.

In a single dose intravenous CV safety study conducted in anesthetized dogs with a premixed combination of insulin glargine and lixisenatide, serum potassium was lowered and heart rate was increased after administration of 0.1 units/kg (<1-times MRHD based on mg/m^2) of insulin glargine. No hemodynamic or myocardial electrical activity changes were reported in the previously reviewed toxicological studies with insulin glargine.

Anti-lixisenatide antibodies were formed in rodents and dogs in a dose and duration-dependent manner and resulted in accumulation of the test-article in the systemic circulation up on multiple dosing. However, results of cell-based assays have indicated that antibody-bound lixisenatide can activate the receptor, although the implication of the cell culture finding in receptor activation in target tissues is unclear.

Lixisenatide has been evaluated for potential toxicity in rodents and dogs for various study durations. The main findings in these studies were decreased body weight gain and food consumption during the period of test-article administration in rodent and dogs and gastrointestinal disturbances in dogs. Gastrointestinal effects are the hallmark toxicity for the class and manifest clinically as nausea and weight loss.

[1] Clinical data also indicated that lixisenatide, but not insulin glargine decreased glucose excursion at week 30 compared to baseline (Study EFC12404).

The testes and epididymis were also targeted at high doses in rats (>160-times the exposure at 20 ug)[2] and mid dose (> 45-times) in dogs after repeat dosing with lixisenatide. These effects were treatment duration-dependent and were reversed after 1-month of drug withdrawal. There were no effects on sperm parameters (count, motility, morphology) in normal men at the recommended therapeutic dose of 20 µg. Consistent with other investigational and marketed GLP-1 agonists, there were no findings of adverse histological changes or signs of drug-related neoplasms in pancreatic tissue in mice or rats administered lixisenatide for up to 2 years, or in dogs for up to 1 year at high multiples of clinical exposure.

Minimal increase in the incidence of subdermal fibrosis/inflammation was noted at the injection site of animals in the 13-week mouse study with lixisenatide. There were also slightly increased incidences of hemorrhage, fibroblastic reaction, and abscess at the injection site for male rats receiving ≥100 µg/kg BID (~ 98-times the exposure at 20 µg in mg/m^2) for 2-weeks. The incidence of increased red focus/area at injection sites, as well as dose-related increase in inflammation and fibrosis was reported at ≥200 µg/kg BID (45-times the exposure at 20 µg based on AUC) in the chronic dog study.

In local tolerability studies, injection site reaction and inflammation were hinted in the original review for insulin glargine, but was not observed in the subsequent studies with injection of a more concentrated product of insulin glargine (Toujeo®). Antibodies were formed in rats against insulin glargine, but not in dogs. This may not be surprising given the similarity of the primary sequence of dog and human insulin compared to that of rat. Local tolerance studies have also been conducted with the pre-mixed clinical formulation and by injection of individual drug formulations on different sides of rabbits via various parenteral routes (SC, PV, IV, and IM). One of the studies evaluated a ready to use aqueous solution of 0.8 mg/m lixisenatide and 3.6 mg/mL insulin glargine. No mortality or adverse clinical signs were noted in the study. Minimally reddened and hemorrhagic appearances were reported equally in control and test-item injected sites. Microscopic examination at the SC injection site showed minimal to mild necrosis of the panniculus muscle that regenerated after 120 hrs.

Reproductive toxicology studies were not conducted with the combination of lixisenatide and insulin glargine. Lixisenatide administered to pregnant rats and rabbits during the period of organogenesis was associated with increased incidences of skeletal and visceral malformations, including closure defects, at systemic exposures that are 1- and 6-times the exposure (AUC) at the 20 µg human Q.D. dose in rats and rabbits, respectively. Since reduced food intake and weight gain was also noted during test-article administration it is plausible that the brief interruption of nutritional status in the dams may have resulted in the adverse skeletal findings, but its contribution to the occurrence of the rare closure defects, including micro- and anophthalmia, diaphragmatic hernia, thoracogastroschisis, and spina bifida, is less obvious. While the very low placental transfer of lixisenatide (0.1% in rats, ≤0.5% rabbits) argues for a

[2] Day 1 AUCs from animals have been compared with AUC value of antibody positive patients in study ACT60011.

more prominent role of maternal factors in the adverse outcome, a direct adverse effect of lixisenatide on developing fetuses cannot be entirely excluded. The effects of insulin glargine in animal reproduction studies (e.g., increased resorptions/fetal loss) do not generally differ from those observed with regular human insulin, and are likely due to hypoglycemia. Since the weight loss and hypoglycemia observed at low multiples of the proposed clinical doses can harm the fetus, the lixisenatide and insulin combination bears potential risk to the fetus during pregnancy.

Carcinogenicity studies of 2-year durations were conducted in CD-1 mice and Sprague-Dawley rats with twice daily subcutaneous doses of 40, 200, or 1,000 µg/kg. Statistically significant increase in thyroid C-cell adenomas were observed in male mice at 2,000 µg/kg/day (>180-times the human AUC achieved at 20 µg). Increased incidence of endometrial adenocarcinoma of the uterus was observed in mice at mid- and high-dose, with statistically significance at the mid-dose group only (~33-times exposure ratio to the recommended dose)[3]. Statistically significant increases in thyroid C-cell adenomas were seen at all doses in rats receiving AVE0010, resulting in systemic exposures that are ≥15-times the human exposure achieved at 20 µg based on AUC[4]. A numerical increase in thyroid C-cell carcinomas was observed at ≥400 mcg/kg/day >56-times AUC of 20 µg dose) in males and females relative to the human AUC at 20 µg).

In vitro mechanistic studies have been conducted to further characterize the potential neoplastic effects of lixisenatide in the thyroids. These studies examined GLP1-R expression in various tissues and species, as well as effects on proliferative genes in calcitonin-expressing cells. The applicant also compared GLP-1R activation by lixisenatide and other marketed GLP-1R agonists in rat vs. human thyroid C-cell lines. The efficacy (E_{max}%) for cAMP for all the GLP-1R agonists was the same, but the relative potencies of exendin-4, lixisenatide & GLP-1(7-36) were the same, but were much greater than that of liraglutide and GIP. E_{max} % for the human thyroid C-cell line was lower for all GLP-1R agonists; relative potency was not calculated because of poor curve fitting. Nonetheless, it appears that GLP-1R activation appears to favor the rat cell line.

In addition, high doses of lixisenatide (~350-times the 20 µg human dose) administered to mice in 13-week mouse study showed a 2-5-fold increase in the expression of GLP-1R and calcitonin in the medullary thyroid. Immunohistochemical examination of thyroid tissue using calcitonin and Ki-67 double staining did not reveal an increase C-cells that were undergoing replication. Another gene expression analyses showed a treatment-related increase in the number calcitonin and GLP-1R mRNA transcripts in thyroid C-cells in mice continuously transfused with the same dose (~ 20-times the 20 µg, based mg/m^2) of lixisenatide and exenatide. No meaningful differences in the expression of

[3] AUC value from Days1/2 in 3-month mouse study (DSE 2005-00443) was compared with human AUC from ADA positive patients.
[4] AUC value from Day 4 in rat carcinogenicity study was compared with human AUC from ADA positive patients.

proliferative genes were observed between treated and control groups, which is consistent with an apparent lack of C-cell proliferation based on histopathology. Thus, in mouse calcitonin secretion and GLP-1R are upregulated after sustained infusion of lixisenatide and exenatide. However, there are no indications that the prolonged activation of the GLP-1R initiates a proliferative effect.

There are warnings for long-acting GLP-1R agonists concerning thyroid c-cell hyperplasia and neoplasia in rodents with undetermined relevance to humans. Incidences of thyroid C-cell adenomas were increased in mice and rats with lixisenatide. However, the neoplastic effects were noted at high multiples of the clinical exposure. Moreover, lixisenatide has a short half-life, and there are no data in many of the mechanistic studies that implicate lixisenatide in thyroid C-cell proliferation. These data suggest that the carcinogenicity profile of lixisenatide is similar to the twice daily formulation of exenatide, although it is developed as a once daily formulation.

Post-marketing reports implicate GLP-1R agonist in pancreatitis. Consistent with other investigational and marketed GLP-1 mimetics, there were no findings of adverse histological changes or signs of drug-related neoplasms in pancreatic tissue in mice or rats administered lixisenatide for up to 2 years, or in dogs for up to 1 year.

In conclusion, since lixisenatide did not affect the pharmacokinetics and mitogenic potential of insulin glargine, and in light of the absence of the potential for cross-receptor activation between the two drug substances, the safety profile of the fixed dose combination was comparable with that of the individual components. However, there may be additional risk of hypoglycemia at the highest proposed clinical doses.

1.3 Recommendations

1.3.1 Approvability

The nonclinical data referenced in this marketing application support the approval of a fixed dose combination of insulin glargine and lixisenatide.

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

The labeling recommendation submitted by the sponsor in PLLR format is acceptable with the suggested changes (see labeling changes at the end of current review).

2. Drug Information

2.1 Drug

CAS Registry Number (Optional): 160337-95-1 and 320367-13-3

Generic Name: Insulin glargine and Lixisenatide FDC

Code Name: HOE901 and AVE0010/ZS42-0010/ZP10

Chemical Name:

Insulin glargine: 21^{A}-Gly-30^{B}a-L-Arg-30^{B}b-L-Arg-human insulin

Lixisenatide: H-His-Gly-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Gln-Met-Glu-Glu-Glu-Ala-Val-Arg-Leu-Phe-Ile-Glu-Trp-Leu-Lys-Asn-Gly-Gly-Pro-Ser-Ser-Gly-Ala-Pro-Pro-Ser-Lys-Lys-Lys-Lys-Lys-Lys-NH2

Molecular Formula/Molecular Weight:

Insulin glargine: $C_{267}H_{404}N_{72}O_{78}S_6$/6063.02
Lixisenatide: $C_{215}H_{347}N_{61}O_{65}S$/4858.5

Pharmacologic Class:

Insulin analog and glucagon-like peptide-1 receptor agonist

2.2 Relevant INDs and NDAs

Table 1: Relevant applications

Application	Product	Dose	Route
Insulin Glargine			
NDA 21,081/ IND 49078	Insulin glargine/HOE90	10 U, Q.D.	SC injection
NDA 206,538	insulin glargine	1- 80 U, Q.D	SC injection
Exendin-4 analogs			
NDA 208.471/IND 62,724	AVE0010 (lixisenatide)	20 µg Q.D	SC injection

2.3 Drug Formulation

Table 2: Insulin glargine/Lixisenatide Solution for Injection

Components[a]	(b) (4)	Composition of formulation with 33 µg/mL lixisenatide			Function	Reference to standards[b]
		Percentage [%]	Per mL [mg]	Per unit (3 mL cartridge) [mg]		
Insulin glargine *[equivalent to U of insulin glargine]*		0.36	3.6378 *[100]*	10.9134 *[300]*	Drug substance	Ph. Eur., USP
Lixisenatide		0.003	0.033	0.099	Drug substance	In-house
Glycerol (b) (4) per cent)		2.0	20.0	60.0	(b) (4)	Ph. Eur.
Methionine		0.3	3.0	9.0		Ph. Eur., USP
Metacresol[c]		0.27	2.7	8.1		Ph. Eur., USP
Zinc (b) (4)				(b) (4)		Ph. Eur., USP
Hydrochloric acid, (b) (4)						Ph. Eur., NF
Sodium hydroxide						Ph. Eur., NF
Water for injection						Ph. Eur., USP
(b) (4)						

a Components are listed according to their pharmacopoeial names, if available. If more than one monograph exists, other names are given in brackets, along with the compendial origin.
b Reference is made to the current edition of the Pharmacopoeia.
c For metacresol, the common chemical name "m-cresol" is also used within this document.
d (b) (4)
e The amount of zinc (b) (4)

2.4 Comments on Novel Excipients

There are no novel excipients in the proposed clinical formulation

2.5 Comments on Impurities/Degradants of Concern

None

2.6 Proposed Clinical Population and Dosing Regimen

The product in this application is a fixed dose combination of insulin glargine and the GLP-1 receptor agonist lixisenatide. The proposed indication is as an adjunct to diet and exercise to improve glycemic control in adults with T2DM when treatment with both insulin glargine and lixisenatide is appropriate.

Sanofi intends to market two fixed-ratio solutions of lixisenatide and insulin glargine to provide insulin glargine daily doses from 10 to 60 units, while limiting the lixisenatide dose to a daily maximum of 20 µg. The dose of lixisenatide ranges from 5-20 µg depending on the dose of insulin glargine that is required by the patient, and the ratio in the pen being used. The formulation is to be delivered by prefilled disposable pen-injectors at 2U:1µg ratio of insulin glargine: lixisenatide (10-40 pen) for doses up to 40 U, and 3U:1µg ratio for insulin glargine doses up to 60 U (30—60 pen).

2.7 Regulatory Background

Insulin glargine is marketed in the United States as parenteral hypoglycemic agent since year 2000 (Sanofi-Aventis NDA 21,081). Administration of a daily single subcutaneous dose of 0.2 U/kg or up to 10 U/day of 100 U/mL is recommended for adults with type 2 diabetes mellitus that are not on insulin already. A more concentrated

(300 U/mL) insulin glargine, (Sanofi-Aventis NDA 206,538) is approved for glycemic control in adult T1DM and T2DM patients. A single daily injection of 1-80 U/day of is recommended depending on the individual's need.

Lixisenatide (AVE0010) is a DPP-4 resistant GLP-1R agonist. It is indicated in glycemia control as an adjunct to diet and exercise in T2DM. Marketing application (Sanofi-Aventis NDA 204,961) was filed for lixisenatide in 2012, but withdrawn with plans to resubmit when the final report CV study (ELIXA) is available. The lixisenatide NDA (NDA 208,471) has been submitted in July 2015 and is currently under review for subcutaneous daily administration of 10 µg for 14-days as initiation dose and 20 µg as maintenance dose.

The schema and table provided by the sponsor below show the clinical studies conducted in the development of the FDC of lixisenatide/insulin glargine.

Figure 1: Clinical studies with the fixed combination product

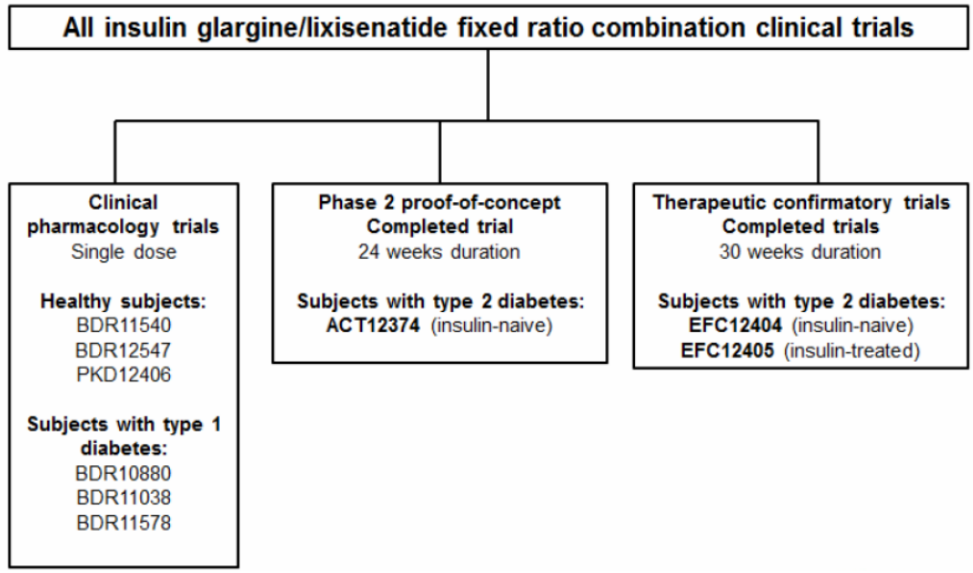


Table 3: Randomized, controlled trials for the demonstration of efficacy in the Phase 3 fixed-ratio combination clinical development program

Trial	Trial description and treatment	Population	Antidiabetic treatment at screening	No. of subjects randomized	Endpoints
EFC12404 (Phase 3): A randomized, 30 week, active-controlled, open-label, 3-treatment arm, parallel group multicenter study comparing the efficacy and safety of insulin glargine/lixisenatide fixed-ratio combination (FRC) to insulin glargine (100 U/mL) alone and to lixisenatide alone, all treatments on top of metformin in patients with type 2 diabetes mellitus	Three arms: -FRC -Insulin glargine (100 U/mL) alone -Lixisenatide alone ***4-week run-in phase:*** Discontinuation of OAD other than metformin. Optimization of the metformin daily dose ***Randomized treatment period:*** Daily FRC dose could be titrated from an initial dose of 10 U/5 µg to a maximum dose of 60 U/20 µg. Daily glargine dose could be titrated from the initial dose of 10 U to a maximum dose of 60 U. The FRC and insulin glargine group doses were adjusted once weekly to achieve target fasting self-measured plasma glucose (SMPG) of 80 to 100 mg/dL (4.4 to 5.6 mmol/L) while avoiding hypoglycemia. Lixisenatide was initiated at 10 µg QD for 2 weeks; a maintenance dose of 20 µg QD was to be used for the duration of treatment, tolerability allowing.	Patients insufficiently controlled for at least 3 months prior to screening with metformin ± a second OAD. ***At screening:*** HbA1c ≥7.5% and ≤10.0% for patients previously treated with metformin alone HbA1c ≥7.0% and ≤9.0% for patients previously treated with metformin plus a second OAD ***At the end of the 4-week run-in period*** (before randomization): HbA1c ≥7.0% and ≤10.0% FPG ≤250 mg/dL (≤13.9 mmol/L)	Metformin ± a second OAD (sulfonylurea [SU], a glinide, a sodium-glucose co-transporter-2 (SGLT-2) inhibitor, or a dipeptidyl peptidase-4 [DPP-4] inhibitor). Discontinuation of OAD other than metformin. Daily metformin dose was increased weekly during the run-in phase to a final daily dose of at least 2000 mg or up to the maximal tolerated dose which had to be ≥1500 mg/day to allow randomization After randomization, metformin dose was to be maintained at a stable dose throughout the study unless safety issues developed.	Randomized 2:2:1 (FRC: insulin glargine: lixisenatide) FRC: N=469 Insulin glargine: N=467 Lixisenatide: N=234	**Co-primary**: - Superiority of the FRC versus lixisenatide - Non-inferiority of the FRC versus insulin glargine in change in HbA1c from baseline to Week 30. If noninferiority was shown, statistical superiority of the FRC compared to insulin glargine was to be tested according to a prespecified testing hierarchy. **Secondary:** Change in 2-hour PPG and blood glucose excursion during a standardized meal test from baseline to Week 30 Change in body weight from baseline at Week 30 Change in 7-point SMPG profiles from baseline to Week 30 (each time point and average daily value) Change in daily dose of insulin glargine at Week 30 (in the FRC and insulin glargine groups) Change in FPG (measured in central laboratory) from baseline to Week 30 Percentage of patients reaching HbA1c ≤6.5% and <7.0% at Week 30 Percentage of patients reaching HbA1c <7.0% with no body weight gain at Week 30 and/or no documented symptomatic hypoglycemia (PG ≤70 mg/dL (≤3.9 mmol/L) during the 30-week randomized treatment period Percentage of patients requiring rescue therapy during the 30-week treatment period
Trial	**Trial description and treatment**	**Population**	**Antidiabetic treatment at screening**	**No. of subjects randomized**	**Endpoints**
EFC12405 (Phase 3): a randomized, 30-week, treat-to-target, active-controlled, open-label, 2-treatment arm, parallel-group, multinational study comparing the efficacy and safety of insulin glargine/ lixisenatide fixed ratio combination (FRC) to insulin glargine alone (100 U/mL) (both treatments with or without metformin)	Two arms: - FRC - Insulin glargine (100 U/mL) alone ***6-week run-in phase***: Switch to insulin glargine (if other basal insulin taken) and dose titration. Discontinuation of OAD other than metformin. ***Randomized treatment period:*** <u>Starting dose:</u> **FRC**: 20 U/10 µg (Pen A) if glargine dose on the day before randomization was <30 U; 30 U/10 µg (Pen B) if glargine dose on the day before randomization was ≥30 U. The dose was to remain stable for 2 weeks. **Insulin glargine**: same dose as the one received on the day before randomization <u>Titration</u> FRC and glargine doses adjusted once weekly to achieve target fasting SMPG of 80 to 100 mg/dL (4.4 to 5.6 mmol/L) while avoiding hypoglycemia. Maximum FRC dose: 60 U/20 µg; Maximum glargine dose: 60U.	Patients insufficiently controlled on a stable basal insulin regimen with a stable (± 20%) daily dose between 15 and 40 U/day for at least 2 months before screening ***At screening:*** FPG ≤180 mg/dL (≤10.0 mmol/L) for patients receiving basal insulin in combination with 2 OADs or with 1 OAD other than metformin FPG ≤200 mg/dL (≤11.1 mmol/L) for patients on basal insulin only or basal insulin plus metformin HbA1c ≥7.5% or ≤10.0% ***At the end of the 6-week run-in period*** (before randomization): HbA1c ≥7.0% or ≤10.0% Mean fasting SMPG ≤140 mg/dL (≤7.8 mmol/L), calculated for the 7 days before randomization Average insulin glargine daily dose ≥20 U or ≤50 U calculated for the last 3 days before randomization	Metformin ± OAD(s) OADs could be 1 to 2 out of a SU, a glinide, a SGLT2-inhibitor, or a DPP-4 inhibitor. Only metformin could be continued during the study. If taken, metformin daily dose had to be ≥1500 mg/day and was to be maintained at a stable dose throughout the study unless safety issues developed	Randomized 1:1 FRC: N=367 Insulin glargine: N=369	**Primary**: Superiority of the FRC versus insulin glargine in change in HbA1c from baseline to Week 30 **Secondary:** Change in 2-hour PPG and blood glucose excursion during a standardized meal test from baseline to Week 30 Change in body weight from baseline to Week 30 Change in 7-point SMPG profiles from baseline to Week 30 (each time point and average daily value) Change in daily dose of insulin glargine from baseline to Week 30 Change in FPG (measured in central laboratory) from baseline to Week 30 Percentage of patients reaching HbA1c ≤6.5% and <7.0% at Week 30 Percentage of patients reaching HbA1c <7.0% with no body weight gain at Week 30 and/or no documented symptomatic hypoglycemia (PG ≤70 mg/dL (≤3.9 mmol/L) during the 30-week randomized treatment period Percentage of patients requiring a rescue therapy during the 30-week open-label treatment period

FRC: fixed-ratio combination; FPG: fasting plasma glucose; OAD: oral antidiabetic drug; PG: plasma glucose; PPG: postprandial plasma glucose; QD: once daily; SMPG: self-monitored plasma glucose; SU: sulfonylurea

3. Studies Submitted

3.1 Studies Reviewed

Table 4: Studies submitted

Pharmacological studies
divt0007 - Functional activity of lixisenatide in thyroid c-cells of rat and human origin *in vitro*
divt0044 - Influence of Lixisenatide on Insulin Glargine stimulated IGF 1 Receptor Autophosphorylation using MEF-IGFR cells
divt0049 - Influence of Lixisenatide on Insulin Glargine stimulated Insulin Receptor Autophosphorylation using CHO-IR cells
divt0056 - Characterization of Insulin glargine and Lixisenatide stimulated AKT- phosphorylation in RTC6-23 cells
divt0057 - Influence of lixisenatide on binding affinity of insulin glargine to the insulin receptor B
divt0063 - Expression of GLP1R, IGFR and INSR mRNA in cell lines 1.1B4, 6-23 and TT
divt0064 - In vitro potency of insulin glargine, lixisenatide, as well as mixtures thereof in the rat thyroid carcinoma cell line RTC 6-23 (clone 6)
divt0065 - Influence of the combination treatment with lixisenatide and insulin glargine on the process of apoptosis in rat thyroid carcinoma cell line RTC 6-23
divt0067 - In vitro potency of insulin glargine, lixisenatide, as well as mixtures thereof in the human pancreatic beta cell line 1.1 B4
divt0068 - Study of SAR213916 and SAR274136
divt0068-abbr - Binding of insulin glargine (SAR274136) to the GLP-1 receptor
divt0070 - Characterization of Insulin Glargine and Lixisenatide stimulated AKT- phosphorylation in 1.1B4 cells
divt0071 - Influence of the combination treatment with lixisenatide and insulin glargine on the process of apoptosis in the human pancreatic beta cell line 1.1 B4
divt0072 - Influence of the combination treatment with lixisenatide and insulin glargine on proliferation in human pancreatic beta cell line 1.1 B4
divv0050 - Acute effects of a subcutaneous injection of low dose lixisenatide mixed into two different concentrations of insulin glargine, U100 and U300, on oral glucose tolerance in male dogs
mvt0010 - Radioligand binding assay with human GLP-1 receptor vs. 0.03 nM 125I GLP1(7-36) amide (*NDA 208, 471 review*)
mvt0010ext - Radioligand binding assay with human GLP-1 receptor vs. 0.03 nM 125I GLP1(7-36) amide (*NDA 208, 471 review*)
mvv0025 - Acute glycemic effects of subcutaneous AVE0010/insulin glargine combination in female db/db mice
mvv0026 - Acute effects of subcutaneous AVE0010/insulin glargine combination on oral glucose tolerance in male dogs
cvr0345 - Insulin glargine/AVE0010 combination – Effect of a single intravenous dose on cardiovascular function in anesthetized dogs
Pharmacokinetic studies
doh1006 - Validation of an LC-MS/MS assay for the quantitation of HOE901, HOE901-M1 and HOE901-M2 in human plasma *(cursory review)*
dos1247 - Validation of an ELISA for the quantitative determination of total AVE0010 concentration in dog K2-EDTA plasma samples using AVE0010 ELISA kit (b) (4) *(cursory review)*
pmh0125-bads01 - Bioanalytical Data Summary AVE0010/D120411 Analysis of lixisenatide in

dog plasma samples from study PMH0125 *(cursory review)*
pmh0125-bapk2 - Plasma concentrations and pharmacokinetic parameters of insulin glargine, -M1 and i-M2 in Beagle Dogs after a single SC application of Lantus U100 or U300 formulation with or without the application of lixisenatide
pmh0125-pk1 - Plasma concentrations and pharmacokinetic parameters of AVE0010 in male Beagle Dogs after a single SC application of AVE0010 with and without the application of 0.3 U/kg Lantus (HOE901) as U100 or U300 formulation
Toxicological studies
tol1122 - AVE0010/Insulin glargine: Combined local s.c, i.m., i.v., p.v. tolerance study in female rabbits
tol1145 - AVE0010/Insulin glargine: Combined local SC, IM, IV and PV tolerance study in female rabbits
txc1539 - HOE901 - 13-week subcutaneous toxicity study in rats for impurity qualification

3.2 Studies Not Reviewed

None

3.3 Previous Reviews Referenced

a. Label for Lantus® (8/2015 revision) and NDA 21,081 nonclinical review by Dr. Herman Rhee, dated January 19, 2000.
b. NDA 204,961review for lixisenatide by Dr. Tim Hummer (DARRTS 22-Aug-2013)
c. IND reviews for insulin glargine and lixisenatide by Dr. Tim Hummer (DARRTs 18-Jun-2009 and 22-Apr-2011).

4. Pharmacology

4.1 Primary Pharmacology

Insulin glargine

Insulin glargine (HOE901, Lantus®) is recombinant human insulin produced in (K12 strain of *Escherichia coli.* The primary structure of human insulin was modified by addition of two arginines in the C-terminus of the B-chain and by replacement of asparagine at position 21 of the A-chain with glycine. These changes alter the isoelectric point of the molecule towards neutral pH (b) (4) after subcutaneous injection, and a prolonged absorption from the injection site. Since the insulin glargine formulation is acidic (pH 4.0), subcutaneous injection results in its local “precipitation” and in turn prolongation of absorption and its associated hypoglycemic action.

Insulin glargine exhibited about two-fold lower affinity of binding to the insulin receptor compared to human insulin, but had a similar association rate and a two-fold faster dissociation rate. Insulin glargine appears to act as a full agonist at the insulin receptor since comparable peak responses were noted *in vitro* despite lower potency (50%) relative to human insulin. There is evidence that higher plasma levels compensate for the 50% *in vitro* potency found for insulin glargine as compared to human insulin.

Depending on the cell type used for testing *in vitro*, insulin glargine had a 1- to 12-fold higher affinity to the IGF-1 receptor compared to human insulin. Insulin glargine had the highest IGF-1 receptor affinity (12-fold) relative to human insulin in osteosarcoma cells. Mitogenicity assays in these cells suggested that insulin glargine might have a mitogenic potential through binding to the IGF-1 receptor. The mitogenic activity of the insulin glargine and lixisenatide combination was tested in the human pancreatic beta cell line 1.1B4 that express both GLP-1 and insulin receptors. Lixisenatide alone did not increase thymidine incorporation, but insulin glargine, IGF1 and human insulin promoted thymidine incorporation in a dose-related manner. The mitogenic potential of insulin glargine is greater than that of the human insulin, and was not altered by lixisenatide.

The primary action of insulin glargine is similar to that of human insulin. It aids glucose metabolism by peripheral tissues and inhibits gluconeogenesis by the liver. In general the glucose lowering activity of insulin glargine at therapeutic doses and its catecholamine-releasing properties at high doses was not different from other marketed insulins. Intravenous clinical testing showed that the glucose-lowering effect of insulin glargine on a molar basis was similar to that for human insulin. Nonclinical findings have suggested that the effects of insulin and insulin glargine were additive. For instance, in adipocytes, equipotent mixtures of insulin glargine and human insulin exerted additive effect on lipogenesis and glucose transport. Nonetheless, evidence for interference of insulin glargine with the actions of human insulin has not been reported.

Lixisenatide

The glucagon-like peptide-1 (GLP-1) is an incretin secreted from the intestine in response to meal ingestion and also as a neuropeptide. GLP-1 (7-36)-amide is the major endogenous biologically active form of GLP-1, and GLP-1 (7-37) is the other minor circulating form in humans. These peptides are inactivated by DPP-4 within a couple of minutes. GLP-1 and other GLP1-related peptides bind the GLP-1 receptor (GPL-1R), a G-protein coupled receptor that uses cAMP as second messenger. GLP-1 related peptides modulate insulin secretion in a glucose-dependent manner and aid in glycemic control, suppress glucagon secretion & endogenous glucose production, delay gastric emptying, and promote early satiety & weight loss (e.g. Kim and Egan, 2008; Combettes)[5,6]. Studies in animal models of diabetes have suggested that GLP-1 increases pancreatic β-cell mass by stimulating cell proliferation and protects the pancreas against glucose-induced apoptosis (Bulotta, et al, 2004; Urusova, 2004)[7,8].

[5] **Kim W**, Egan JM (2008). The role of incretins in glucose homeostasis and diabetes treatment, Pharmacol Rev 2008; **60**: 470-512

[6]Combettes MM (2008). GLP-1 and type 2 diabetes: physiology and new clinical advances, Curr Opin Pharmacol **6**: 598-605.

[7] Bulotta A, Farilla L, Hui H, Perfetti R. The role of GLP-1 in the regulation of islet cell mass (2004). Cell Biochem Biophys. 40(3 Suppl):65-78.

[8] Urusova IA, Farilla L, Hui H, D'Amico E, Perfetti R (2004).GLP-1 inhibition of pancreatic islet cell apoptosis. Trends Endocrinol Metab. 15(1):27-33.

Lixisenatide (AVE0010) is a DPP-4 resistant GLP-1R agonist that is structurally related to Exendin-4, a 39 amino acid peptide. Lixisenatide is a synthetic 44 amino acid peptide; the first 37 amino acids are identical to exendin-4. Proline at position 38 in exendin-4 was removed and the final 6 amino acids are all lysines. Marketing application (Sanofi-Aventis NDA 204,961) was filed for lixisenatide in the year 2012, but withdrawn with plans to resubmit when the final report CV study (ELIXA) is available. The NDA (NDA 208,471) is lixisenatide been submitted in July 2015 for T2DM indication and is currently under review for subcutaneous daily administration of 10 µg for 14-days as initiation dose and 20 µg as maintenance dose.

Four other GLP-1 receptor agonists have been licensed in the United States (albiglutide & dulaglutide for weekly, exenatide for twice daily & weekly, and liraglutide for daily) dosing. Publically available information indicates considerable heterogeneity in terms of structure, pharmacokinetics, dose, efficacy & tolerability within these GLP-1 agonists.

Nonclinical studies submitted in NDA 204,961 have been reviewed by Dr. Tim Hummer. Thus, the reader is referred to that review for details. The summary below is an extract from Dr. Hummer's review:

Like exendin-4, lixisenatide is resistant to degradation by dipeptidyl peptidase-4 (DPP4) thereby extending its half-life compared with endogenous GLP-1. In animal models for diabetes, lixisenatide was shown to improve oral glucose tolerance, basal blood glucose and HbA1c with a rapid onset and sustained duration of action. Enhancement of glucose-stimulated insulin secretion occurred in a glucose-dependent manner.

Insulin glargine and Lixisenatide

In vitro

[Study# divt0068-abbr]: Binding of insulin glargine (SAR274136) to the GLP-1 receptor

Study # divt0063 - Expression of GLP1R, IGFR and INSR mRNA in cell lines 1.1B4, 6-23 and TT

The binding of insulin glargine to the GLP-1 receptor was evaluated *in vitro* in an insulin-secreting cell line derived from pancreatic tumor (βTC6 cells). Insulin glargine at 100 nM did not inhibit the specific radioligand binding of 25 pM [$I^{125}I$]GLP-1(7-36) to the GLP-1 receptor.

The expression of GLP1-R, IGF-receptor (IGFR) and the insulin receptor (INSR) was measured in human cell lines that were used to evaluate cross-receptor activation and other functional tests with PCR. The mRNA expression of GLP-1R, IGF1R and INSR

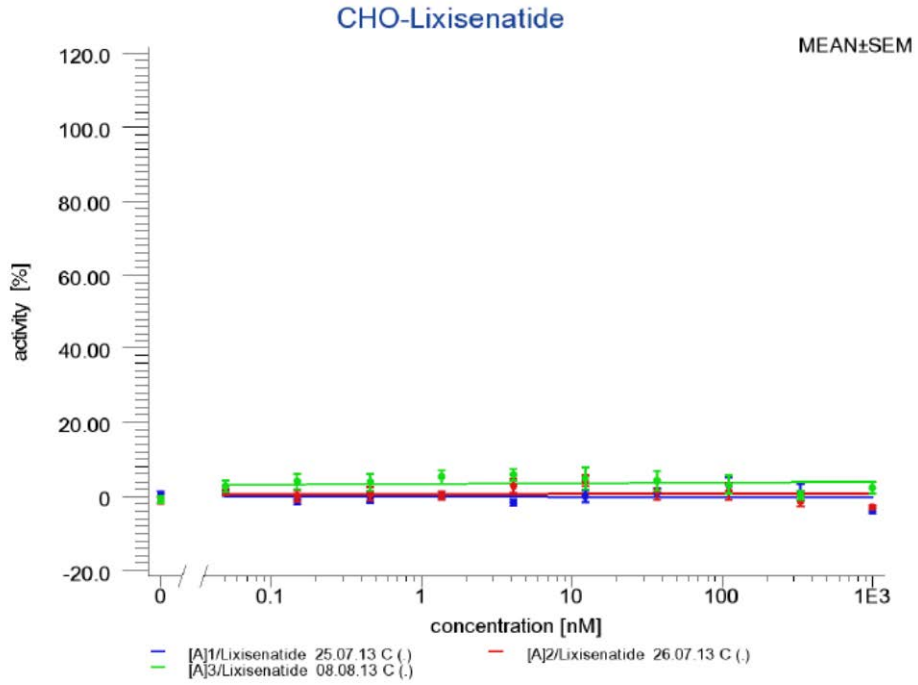
could be detected in the cell lines 1.1B4, rat thyroid c-cell line 6-23, and TT except for the mRNA expression of IGF1R that was not detectable in 6-23 cells.

[Study # divt0049]: Influence of lixisenatide on insulin glargine stimulated insulin receptor autophosphorylation using CHO-IR cells

[Study #: divt0057]: Influence of lixisenatide on binding affinity of insulin glargine to the insulin receptor B

The potential cross-activation of the INSR by lixisenatide alone and in combination with insulin glargine was tested at 100 nM in CHO-IR cells that overexpress the human INSR. EC_{50} values were compared from lixisenatide, lixisenatide + insulin glargine and insulin glargine alone. Lixisenatide did not activate the INSR, nor did it modulate the actions of insulin glargine on the receptor as shown in the sponsor **Figure 2** and **Table 5** below:

Figure 2: Insulin receptor autophosphorylation by lixisenatide



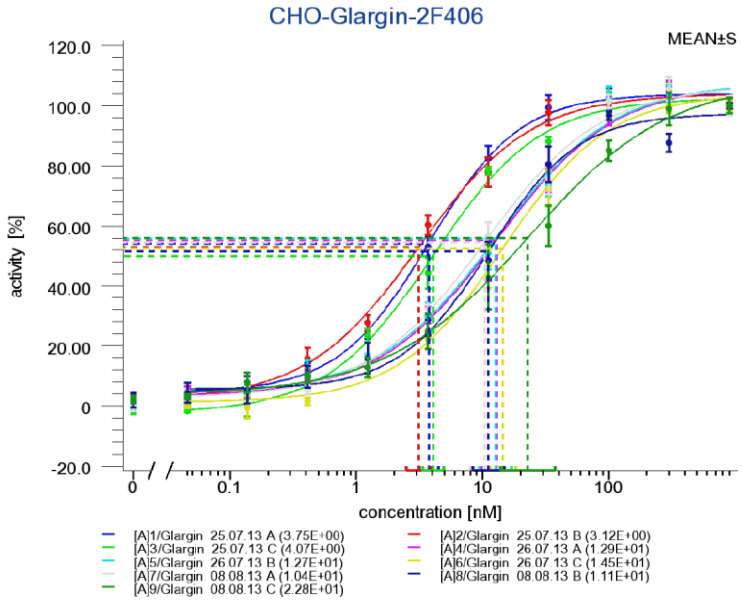


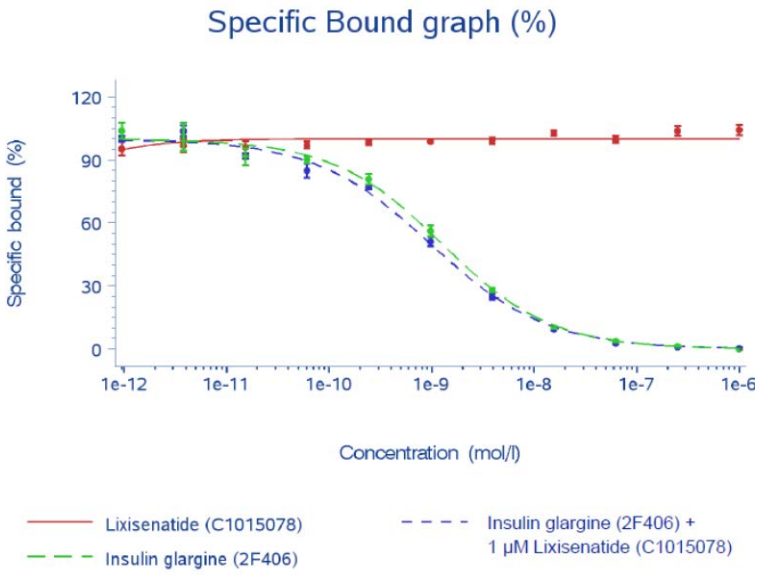
Table 5: In vitro activity of insulin glargine in the presence of lixisenatide on human insulin receptor-B expressing cells

Compound	Reference	Ratio of EC_{50} values (lixisenatide + insulin glargine vs. insulin glargine)	
		Ratio	90% confidence interval
Lixisenatide + insulin glargine	linsulin glargine	1.034	[0.921; 1.162]

Mixed model on log-transformed EC_{50} with plate considered as random effect.

Another study was conducted to test the affinity of lixisenatide to the human INSR-B, and whether it affects the affinity of insulin glargine to the receptor in CHO F1P-IN cell line stably transfected with human INSR-B. Lixisenatide (C1015078) was analyzed for its potency to inhibit the binding of [^{125}I]-labeled insulin to human INSR-B *in vitro* or to influence the binding affinity of insulin glargine (2F406) to INSR-B in the presence of 1 µM lixisenatide. Insulin glargine was used as reference. Lixisenatide did not show binding to the receptor, as shown in the sponsor provided **Figure 3** and the ratio of mean IC_{50} values between insulin glargine alone and in the presence of 1 µM lixisenatide was close to 1 with a 90% confidence interval of [0.87- 1.277].

Figure 3: Binding curves of lixisenatide, insulin glargine and insulin glargine + lixisenatide to the human insulin receptor (1 out of 4 experiments)



[Study # divt0044]: Influence of lixisenatide on insulin glargine stimulated IGF 1 receptor autophosphorylation using MEF-IGFR cells

Mouse embryonic fibroblast (MEF) cells over expressing the human IGF-1 receptor (MEF-IGF1R) were used in the study. Potential autophosphorylation of the receptor was assessed in the presence and absence of 100 nM lixisenatide. The results of this study are summarized in the sponsor provided in **Figure 4** and **Table 6**. Lixisenatide did not activate the IGF1 receptor.

Figure 4: Potential autophosphorylation of IGF1 receptor by lixisenatide

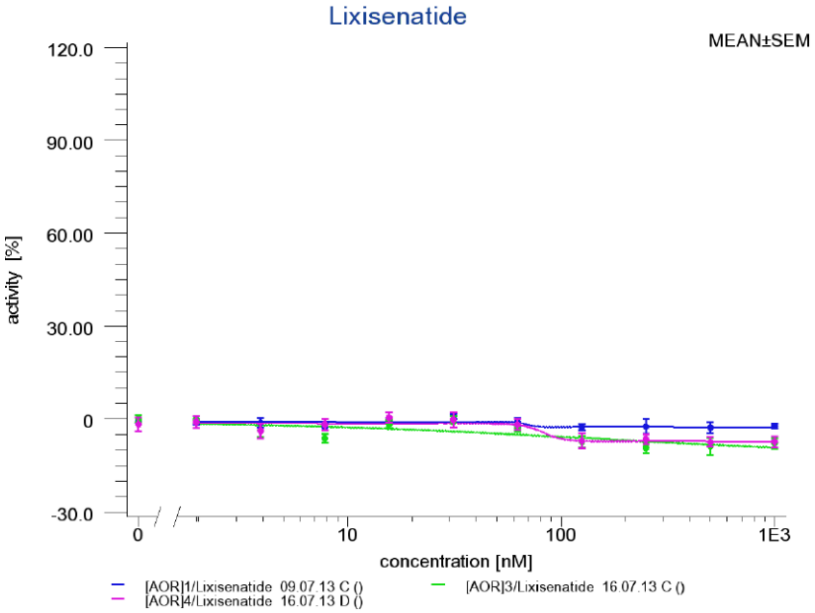


Table 6: Potential autophosphorylation of human recombinant IGF-1R by lixisenatide

Compound	EC50 geometric mean [nM]	95% confidence interval [nM]
Human IGF-1	9.150	[7.423 ; 11.28] (n=11)
Insulin Glargine (batch 2F406)	166.3	[109.7; 252.1] (n=7)
Insulin Glargine (batch 2F406) + 100nM LIXISENATIDE (batch C1015078)	138.7	[91.5 ; 210.2] (n=7)
LIXISENATIDE (batch C1015078)	n.a.	

Mixed model for glargine and insulin glargine + lixisenatide on log-transformed EC50 with plate considered as random effect.

[Study divt0067]: In vitro potency of insulin glargine, lixisenatide, as mixtures thereof in the human pancreatic beta cell line 1.1 B4

[Study divt0070]: Characterization of insulin glargine and lixisenatide stimulated AKT- phosphorylation in 1.1B4 cells

[Study # divt0056]: Characterization of insulin glargine and lixisenatide stimulated AKT- phosphorylation in RTC6-23 cells

Study divt0067 examined the potential agonistic activity of the individual test-articles and the combination at the GLP-1R by measuring the cAMP formation in human

pancreatic cell line 1.1 B4[9]. The result s of the studies indicated that insulin glargine was without any effects as shown in the sponsor provided **Table 7** below.

Table 7: Agonist-activity at the GLP-1R with insulin glargine and lixisenatide combination

Compound / combination	EC50 - individual experiments [pM]				EC50 - geometric means [pM]	95% confidence interval [pM]
	1	2	3	4		
Insulin glargine	-	-	-	-	-	- (n=4)
AVE0010	6.88	5.27	7.58	9.77	7.20	[5.34; 9.70] (n=4)
AVE0010 + 1 µM fc insulin glargine	6.69	5.75	8.69	6.26	6.76	[5.02; 9.11] (n=4)
AVE0010 + 1 nM fc insulin glargine	6.08	6.30	7.31	11.0	7.45	[5.53; 10.0] (n=4)
AVE0010 + 1 pM fc insulin glargine	6.89	4.94	9.51	8.02	7.14	[5.30; 9.62] (n=4)
AVE0010 + 1 fM fc insulin glargine	6.30	5.46	12.1	11.0	8.23	[6.10; 11.1] (n=4)
AVE0010 + 1 aM fc insulin glargine	6.41	6.24	6.18	10.4	7.12	[5.28; 9.59] (n=4)

Mixed model on log-transformed EC50 with experiment considered as random effect; concentrations in pM

The insulin receptor tyrosine kinase (IR) activation of the insulin receptor substrate (IRS) starts a signaling cascade that activates Akt (PKC-B) through phosphorylation at Ser^{473} and Thr^{308}. The activated AKT facilitates glucose uptake in adipocytes & muscle cells by enabling translocation of GLUT4 to the plasma membrane, and inhibits PKA activation. AKT phosphorylation stimulated by insulin, insulin glargine and insulin glargine-M1 in the absence or presence of 10 nM lixisenatide was assessed in 1.1B4 cells. Lixisenatide alone was not able to phosphorylate Akt as shown in the sponsor provided **Figure 5** below. Moreover, the same concentration of lixisenatide did not affect the EC_{50} values for Akt phosphorylation as shown in the sponsor **Table 8.**

[9] 1.1B4 cells have been shown to express insulin, glucokinase and islet amyloid polypeptide and GLUT (McClusky et al (2011), J Biol Chem., 286:21982-21992.

Figure 5: Lixisenatide and AKT phosphorylation in 1.1B4 cells

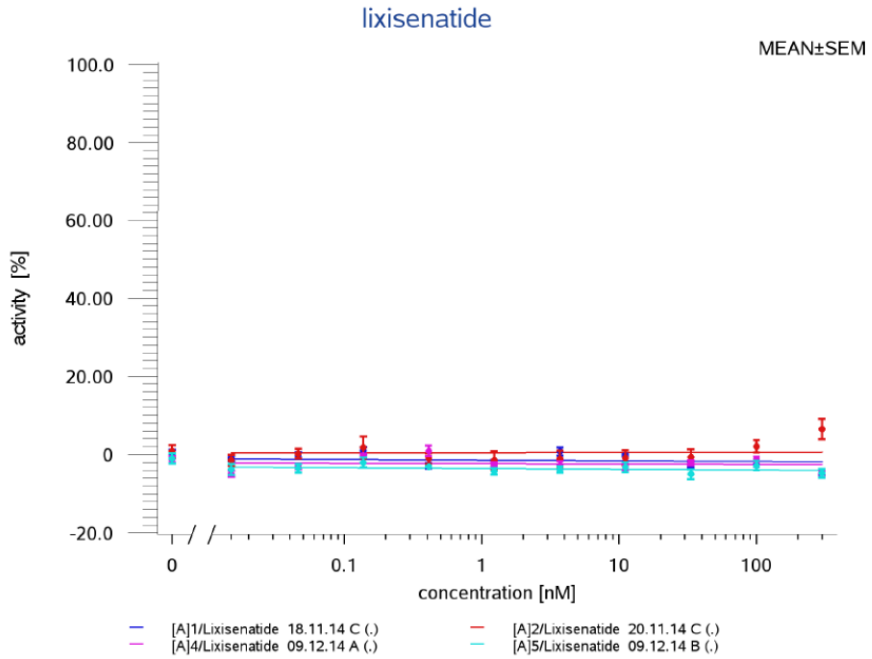


Table 8: Ratio of EC_{50} values for the activity on AKT phosphorylation between insulin, in the presence and absence of lixisenatide.

compound	Reference	Ratio of EC_{50} values (compound vs. reference)	
		Estimate	90% confidence interval
Glargine and Lixisenatide	Glargine	1.110	[0.934; 1.319]
Glargine-M1 and Lixisenatide	Glargine-M1	1.049	[0.887; 1.241]
Insulin and Lixisenatide	Insulin	1.144	[0.942; 1.389]

Mixed model for compounds on log-transformed EC_{50} with plate considered as random effect.

Another cell line, the rat thyroid (RTC6-23) was used for testing Akt phosphorylation with 100 pM lixisenatide alone or human insulin, insulin glargine and insulin glargine-M1. Lixisenatide did not induce Akt activation alone and failed to modulate phosphorylation by insulin and insulin glargine as summarized in the sponsor **Table 9.**

Table 9: Mean EC_{50} values [nM] with 95% confidence intervals for the activity on AKT phosphorylation of lixisenatide and of mono treatments

Compound	EC_{50} geometric mean [nM]	95% confidence interval [nM]
LIXISENATIDE (batch C1015078)	-	- (n=3)
Insulin (batch PDB000151-164)	0.709	[0.504; 0.995] (n=11)
Insulin + 100 pM LIXISENATIDE	0.704	[0.495; 1.002] (n=8)
Insulin glargine (batch 2F406)	1.035	[0.874; 1.224] (n=8)
Insulin glargine + 100 pM LIXISENATIDE	1.184	[1.001; 1.401] (n=8)
Insulin Glargine M1 (batch PDB00079-120)	1.120	[0.946; 1.326] (n=7)
Insulin glargine M1 + 100 pM LIXISENATIDE	1.291	[1.090; 1.528] (n=7)

In vivo

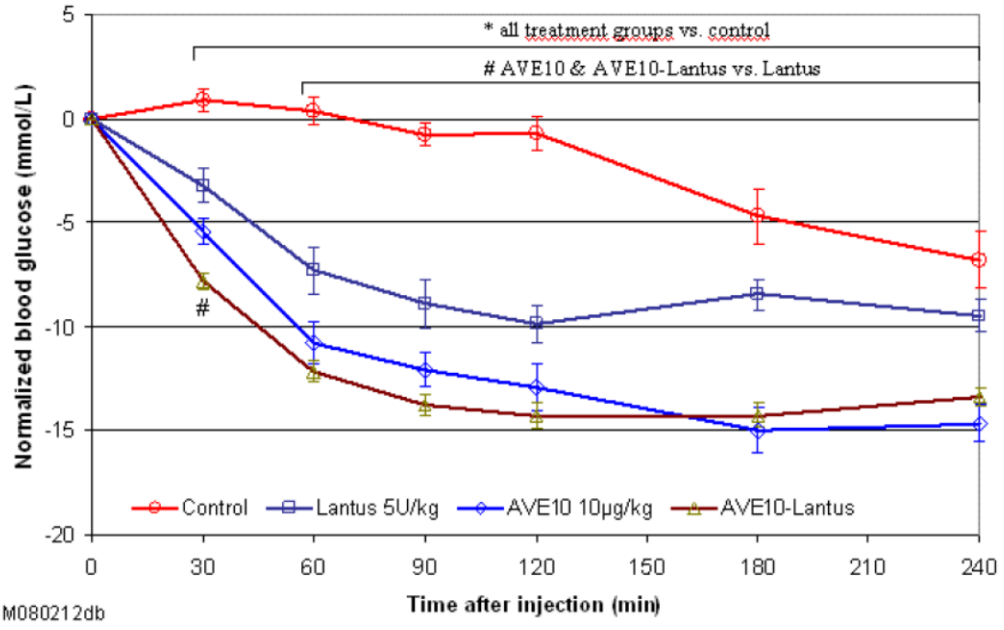
[Study # MVV0025]: Acute glycemic effects on db/db mice after subcutaneous injection.

[Study # MVV 0026]: Acute glycemic effects on oral GTT in male dogs.

Acute effects of the combination of insulin glargine and AVE0010 was compared in female db/db mice and dogs. Diabetic mice (db/db mice (BKS.Cg-m +/+ $Lepr^{db}$/J, (b) (4); n=10/group) received a single subcutaneous dose of AVE0010 (10 µg/kg), insulin glargine (5 U/kg), or a preformulated combination of both agents. The animals were fasted for 2 hrs before injection. Blood glucose was determined before the first injection and thereafter for up to 4 hrs. In a second arm of the experiment, an acute OGTT was conducted in mice fasted overnight by administering 2g/kg glucose by oral gavage 30 minutes after test article administration. Blood glucose was determined before the first injection, before oral glucose challenge, and thereafter for up to 4 hrs.

The change in basal blood glucose relative to baseline was significantly decreased versus placebo by all three treatments between 30 and 240 minutes after dosing. Compared with insulin glargine treatment alone, basal blood glucose was significantly lowered by AVE0010 between 60 and 240 minutes. The glucose concentration with combination treatment was statistically lower relative to insulin glargine alone, but not for AVE0010 from 30 to 240 minutes after injection (see sponsor **Figure 6**).

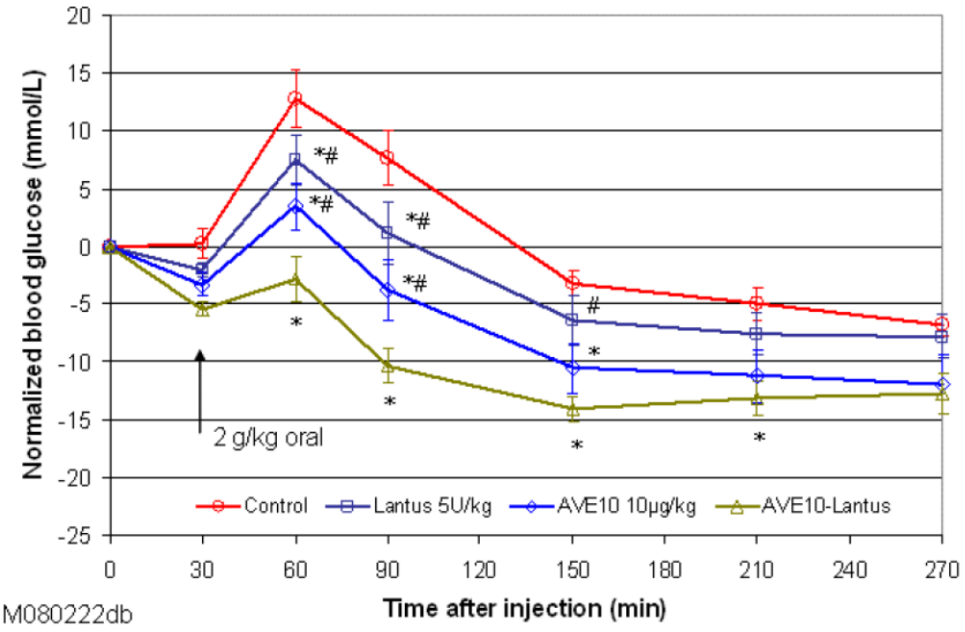
Figure 6: Effect of AVE0010/insulin glargine on basal blood glucose in db/db mice



Normalized blood glucose concentration following treatment with AVE0010, insulin glargine (Lantus), AVE0010/insulin glargine combination or placebo; mean +/- SEM, female db/db mouse, n = 10
* $p<0.05$ vs. placebo, # $p<0.05$ vs. Insulin glargine; two-way ANOVA on normalized glucose concentrations followed by Newman-Keuls posthoc test for pairwise comparisons

Both AVE0010 and insulin glargine alone had a significant effect on blood glucose excursion versus placebo at 60 and 90 minutes after treatment. The AVE0010 effect lasted up to 150 minutes after treatment, and the AVE0010/insulin glargine combination resulted in a statistically significant reduction of blood glucose excursion versus placebo from the 60 minute time point to the 210 minute time point. Additionally, the effect of the combination was significantly different from AVE0010 alone at 60 and 90 minutes and to insulin glargine at 60, 90, and 150 minutes (see sponsor **Figure 7**).

Figure 7: Effect of subcutaneous AVE0010/insulin glargine on oral glucose tolerance in db/db mice



Normalized blood glucose concentration following treatment with AVE0010, insulin glargine (Lantus), AVE0010/insulin glargine combination or placebo; mean +/- SEM, female db/db mouse, n = 10
* p<0.05 vs. placebo, # p<0.05 vs. AVE-Lantus; two-way ANOVA on normalized glucose concentrations followed by Newman-Keuls posthoc test for pairwise comparisons

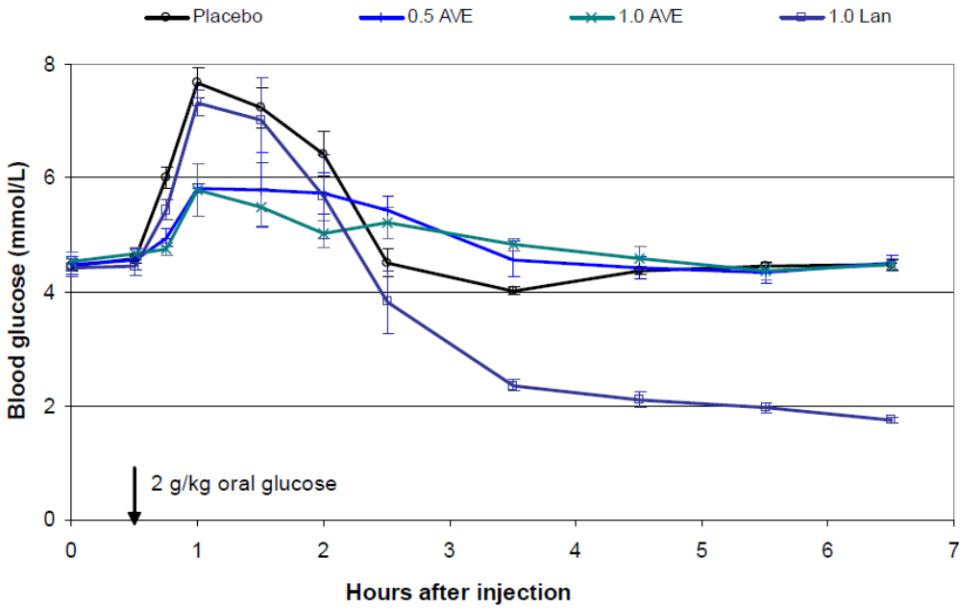
An acute OGTT was also performed in normoglycemic Beagle dogs ((b) (4), weight: 13-17 kg) fasted overnight. Animals received a single subcutaneous injection of AVE0010 (0.5 µg/kg or 1.0 µg/kg, batch # FRA-00264), or insulin glargine (1.0 U/kg – batch E249 and B036) or premixed AVE0010/insulin glargine (0.15 µg/kg/0.3 U/kg, 0.5 µg/kg/0.3 U/kg, 1.0 µg/kg/0.3 U/kg, 1.5 µg/kg/0.3 U/kg, or 1.0 µg/kg/1.0 U/kg). The compounds were dissolved in 6-60 µL/kg of insulin glargine formulation at pH 4.0-4.5.

Treatment was initiated 30 minutes before 2 g/kg glucose challenge by oral gavage. Glucose, insulin and C-peptide were monitored in blood and serum obtained from the jugular vein. The sponsor has submitted that the insulin and C-peptide values may not be quantitative because of the cross reactivity with the endogenous molecules.

Results of the OGTT studies with monotherapy are summarized in the sponsor provided **Figure 8, Figure 9**. Blood glucose reached maximum after 0.5 hrs and returned to baseline after 2 hrs of the bolus. Basal glucose was not affected by either agent alone before glucose surge. This return to baseline was marginally delayed for the AVE0010-treated dogs in both the absorptive and post-absorptive phases. Similar effect was only seen at post-absorptive phase for insulin glargine. However, when AVE0010 and insulin glargine were injected in combination there was a decrease in blood glucose levels during both absorptive and post-absorptive phase of the OGTT that was statistically

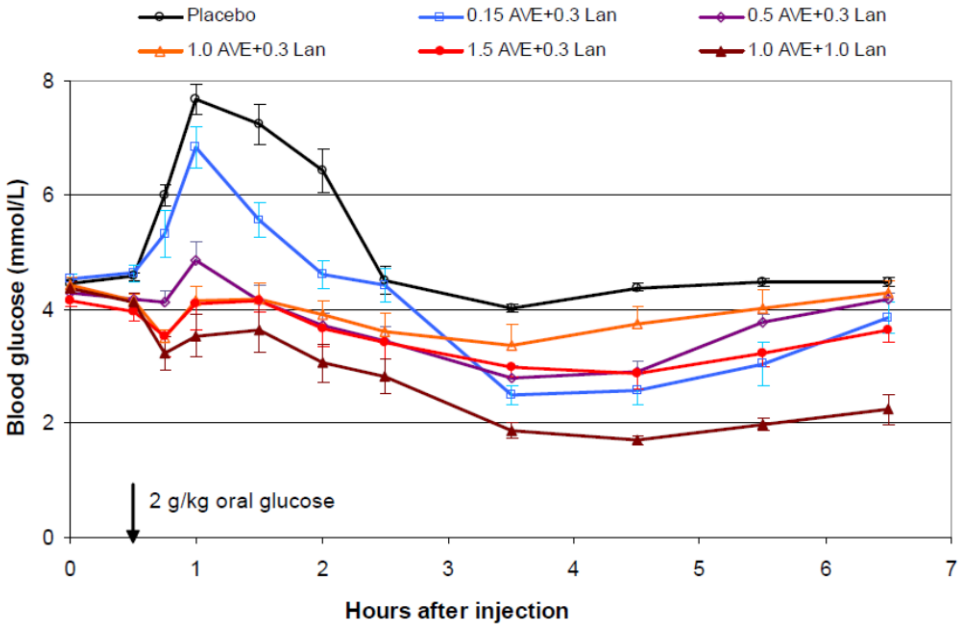
significant up to 6.5 hrs when compared to the effects obtained by same doses of AVE0010 and insulin glargine.

Figure 8: Effect of AVE0010 and insulin glargine on blood glucose in OGTT in dogs



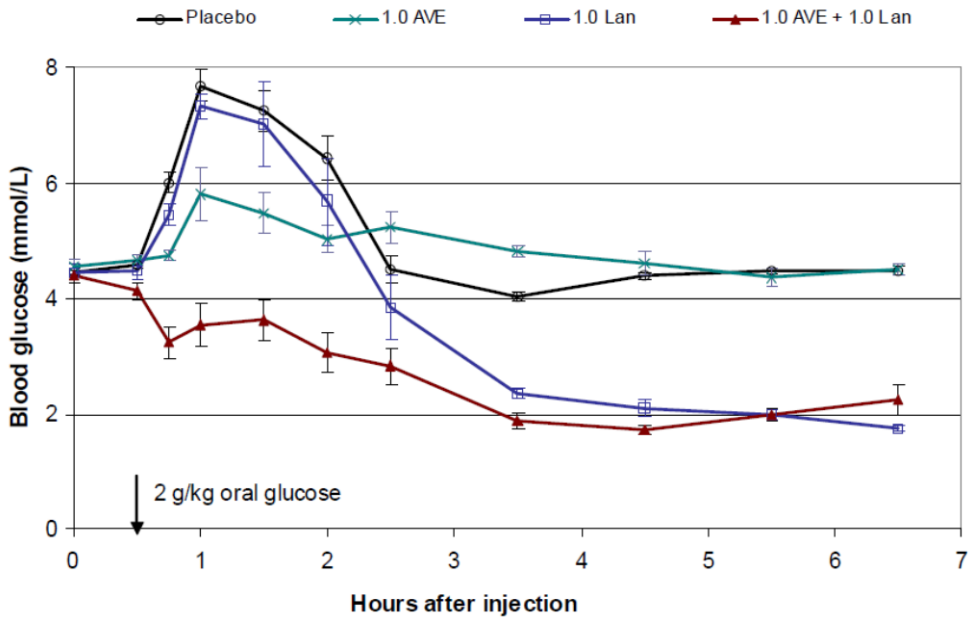
In the study that used various doses of the preformulated combination dose, blood glucose was statistically significantly decreased compared to vehicle treated animal starting 15 minutes after glucose challenge to 4.5 hrs to the end of the observation period depending on the dose (see sponsor provided **Figure 9**).

Figure 9: Dose-dependence of AVE0010 and insulin glargine combinations on blood glucose in OGTT in dogs



At the top evaluated doses as monotherapy or when applied together as combination (see sponsor provided **Figure 10** below), glucose levels were significantly lower for the combination relative to the monotherapy from 15 minutes after glucose challenge to 4.5 hrs after test article administration.

Figure 10: Comparative effect of high dose monotherapy and combination on blood glucose in OGTT in dogs



Endogenous canine insulin and C-peptide levels were measured semi-quantitatively in serum samples in the study groups. As can be noted in the sponsor provided figures (**Figure 11** and **Figure 12**), insulin values during the absorptive phase (0.5 to 2.5 hrs after glucose challenge) were similar in placebo and animals treated with AVE0010 alone, insulin alone and the low-dose combination. Marginal suppression was noted in the groups receiving AVE0010 alone, perhaps due to its effect on GI motility. In animals that received AVE0010 and insulin glargine combination, insulin showed a declining trend from about 1 hr to 5.5 hrs post challenge without clear dose dependency. The higher doses of AVE0010 combined with the low dose of insulin glargine (AVE/Lan 1.0+0.3 and AVE/Lan 1.5+0.3) showed the lowest serum insulin levels during OGTT. This effect was not reported in the AVE/Lan 1.0+1.0 group, which was also effective in lowering blood glucose.

Serum C-peptide rose immediately after glucose challenge in the placebo group and returned to baseline after 3.5 hrs. Insulin glargine monotherapy had a slight influence on the C-peptide peak, but due to rising insulin levels from exogenous insulin glargine, serum C-peptide was already back to baseline at 2 hrs. In contrast, with AVE0010 alone, the return of C-peptide to baseline was slowed compared with placebo. In the absorptive phase of the OGTT, the lowest dose of AVE0010 & insulin glargine combination showed the lowest suppression of C-peptide, while the highest dose of

both compounds in combination (1.0 + 1.0) showed the highest suppression of C-peptide. All AVE0010 & insulin glargine combination groups had lower C-peptide compared with placebo. The sponsor suggested that the suppression of endogenous insulin release in groups that received insulin glargine, irrespective of whether alone or in combination with AVE0010 was most likely due to the presence of exogenous insulin glargine.

Figure 11: Serum insulin in OGTT dogs after administration of monotherapy and combination

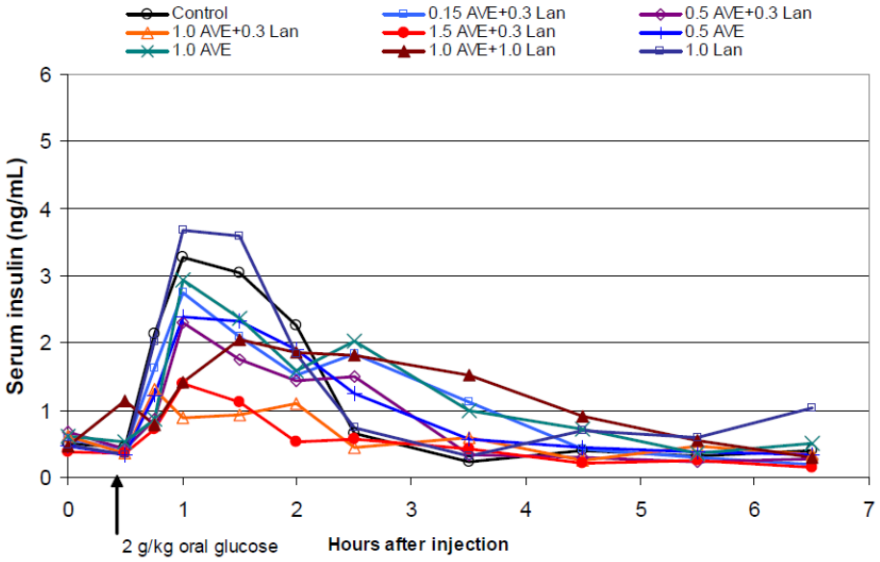
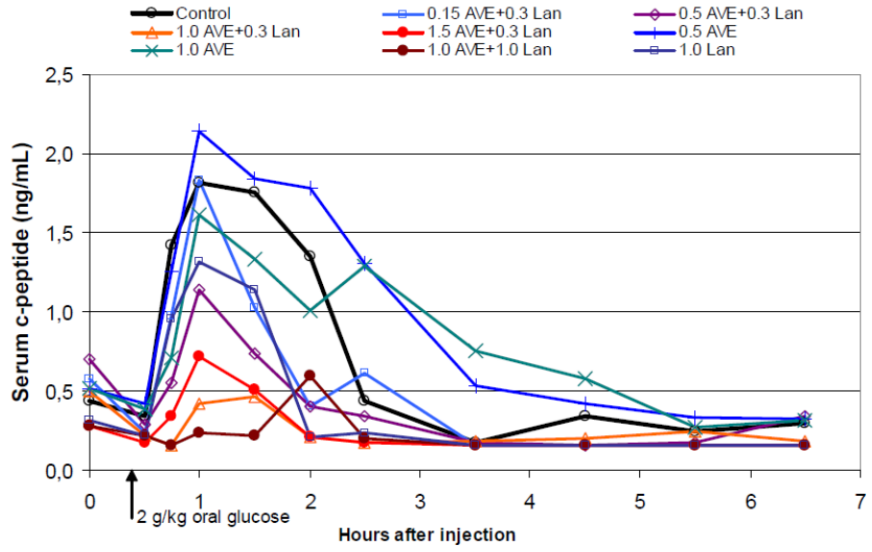


Figure 12: Serum C-peptide in OGTT dogs after administration of monotherapy and combination



[Study divv0050]: Acute effects of a subcutaneous injection of low dose lixisenatide mixed into two different concentrations of insulin glargine, U100 and U300, on oral glucose tolerance in male dogs

This OGGT in normoglycemic male Beagle dogs (n=6-8/group) compared a low dose lixisenatide (0.15 µg/kg) combined with 0.3 U/kg insulin glargine injected at two different strengths (100 U/mL and 300 U/mL). Equal doses of individual drugs were injected for comparison. The dogs were treated 0.5 hrs prior to an oral challenge of 2 g/kg glucose.

Test: Oral glucose tolerance test (OGTT)	**Test Article (batch):** Placebo (HOE901_09_142); Lixisenatide/insulin glargine U100 (C1016632); Lixisenatide/insulin glargine U300 (C1018669); Lixisenatide (1F001); Insulin glargine (A0164)	**Doses:** 0.15 µg/kg lixisenatide, 0.3 U/kg insulin glargine
Species/Strain: Dog/Beagle (HsdRcc:DOBE)		**Administration:** SC injection
Gender/No. per Group: Male/n=6-8	**Vehicle**: Placebo (HOE901_09_142)	**Treatment Schedule**: Single dose administration of test article at 0h, oral glucose at 0.5h, blood collection 0-8h
Weight/Age: 8-14 kg/22-37 m		**Study Period:** 11-Apr-2012-15-May-2012

Glucose surged within 0.5 hr in the vehicle animals, peaking at about 1 hr post challenge and returned to baseline within 3 hrs. As shown in the sponsor provided **Figure 13** and **Figure 14**, both the monotherapy and the combination treatments reduced the glucose surge and maintained it for the duration of measurement. The effects of the combination on blood glucose were similar to that of lixisenatide alone during the absorptive phase and to that of insulin glargine in the post-absorptive phase.

Figure 13: Effect of 0.15 µg/kg lixisenatide and 0.3 U/kg insulin glargine vs. placebo on blood glucose in OGTT dogs *(reproduced from the report by sponsor)*

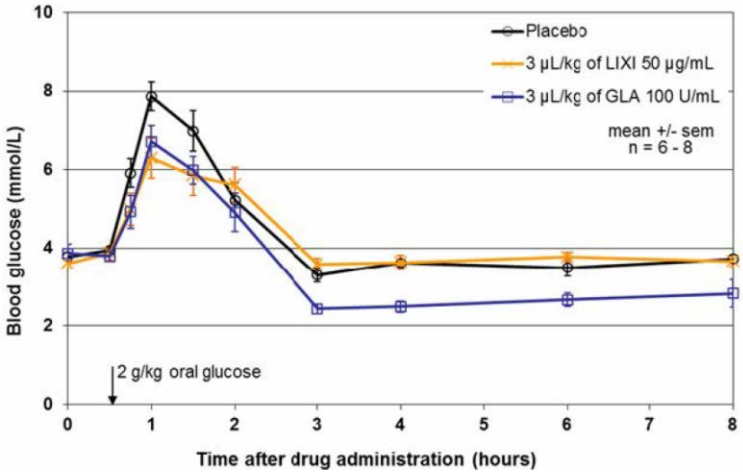
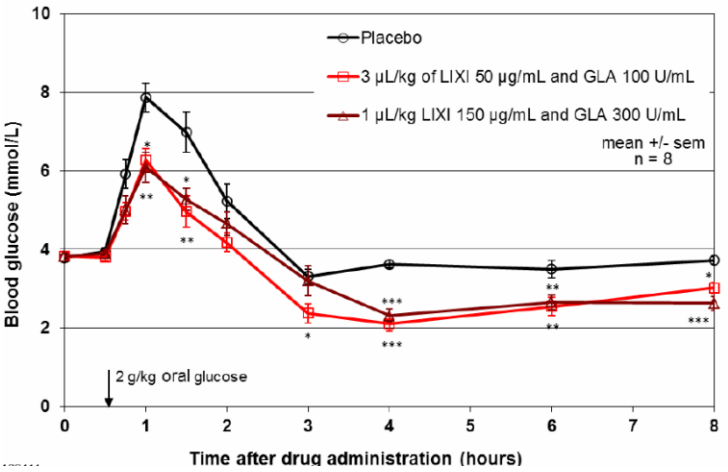


Figure 14: Effect of two premixed combinations with different concentrations of lixisenatide and insulin glargine vs. placebo on blood glucose in OGTT in dogs *(sponsor provided)*



Note that reports of pharmacokinetic studies in dogs at the doses evaluated in this study are available (see page **43).**

Summary of the acute OGTT studies

The lixisenatide and insulin glargine combination improved glucose tolerance in db/db mice better than monotherapy. When db/db mice receiving the vehicle were challenged with glucose, an abrupt surge in glucose peaking after 1 hr and declining by 150 hrs was noted. Significantly lower glucose surge was noted for both the monotherapy favoring AVE0010 and the combination, but greater lowering was reported for the combination. Thus, it can be concluded that the premixed combination had better pharmacodynamic profile after single dose. The effect in the absorptive phase (0.5 – 1 hr) appears to be mainly due to lixisenatide, perhaps through its effect on GI motility, or may have been masked by higher insulin levels in the immediate post-absorptive phase (see **Figure 11).** An effect that appears to be due to exogenous insulin was noted in the post-absorptive phase. Thus, the enhanced PD effects in the post-absorptive phase in the combination may be a reflection of the additive effect of exogenous and endogenous insulin.

Similar oral glucose tolerance studies were conducted in normoglycemic dogs after single dose administration of the premixed formulation and individual compounds. Dogs can model the clinical PD effects better because the depot effect for insulin glargine in dogs is similar to humans; very small for rodents. In addition to improvement in glucose tolerance, serum insulin and C-peptide levels were measured in blood samples obtained from dogs at different intervals.

Dose-dependent effects were seen during OGTT in dogs, where the return to baseline was marginally delayed for the AVE0010-treated dogs in both the absorptive and post-absorptive phases. The surge in blood glucose was suppressed in a dose-related manner when the dose of AVE0010 was escalated from 0.15-1.5 µg/kg keeping the insulin glargine at 0.3 U/kg, lasting up to 5 hrs after glucose challenge. Further improvement in glucose tolerance was noted when the insulin glargine dose in the combination was increased to 1.0 U/mL. Serum insulin was not significantly different in animals receiving AVE0010 alone compared to controls during the absorption phase, but was lower in the animals receiving the combination. Post absorptive insulin concentration was lowest in animals receiving AVE0010 & insulin glargine doses of 1.0 µg/kg/0.3 U/kg and 1.5 µg /0.3 U/kg, but was higher than the control level at 1.0 µg/kg/1.0 U/kg. Serum C-peptide mirrored the insulin values in the groups that received insulin glargine alone or in combination. However, C-peptide was higher than controls in the post-absorptive phase in the groups that received AVE0010 alone, except at 0.15 µg/kg of AVE0010.

Another OGGT study in normoglycemic male Beagle dogs compared the individual compounds to equal combined doses of lixisenatide (0.15 µg/kg) and insulin glargine (0.3 U/kg) injected at two different strengths (100 U/mL and 300 U/mL). The applicant also determined the plasma level of AVE0010 in the same study. The half-life of AVE0010 was lowered when the premixed formulation was injected; other PK parameters were not significantly altered. The effect on the t½ of AVE0010 may not be due to interaction at the injection site since the Tmax and exposure in terms of C_{max} and AUC were not significantly altered.

Given the C-peptide data discussed above, and based on what is known about GLP-1 receptor agonists and GI motility, it appears both the suppression of glucose surge and maintenance of glucose level appear to be dependent on AVE0010 at the lower dose of insulin glargine. The post-absorptive effect appears to be a reflection of the additive effect of endogenous insulin due to AVE0010 and insulin glargine injection; this is reflected in enhanced complementary effects of on blood glucose (**Figure 10**), and increased insulin in the 1.0 µg/kg/1.0 U/kg group. Since insulin level was higher in this group, the suppression of C-peptide is a reflection of the relatively higher dose of insulin glargine in this group.

The sponsor has also concluded that "*compared to placebo treatment, the endogenous insulin levels were nearly unchanged by monotreatment with insulin glargine and tended to be suppressed and retarded by AVE0010 alone. However, when looking to serum C-peptide levels it is obvious that in all groups that received insulin glargine, whether alone or in combination with AVE0010, the C-peptide levels were markedly decreased or were below detection level at 3.5 hour and beyond. This implies that endogenous insulin was suppressed, most probably by exogenous insulin glargine. It can therefore not be ruled out that the higher insulin levels detected in the AVE/Lan 1.0+1.0 group could be due to any cross-reactivity of the human insulin assay with higher levels insulin glargine or its metabolites.*"

4.2 Secondary Pharmacology

Lixisenatide

Lixisenatide was profiled *in vitro* for binding against a broad panel of receptors and in two cellular assays. Only the N-type calcium channel showed > 50% inhibition by lixisenatide. In a follow up patch-clamp study in cultured primary rat dorsal root ganglion neurons, lixisenatide exerted weak and selective blockade of the N-type Ca^{2+}-channel. Investigations of the cardioprotective effects of lixisenatide using isolated perfused heart and in rats with heart failure by ischemia/reperfusion were conducted. The study suggested protection against myocardial ischemia-reperfusion injury at 0.3 nM in isolated heart and 10 µg/kg subcutaneous lixisenatide. Based on chronic subcutaneous infusion of lixisenatide in ApoE KO mice, the sponsor has submitted that lixisenatide's reducing of serum cholesterol has beneficial effect on atherosclerotic plaque progression in the aorta.

Secondary pharmacodynamics studies were not conducted for insulin glargine/lixisenatide combination. However, (b) (4) report [study number 9721373 (N° MET-023-10C)] was submitted in this NDA examining SAR213916 (lixisenatide) and SAR274136 (insulin glargine). The two compounds were profiled *in vitro* for receptor binding and functionality concurrently with reference compounds. The results were expressed as % of control specific binding (measured specific binding/control specific binding) X100 and as % inhibition of control specific binding (100-% of control specific binding). The results of these studies are summarized in the sponsor provided tables below.

Table 10: Receptor profiling with insulin glargine and lixisenatide *in vitro*

Assay (b) (4) Compound I.D.	Client Compound I.D.	Test Concentration (M)	% Inhibition of Control Specific Binding
CT (calcitonin) *(h)* (agonist radioligand)			
(b) (4)	SAR213916	1.0E-07	-1
glucagon *(h)* (agonist radioligand)			
(b) (4)	SAR213916	1.0E-07	9
GLP-1 (agonist radioligand)			
(b) (4)	SAR274136	1.0E-07	-3
secretin *(h)* (agonist radioligand)			
(b) (4)	SAR213916	1.0E-07	15
PAC_1 (PACAP) *(h)* (agonist radioligand)			
(b) (4)	SAR213916	1.0E-07	3
$VPAC_2$ (VIP_2) *(h)* (agonist radioligand)			
(b) (4)	SAR213916	1.0E-07	0

Assay (b) (4) Compound I.D.	Client Compound I.D.	Test Concentration (M)	% of Control Specific Binding 1st	2nd	Mean
CT (calcitonin) *(h)* (agonist radioligand)					
(b) (4)	SAR213916	1.0E-07	102.1	100.5	**101.3**
glucagon *(h)* (agonist radioligand)					
(b) (4)	SAR213916	1.0E-07	90.4	91.7	**91.1**
GLP-1 (agonist radioligand)					
(b) (4)	SAR274136	1.0E-07	102.6	102.8	**102.7**
secretin *(h)* (agonist radioligand)					
(b) (4)	SAR213916	1.0E-07	83.6	86.8	**85.2**
PAC_1 (PACAP) *(h)* (agonist radioligand)					
(b) (4)	SAR213916	1.0E-07	99.2	94.5	**96.9**
$VPAC_2$ (VIP_2) *(h)* (agonist radioligand)					
(b) (4)	SAR213916	1.0E-07	102.6	96.5	**99.5**

Table 11: Cellular functional assays with insulin glargine and lixisenatide *in vitro*

Assay (b) (4) Compound I.D.	Client Compound I.D.	Test Concentration (M)	% Inhibition of Control Agonist Response
GHRH *(h)* (antagonist effect)			
(b) (4)	SAR213916	1.0E-07	2
PTH1 *(h)* (antagonist effect)			
(b) (4)	SAR213916	1.0E-07	-11

Assay (b) (4) Compound I.D.	Client Compound I.D.	Test Concentration (M)	Agonist Response (% of Control) 1st	2nd	Mean
GHRH *(h)* (antagonist effect)					
(b) (4)	SAR213916	1.0E-07	109	86	**97.5**
PTH1 *(h)* (antagonist effect)					
(b) (4)	SAR213916	1.0E-07	113	109	**111.3**

4.3 Safety Pharmacology

Effects of lixisenatide on the nervous, cardiovascular and respiratory systems were evaluated. The mouse nervous system study was conducted using a formulation prepared in 50 mM sodium citrate (pH 5.3) and diluted in saline for subcutaneous injection. Lixisenatide doses of 20, 200 and 2,000 µg/kg were administered to CD-1 mice for evaluation of general behavior, and autonomic and motor functions in Irwin test. No significant effects were reported at any of the doses. In rats, reversible neurobehavioral and locomotor activity changes were noted at single intravenous doses ≥50 µg/kg.

The review for Lantus® by Dr. Rhee indicated that HOE901 at subcutaneous doses of 3, 10 & 30 U/kg caused sedation in mice starting 20-25 minutes of dosing. However, it did not potentiate ethanol- or phenobarbital-induced sleeping. In a cardiovascular study with anesthetized rat, the above doses did not alter heart rate and hemodynamic

parameters, except marginal decrease in diastolic blood pressure. In anesthetized dogs, intravenous injection of 0.3, 1.0 and 3 U/kg caused a slight and transient decline in hemodynamic parameters, while dp/dt max, cardiac output and heart rate were increased over the test period.

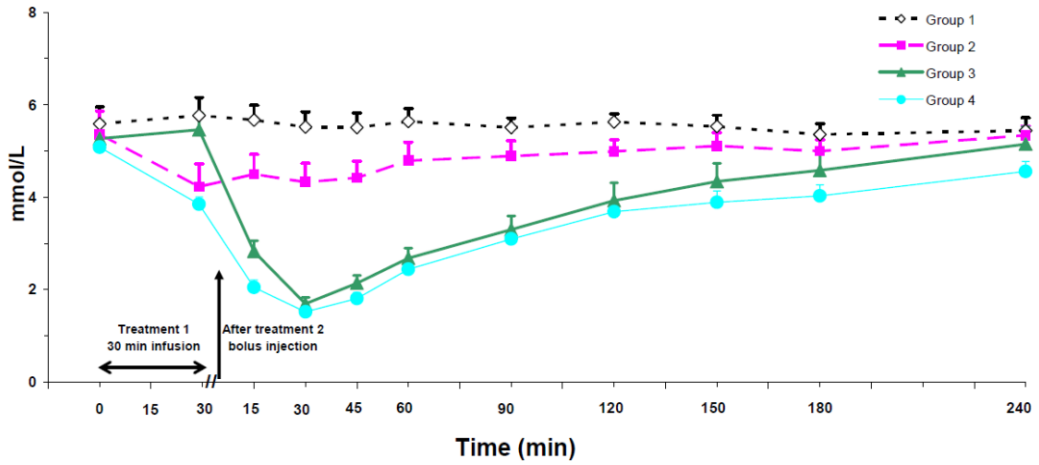
[Study cvr0345]: Insulin glargine/AVE0010 combination – Effect of a single intravenous dose on cardiovascular function in anesthetized dogs

Cardiovascular effects of single intravenous doses of insulin glargine & AVE0010 alone and in combination were tested in anesthetized normoglycemic dogs (study CVR0345). Lixisenatide was infused for 30 minutes and insulin glargine was give as a bolus dose in a cross-over design shown below.

Treatment group codification	No. of animals per group	First Treatment : AVE0010 or its vehicle	Second treatment : insulin glargine or its vehicle	Dose of AVE0010 (µg/kg)	Dose of insulin glargine (IU/kg)
G1	8	AVE0010 vehicle	Insulin glargine vehicle	0	0
G2	8	AVE0010	Insulin glargine vehicle	10	0
G3	8	AVE0010 vehicle	Insulin glargine	0	0.1
G4	8	AVE0010	Insulin glargine	10	0.1

Glucose concentration was measured in the dogs and the result is summarized in the sponsor provided figure below (**Figure 15**). In addition to ECG parameters, heart rate, serum potassium and temperature were measured.

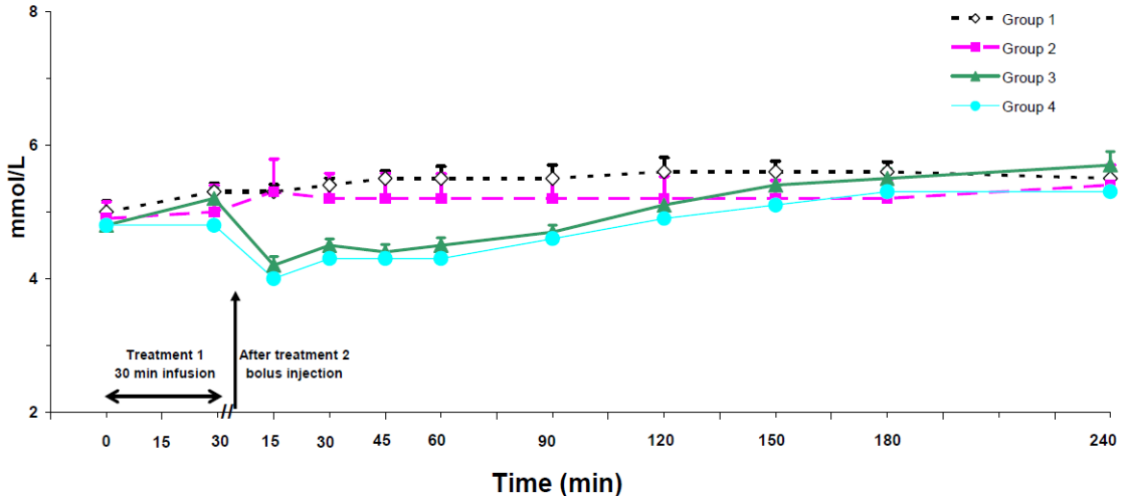
Figure 15: Glucose concentration in anesthetized dogs with insulin glargine and lixisenatide combination



As can be seen from the sponsor provided figure below (**Figure 16**), AVE0010 alone did not affect serum potassium. Decline in serum K^+ occurred immediately after insulin

glargine injection peaking at 15 minutes. This effect has been reported clinically with insulins and is proposed to be due to the shifting of K^+ to the intracellular compartment. Sponsor has reported changes in ECG morphology, and the effect on this ion may be responsible for the observations.

Figure 16: Effect of a single insulin glargine/AVE0010 combination on potassium serum concentration in anesthetized dogs



Heart rate was marginally and transiently increased by AVE0010; the increase was statistically significant when AVE0010 (G2+G4) was compared with vehicle group (G1+G3), reaching maximum of +5±1.6 bpm at mid infusion. Insulin glargine increased heart rate (+15±5.2 bpm) at 45 minutes of injection. The recovery from these changes was slow compared to the changes with AVE0010 that was transient. Insulin glargine increased heart rate when administered after AVE0010. The increase was maximal (+16±5.3 bpm, $p<0.0119$) at 45 minutes, and was significant at 45 and 60 minutes after the injection.

Figure 17: Effect of a single insulin glargine/AVE0010 combination on corrected QT interval duration (Frederica's formula) in anesthetized dogs.

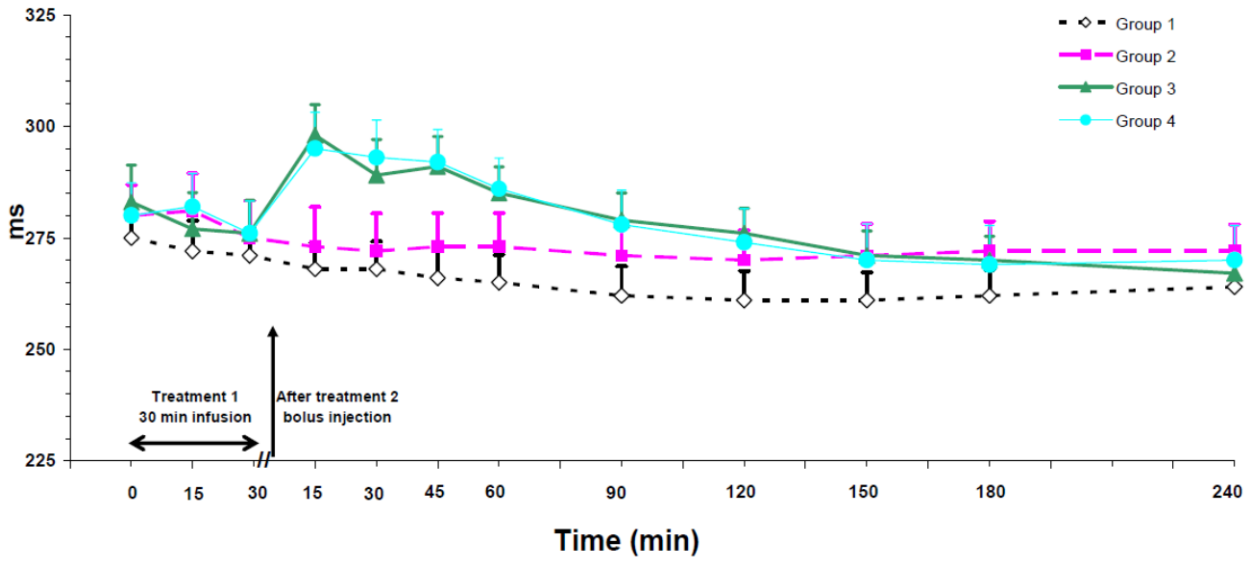
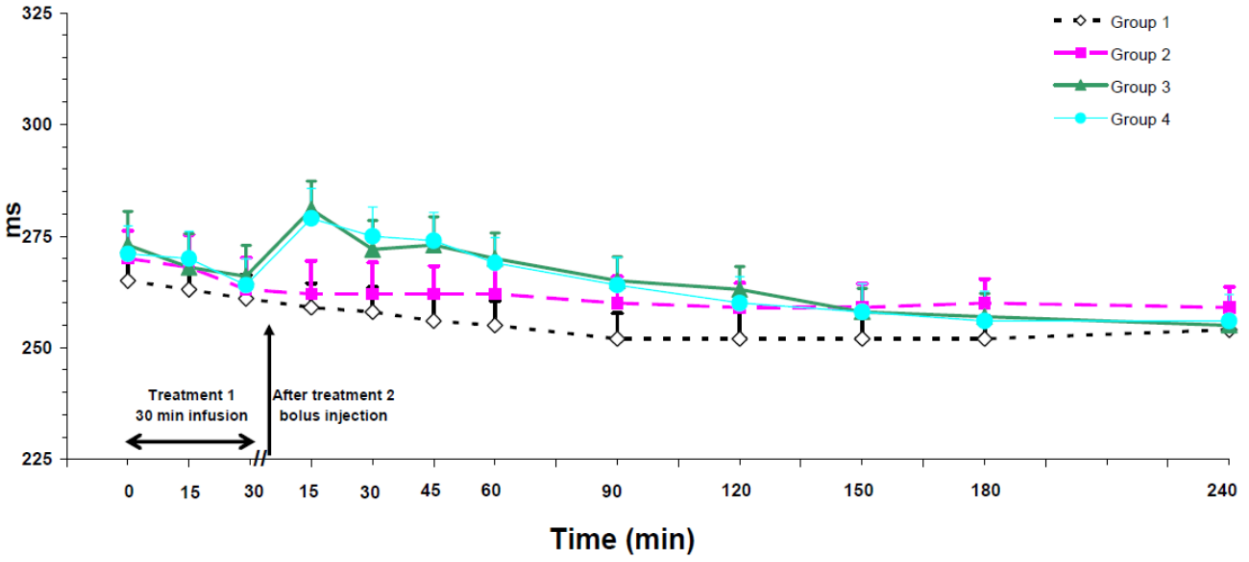


Figure 18: Effect of a single insulin glargine/AVE0010 combination on corrected QT interval duration (Van de Water's formula) in anesthetized dogs



The following is reproduced, verbatim from the submission:

AVE0010 induced a small global statistically significant increase (3±1 ms) only for QTcF[10] interval durations when the AVE0010-groups (G2 +G4) pooled data were compared to the pooled vehicle group data (G1 +G3). No global increase was revealed

[10] QTcF = Frederica's formula; QTcW = Van de Water's formula

for QTcW interval durations. Insulin glargine induced a clear significant increase in QTcF and QTcW interval duration when G3 was compared to vehicle group G1 15 minutes after injection (+23±3.5 ms, p<0.0001 and +17±3.2 ms, p<0.0001 for QTcF and QTcW, respectively). A slow recovery occurred thereafter as QTcF and QTcW interval duration increase remained significant until 90 and 60 minutes post dosing for QTcF and QTcW respectively. The effect on QTc with combination was due to the insulin bolus, where maximal and significant effect was noted after 15 minutes (+26±3.5 ms, p<0.0001 and +19±3.2 ms, p<0.0001 for QTcF and QTcW respectively). Insulin glargine had no effect on body temperature.

Table 12: Summary of cardiovascular effects of lixisenatide/insulin glargine in anesthetized dogs *(reproduced from the sponsor)*

Parameter	AVE0010	Insulin glargine	Co-administration: AVE0010/insulin glargine
Heart rate	+ 5 ± 1.6 bpm [a]	+15 ± 5.2 bpm [b]	+16 ± 5.3 bpm [b]
QT interval duration	No effect	+15 ± 4.5 ms [c]	+ 15 ± 4.5 ms [c]
QTcF interval duration	No effect	+ 23 ± 3.5 ms [c]	+26 ± 3.5 ms [c]
QTcW interval duration	No effect	+17 ± 3.2 ms [c]	+ 19 ± 3.2 ms [c]
Serum glucose concentration	-1.43 ± 0.22 mmol/L [d]	-3.69 ± 0.32 mmol/L [e]	-3.78 ± 0.32 mmol/L [e]
Serum potassium concentration	no AVE0010-related effect	-1.0 ± 0.25 mmol/L [c]	-1.1 ± 0.25 mmol/L [c]

a at mid infusion
b 45 min after bolus injection
c 15 min after bolus injection
d End of infusion
e 30 min after bolus injection

Insulin glargine-related changes in ECG parameters were not reported in the 3- and 6-month dog subcutaneous studies reviewed under NDA 21,081 that evaluated doses of up to 228-times the 0.1 U/kg dose administered in the current study. Nonetheless, all dogs survived the treatments, and the effects on both blood glucose and serum potassium were similar when the order of AVE0010 was changed. The hypokalemia is due the insulin glargine and it appears that the bolus injection of insulin increases QT interval and activates the adrenergic system because of the hypoglycemia. Publically available information supports this notion (e.g. Gastaldelli, *et al*, 2000; van Noord, *et al*, 2010)[11,12].

[11] Gastaldelli A1, Emdin M, Conforti F, Camastra S, Ferrannini E (2000). Insulin prolongs the QTc interval in humans. Am J Physiol Regul Integr Comp Physiol. 279:R2022-5.
[12] Charlotte van Noord, Miriam C J M Sturkenboom, Sabine M J M Straus, Albert Hofman, Jan A Kors, Jacqueline C M Witteman, Bruno H Ch Stricker (2010). Serum glucose and insulin are associated with QTc and RR intervals in nondiabetic elderly, European Journal of Endocrinology, 162: 241–248.

5. Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME/TK

The information summarized for the individual drugs is from the reviews by Dr. Rhee (insulin glargine; NDA21081) and Dr. Hummer (lixisenatide; NDA 204,961). Reference should be made to these reviews for details. The sponsor has also reported single dose PK data from the PD study conducted in dogs after subcutaneous administration (see **Table 13** & **Table 14**).

Insulin glargine

Studies have been conducted in rats to evaluate the bioavailability of insulin glargine relative to human insulin after subcutaneous administration. High levels of radioactivity was first detected in 1 hr and was present at the injection site for up to 24 hrs of [^{125}I]-HOE901 administration. Plasma radioactivity was also sustained for a longer duration compared to intravenous administration, indicating that its release from the injection site was relatively slow compared to human insulin. The toxicokinetic studies in rats, mice and dogs showed that the depot effect increased with increasing dose. Radioactivity was noted in many organs and tissues, except the CNS after intravenous and subcutaneous administration. Organs and contents with the highest radioactivity were kidneys, thyroid gland, urinary bladder and GI contents. Plasma protein binding study was not conducted with insulin glargine, but is not expected to bind plasma proteins since insulin is not known to do so.

Metabolic studies in multiple species, including humans have suggested that insulin glargine may undergo similar biodegradation as human insulin. The main route is the successive removal of three amino acids at the carboxy-end of the B-chain of insulin glargine. The intermediate compound 21^{A}-Gly-30^{B}-L-Arg-insulin and the biologically active metabolites 21^{A}-Gly-insulin (M1) and 21^{A}-Gly-des-30^{B}-Thr-insulin (M2) were recovered from human plasma and at the injection site. There is evidence from human studies that about 50% of the subcutaneous dose in the depot may be biodegraded insulin glargine.

Following intravenous single dose injection to rats and dogs, HOE901 is cleared from circulation with initial t½ of 0.21 and 0.18 hrs and terminal t½ of 1.2 and 0.6 hrs, respectively. After subcutaneous dose, maximum concentration of HOE901 was achieved within 2 and 4 hrs after dosing in rat and dogs, respectively. The plasma half-lives were 4.3 hrs in rats and 5.4 hrs in dogs, respectively. Minimal renal excretion occurred in both rats and dogs; it appears that insulin glargine undergoes hydrolysis to amino acids and is salvaged.

Lixisenatide

The pharmacokinetics of lixisenatide was evaluated in multiple animal species via subcutaneous, intravenous and intraperitoneal routes. Lixisenatide was rapidly

absorbed following subcutaneous or intraperitoneal administration with C_{max} between 0.25 and 3.75 hrs of dosing. After subcutaneous dosing, the absolute bioavailability was about 90% in dogs, 70% in pigs, 36-50% in db/db mice, >30% in rabbits and 3% in rats. The terminal half-lives of lixisenatide ranged between 0.5 and 6.5 hrs after IV administration in different animal species (mouse, rat, rabbit, dog, and pig).

Lixisenatide showed relatively comparable plasma protein binding to human (55%), dog (49%) and rat (62%) plasma proteins. After subcutaneous administration, tissue distribution was limited early to the injection site and increased within 0.25 hrs in the pancreas, renal cortex, lungs and glandular tissues. Distribution to adipose tissue, skeletal muscle, testis and the spinal cord was negligible. Lixisenatide may not enter the brain. Placental transfer studies in rat and rabbits indicate a very low placental transfer (0.1% in rat and < 0.01% to 0.3% in rabbit), and a small amount of lixisenatide is secreted in milk.

Lixisenatide was extensively metabolized after 1 hr incubation in microsomal fractions of liver and kidneys from humans, dogs, and rabbits. In human S9 fractions, 28 metabolites were detected; all of them were degraded peptide products of lixisenatide. Terminal t½ of lixisenatide ranged between 0.5 and 6.5 hrs after IV administration in different animal species (mouse, rat, rabbit, dog, and pig). Terminal plasma half-lives after subcutaneous dosing tend to be shorter in the smaller animals (mouse, rat) and longer in the larger species (rabbit, dog, pig), perhaps a reflection of allometric differences in absorption.

The effects of anti-drug antibodies on exposure and biological activity were evaluated in CD-1 mice after daily subcutaneous injection (Study DSE 2005-0443). Aqueous solution of sterile isotonic saline (50 mM sodium citrate, pH 5.3 + 0.9% NaCl (vehicle control group) or aqueous solutions of AVE0010 in vehicle at 200, 1000, or 2,000 µg/kg BID by subcutaneous injection for 3-months were evaluated. Animals were divided into 4 groups, 20/sex for antibody measurement, and 12/sex for TK measurement.

Body weight gains were statistically significantly increased in low/mid/high dose animals, i.e., approximately 33%/39%/39% and 47%/59%/55% above control for males and females, respectively. Food consumption was dose-dependently decreased within the first two and three weeks of the study in female and male mice, respectively.

A monoclonal antibody directed against the last 13 amino acids of the peptide at the C-terminus (monoclonal anti-WAVE002) of AVE0010 was used for the antibody screening. A total of 40%/75%/78% animals tested positive at low/mid/high dose, respectively. Mean exposure for mid and high dose was increased by 10- and 22-times at day 92 compared to day 1, and is attributed to antibody formation. Food consumption was dose-dependently decreased for 2-3 weeks, although increased weight gain was noted for treated animals. Active AVE0010 was determined using an in vitro cell-based assay measured by cumulative cAMP formation. Evaluation of the AVE0010 exposures in light of the active versus total AVE0010 suggested that neutralizing antibodies were not present after dosing up to 13-weeks.

Similar study was conducted in Beagle dogs exposed to AVE0010 for 13-weeks. ADAs were detected in dogs starting day 27; in week 13 all animals at low and mid dose and 83% animals at the HD were ADA positive. In the recovery arm, 50% of animals were ADA positive after 4-weeks. The results of these studies suggest that the majority of the AVE0010 in dog plasma is biologically active even in the presence of anti-AVE0010 antibodies based on cell-based study. Drug exposure in terms of AUC was increase at termination by about 12-times in males and 5-times in females at the low dose (20 µg/kg) administered twice per day compared to day 1. During the chronic dog toxicity study, all animals treated at ≥200 µg/kg BID were positive for anti-AVE0010 antibodies at 6 and 12-months. In the 2 µg/kg BID group, 67% and 75% of the animals were positive for anti-AVE0010 antibodies at 6-months and at the end of the study, respectively, indicating dose- and duration-related production of anti-AVE0010 antibodies.

In a chronic study conducted in SD rats, animals at the MD and HD developed antibodies after day 28, and about 50% males and 20% females at LD tested positive at the next measurement time (day 154). All animals at the HD tested positive after 4-weeks of drug withdrawal. Exposure ($AUC_{0\text{-}24h}$) increased by approximately 20-times between day 7 and day 183 for the MD and HD groups.

Insulin glargine and lixisenatide

[Study # pmh0125-pk1]: Plasma concentrations and pharmacokinetic parameters of AVE0010 in male Beagle dogs after a single SC application of AVE0010 with and without the application of 0.3 U/kg Lantus (HOE901) as U100 or U300 formulation.

[Study # pmh0125-bapk2]: Plasma concentrations and pharmacokinetic parameters of insulin glargine, -M1 and i-M2 in Beagle Dogs after a single SC application of Lantus U100 or U300 formulation with or without the application of lixisenatide.

The pharmacokinetic profile of lixisenatide in male Beagle dogs after a single subcutaneous application of 0.15 µg/kg AVE0010 with and without the application of 0.3 U/kg Lantus (HOE901) as U100 or U300 formulation has been evaluated. The plasma concentration-time profile curve provided by the sponsor is shown below (**Figure 19)**. Lixisenatide exposure in terms of both C_{max} and AUC was comparable, but the half-life was marginally reduced when administered as a premixed formulation. The reason for this change is unclear in light of the absence of differences in bioavailability.

Figure 19: Mean plasma concentrations of lixisenatide

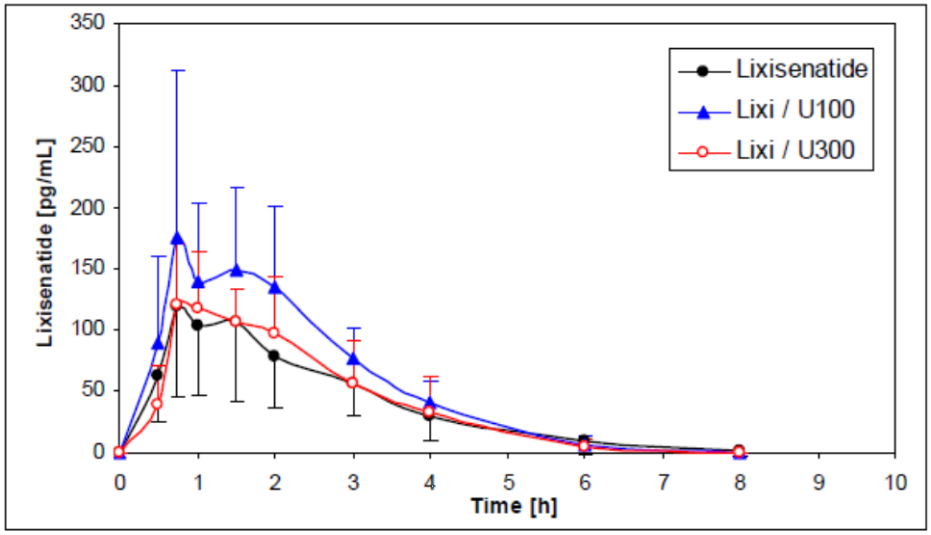


Table 13: Mean PK parameters for lixisenatide after single dose subcutaneous administration of lixisenatide and combination to dogs

	Lixi	Lixi + U100	Lixi + U300
T_{max} (hr)	1.0	1.09	1.19
C_{max} (ng/ml)	138	223	137
$AUC_{0\text{-}8h}$ (pg.hr/mL)	309	457	327
$AUC_{(0\text{-}\infty)}$ (pg-hr/ml)	365	471	361
t½ (hr)	1.86	1.1	1.12

Another pharmacokinetic study [PMH0125-Pk2 (D120411)] evaluated single dose subcutaneous pharmacokinetic profile of insulin glargine and the active metabolites of insulin glargine-M1 and insulin glargine–M2 after administration of 0.3 U/kg Lantus® U100 or U300 formulation with or without 0.15 µg/kg lixisenatide.

Plasma concentrations of insulin glargine were not above the LLOQ (0.2 ng/mL) for most samples, thus PK parameters were not calculated. Similarly, no PK parameters were calculated for insulin glargine-M2. The T_{max} values for insulin glargine-M1 varied between 0.5-8 hours, and the mean C_{max} values were 2.13 ng/mL and 2.07 ng/mL after administration of insulin glargine as U100 formulation with or without co-administration of lixisenatide, respectively. The mean $AUC_{0\text{-}8h}$ values were 7.83 ng*h/mL and 7.34 ng*h/mL after administration of insulin glargine as U100 formulation with or without co-administration of lixisenatide, respectively.

Figure 20: Mean plasma concentrations of insulin glargine-M1 in male Beagle Dog *(reproduced from the sponsor)*

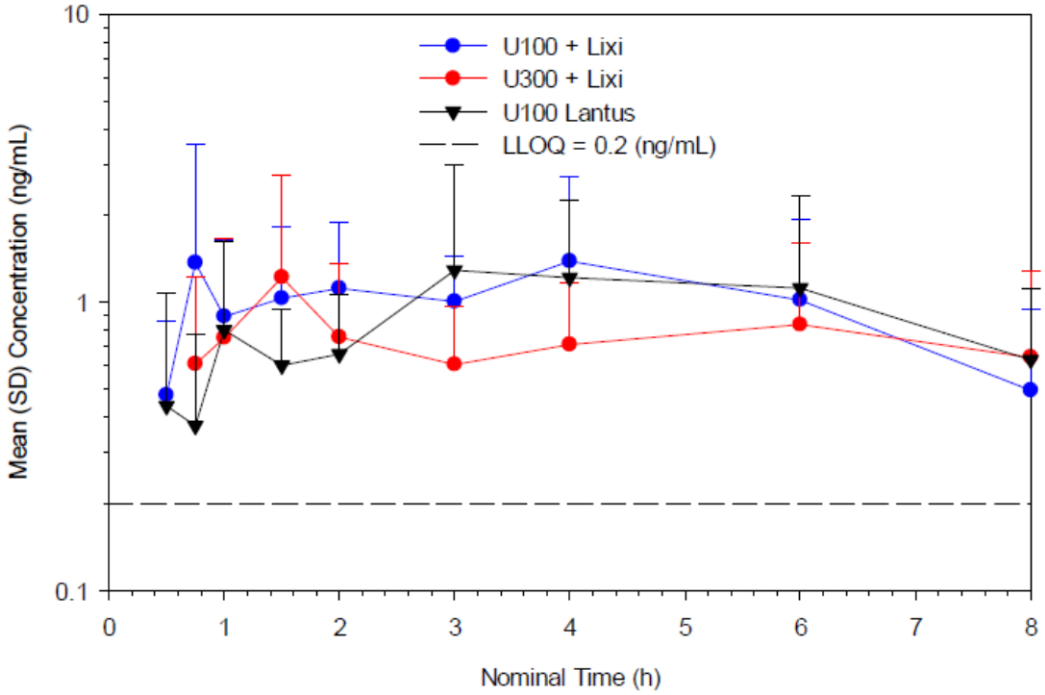


Table 14: PK parameters of insulin glargine-M1 in dogs after coadministration with lixisenatide

	U100 Lantus	U100 + Lixi	U300 + Lixi
T_{max} (hr)	4.67	2.47	3.13
C_{max} (ng/ml)	2.07	2.13	1.52
AUC_{0-8h} (pg.hr/mL)	7.34	7.83	5.65
t½ (hr)	-	-	-

6. General Toxicology

6.1 Single-Dose Toxicity

The acute toxicity of HOE901 (insulin glargine) has been evaluated in mice and dogs subcutaneously and intravenously in rats. The LD_{50} was >100 Units (~36 mg) in rodents. Animals showed clinical signs of severe hypoglycemia, but postmortem examination did not reveal organ toxicity. Dogs were more sensitive to the acute effects of insulin glargine than rodents. Dogs injected with 10 U/kg (0.364 mg) of insulin glargine died within 24 hrs, and postmortem findings indicated that death occurred due to severe hypoglycemia.

Single dose toxicity of AVE0010 (lixisenatide) was evaluated in rodents and dogs after intravenous and subcutaneous administration with a 14-day observation. CD-1 mice

tolerated 500 µg/kg injected by subcutaneous and intravenous routes. Mortality, adverse clinical signs, weight loss and macroscopic findings were not reported. An intravenous bolus dose of 5,000 µg/kg administered to Wistar rats were also tolerated, except 1 male animal was lethargic for about 2 hrs. In a single ascending dose study in Wistar rats with doses ranging from 1 – 500 µg/kg, packed cell and spleen weight were reduced, and bilirubin was increased in males receiving at 125 and 500 µg/kg. There were no changes in body weight and postmortem, and macroscopic microscopic findings were absent.

Increased vacuolation of the pancreatic islet cells was noted in female dogs receiving the HD in subcutaneous single ascending dose study of 10-250 µg/kg. There were no other changes in this study or another intravenous study that evaluated given 5, 20 and 100 µg/kg AVE0010.

6.2 Repeat-Dose Toxicity

Multiple dose toxicity studies were not performed with the insulin glargine and lixisenatide combination product. The information summarized below is from previous reviews and submissions for the individual drugs.

Insulin glargine

Toxicological studies submitted during the nonclinical development of insulin glargine include short-tem, subchronic and chronic studies conducted in rodent and dogs. Subcutaneous daily doses of 0.1455, 0.4547, 1.455 mg/kg (1U=0.0364 mg), and 0.4547 mg/kg human insulin were administered to Wistar rats for 3-months. Nineteen animals receiving the high dose of HOE901 and 3 animals on human insulin group, died perhaps due to hypoglycemia. However, these animals did not show remarkable clinical signs due to the hypoglycemic events. Significant decrease in erythrocyte count and hematocrit in both sexes, and increase in leukocyte count in females was reported in the HD group. Increased incidence of degranulation of the β-cells of the Islets of Langerhans was noted in the mid and high dose animals (this was also true in the 1-month study).

The potential toxicity of daily doses of 0.073, 0.23, 0.73 mg/kg HOE901 was tested in the chronic Wistar rat study. Nine rats receiving the HD died in the study, perhaps due to hypoglycemia, although animals didn't show clinical signs. Necrosis of the ganglion cells within the hippocampus was noted in six of the animals that died in the interim. Clotting time and reticulocyte counts were decreased in male animals in a dos-related manner. Erythrocyte count and hematocrit were low in females at the HD. Increased incidence of degranulation of the β-cells of the islets of Langerhans was also noted in this study, but was reversed after drug withdrawal.

In a 3-month Beagle dog study, daily doses of 0.036 and 0.182 mg/kg were assessed for toxicity after subcutaneous administration. All animals survived to the end of the study and ECG parameters were not affected. There were no remarkable findings in the study except β-cell degranulation upon microscopic examination.

All animals at the HD were prematurely sacrificed because of poor clinical conditions in a 6-month study with subcutaneous doses of 0.036 & 0.109 mg/kg/day. Animals showed severe signs of hypoglycemia, including tonic-colonic convulsions and coma. No adverse findings were reported in hemodynamic and ECG parameters in the low dose, although the data from the HD may have been confounded by the hypoglycemia. Degranulation of the β-cell of the islets was noted in the animals receiving the test-article.

Local tolerance study (TOL 1099) has been submitted under NDA 206,538 (Toujeo®) for insulin glargine. Insulin glargine and the high concentration product, Toujeo® were administered parenterally [subcutaneous (0.1 mL), intramuscular (0.5 mL), intravenous (0.5 mL) and paravenous (0.1 mL)] over a 6-day period. Saline was injected for control, and animals received 20% glucose solution immediately and after 4 hrs to prevent hypoglycemic events. Two rabbits died, and decreased activity of some animals was also apparent. Microscopic observations revealed focal hemorrhage, necrosis and mixed inflammatory cell infiltration with similar incidence and severity in control and test-article injected animals. Thus, it was concluded that insulin glargine was well tolerated.

In summary, the effects reported in the toxicological study in rats and dogs with insulin glargine appear to be related to the pharmacodynamic effect of the test-article. Although inconsistent, there were clinical signs related to hypoglycemia, such as irregular respiration, tremor, unsteady or ataxic gait, convulsions and coma. The effects on hematological parameters may also be related to hypoglycemia. In local tolerability studies, injection site reaction and inflammation were hinted in the original review for Lantus®, but was not observed the subsequent study. Unlike the ECG parameter changes noted with 0.1U of insulin glargine no hemodynamic or electrical activity changes were reported in the toxicological studies. Reversible degranulation of the β-cells of the islets of Langerhans was noted in both rats and dogs.

Lixisenatide

AVE0010 has been evaluated for potential toxicity in rodents and dogs for various study durations. The mouse studies evaluated twice daily doses ranging from 20 - 2,000 µg/kg administered subcutaneously. Two subchronic studies were conducted; the first one tested twice daily doses of 20, 200, 1,000 & 2,000 µg/kg after SC administration. All standard toxicological endpoints were assessed in this study. A second 13-week study with the same doses was conducted for antibody screening and measurement of total drug concentration; mortality, clinical signs, body weight and food intake were also assed. Mortality was reported in the second study although the cause was not determined. Weight loss was reported at all doses in the 2-week and the first 13-week studies. Minimal increase in the incidence of subdermal fibrosis/inflammation was noted at the injection site of animals in the 13-week study. Exposure was dose-proportional in the 2-week study, but was highly variable and more than dose proportional between the low and mid dose, due to dose-related increase in ADA. A second 13-week subcutaneous study evaluated the same doses and further demonstrated ADA formation and quantified exposure. Early dose-related death occurred in the second

study and food intake was lower in the first 2-3 weeks. But, mean body weight was paradoxically higher for treated animals compared to controls. More than dose-proportionality was observed in total drug exposure in the study. ADA was formed in a dose-dependent manner contributing to drug accumulation of 10- to 20-times on Day 92 compared to Day 1.

Sprague-Dawley rats were administered twice daily SC doses of 5, 20, 200, 1,000 and 2,000 µg/kg for 13-weeks. There was no mortality in the study, but weight gain was decreased in the first 4-weeks, but recovered after drug withdrawal for 4-weeks. Reversible decline in total leukocytes and lymphocytes was reported after 4-weeks. Decline in urinal Na^+ and K^+ was reported despite decreased urine volume in male animals. Non-reversible increase in kidney weights was recorded in females in all doses. Increased incidences of papillary and corticomedullary mineralization, and inflammatory cell foci were noted in females at higher dose. ADAs were detected in greater than 80% of animals in week-14.

A chronic rat study was also conducted in SD rats after twice daily administration of 5, 100 and 2,000 µg/kg subcutaneously. There was no mortality, but decline in body weight that reversed after recovery period was noted in all groups. Increased incidence and severity of microscopic findings in the testis (seminiferous tubule atrophy and necrosis, spermatid stasis, mineralization), seminal vesicle (atrophy), and epididymis (oligospermia, aspermia, lymphocyte infiltrate) were observed at 2,000 µg/kg BID. These effects in general were reversible. There was also a slightly increased incidence of hemorrhage, fibroblastic reaction, and abscess at the injection site for males receiving ≥100 µg/kg BID dose. Anti-AVE0010 antibodies formed after 28-days at MD and HD, but later (Day 154) in the LD. Exposure based on total drug AUC increased by about ~230-times between the MD and LD, and 13-times between the MD and HD. The NOAEL was determined to be 2,000 µg/kg BID for females, but was 100 µg/kg BID for males based on the testicular findings.

The potential toxicity of AVE0010 was evaluated in 4-, 13- and 52-week studies in Beagle dogs. Twice daily doses of up to 200 µg/kg were tested in a 4-week study. Mortality was absent, but female animals in all groups gained less weight in the first week, and both sexes had decreased weight gain associated with less food intake in the second week. The weight of epididymis and testes was low in the HD group. A slight increase in the proliferation of fibroblasts was noted at the injection site. In a 13-week dog study with 4-week recovery arm, twice daily doses of 20, 300/100 and 1,000/400/250 were tested. Dose adjustments were made because of an acute decline in body weight associated with low food intake at the mid and high dose. There was no mortality, but there was dos-related emesis and diarrhea. Heart rate was lower in treated animals, and reversible microscopic findings of glycogen depletion in the liver and sperm stasis were reported at the MD and HD, and injection inflammation/fibrosis in all treatment groups. AntiAVE0010 antibodies were detected in all treatment groups at >70% starting in week-4 of dosing.

Doses of 2, 200 and 1,000 µg/kg given twice daily were tested in the chronic dog study via the SC route. There was no mortality, but significant weight loss accompanied by low food intake was reported at ≥ 200 µg/kg. Increased mean absolute and relative uterine and ovary weights were noted at ≥200 µg/kg BID and decreased mean absolute and relative thymus weights were observed for HD females. The incidence of increased red focus/area at injection sites, as well as dose-related increase in inflammation and fibrosis were reported at ≥200 µg/kg BID. Moreover, moderate to severe hypo-spermatogenesis in seminiferous tubules; epididymal dilation, degeneration, oligospermia or aspermia in testis were noted at ≥200 µg/kg BID. All animals treated at ≥200 µg/kg BID were positive for anti-AVE0010 antibodies in dose- and duration-related manner. TK data showed significantly increased AUC values from Day 1 to 6-months (up to 12X), which was due to anti-AVE0010 antibody formation. The NOAEL for males was considered to be 2 µg/kg BID based on treatment-related effects on testes at ≥200 µg/kg BID. The NOAEL for females was 1,000 µg/kg BID.

7. Genetic Toxicology

The combined formulation of insulin glargine and lixisenatide has not been tested for potential genotoxicity. The applicant referenced the studies conducted with the individual drug substance

HOE901 tested negative in a bacterial mutagenic tests with or without metabolic activation at doses up to 50 mg/mL (5,000 µg/plate). The compound also tested negative in an *in vitro* mammalian gene mutation and chromosomal aberration tests when tested at concentrations of ≤ 100 µg/mL with and without metabolic activation. HOE901 was not clastogenic in the *in vivo* cytogenetic test in bone marrow cells isolated from CHO cells receiving single dose of 750 U/kg (27 mg/kg).

AVE0010 was tested in a standard battery of genotoxicity tests. The reverse mutation test in bacteria was performed using modified preincubation method up to the maximum recommended concentration of 5,000 µg/mL. The *in vitro* mammalian chromosome aberration test was conducted in human lymphocyte cell line with and without S9 fraction at concentrations of up to 50 µM. AVE0010 tested negative in both the Ames and the chromosomal aberration assays. Moreover, AVE0010 did not induce micronucleated erythrocytes in the bone marrow of rats receiving single intravenous doses of up to 5 mg/kg AVE0010.

8. Carcinogenicity

The insulin glargine lixisenatide combination has not been evaluated for carcinogenicity. The applicant made reference to the studies conducted with the individual agents as agreed.

Insulin glargine

A two-year carcinogenicity study with insulin glargine was performed in NMRI mice at HOE901 doses of 0.073, 0.182 and 0.455 mg/kg, controlling with saline, placebo and human insulin (HOE36H). The study in female mice was confounded by excessive mortality in all dose groups. Thus, Dr. Rhee concluded that the female study was invalid. The incidences of histiocytomas were increased at injection sites in male mice, but were not statistically significant. Thus, HOE901 is not tumorigenic in male mice up to the high dose, which is approximately 1-times the recommended human subcutaneous high dose of 60 units/day (0.0364 mg/kg/day), based on body mass comparison.

The same doses tested in mice were subcutaneously administered in Sprague-Dawley rats to evaluate the carcinogenicity potential of HOE901. Saline, placebo and human insulin (HOE36H) injections were used concurrently in control groups. Excessive mortality was reported for male rats in placebo and other groups except the group receiving saline. Increased mortality was also reported in females in the HD group and HOE36H. The nonclinical reviewer questioned the validity of the study based on the number of animals available at the end of the study for statistical testing. Malignant fibrous histiocytomas at the injection site occurred in higher incidences in all mall rats except the group receiving saline and human insulin. (b) (4) The reviewer disagreed because of the absence of such finding in females. Malignant fibrous histiocytomas at the injection site occurred at statistically significant level in male rats in the pairwise testing, but was not significant in trend analysis. Thus, it was concluded that HOE901 is not tumorigenic when tested up to 0.455 mg/kg, approximately 2-times the proposed human high dose of 60 units/day based on allometric scaling.

Lixisenatide

Carcinogenicity studies of 2-year durations were conducted in CD-1 mice and Sprague-Dawley rats with twice daily subcutaneous doses of 40, 200, or 1,000 µg/kg.

The following summary is from Dr. Hummer's review:

Thyroid c-cell adenomas were noted in 1 mouse each at the low and mid doses, and in 4 animals at the HD compared with none in the two control groups. The increase was only found to be statistically significant for the high-dose group. The mid-dose group had a slight increase in c-cell hyperplasia whereas the low-dose group did not. Because of the associated hyperplasia, it is possible that the tumor observed in a single mid-dose animal was treatment-related, but a definitive treatment-related effect cannot be concluded. A statistically significant increase in c-cell adenomas was not observed in females at any dose level.

Additionally, a slight increase in endometrial adenocarcinoma of the uterus was observed for mid- and high-dose females, but the increase was found to be statistically significant for the mid-dose group only. The most noteworthy non-tumor finding was a large increase in basophilia of the parotid salivary gland for all treated groups. Other microscopic findings that may have been treatment-related included a slight increase in squamous cell hyperplasia and metaplasia of the cervix (HD females); slight increase in adrenal gland hyperplasia of the medulla (HD females); gall bladder hyperplasia (HD female); fibro-osseous lesion of the sternum (MD and HD females); diffuse hepatocyte hypertrophy (MD and HD males); and pancreatic acinar cell hyperplasia (MD and HD females).

Exposure increased in a greater than dose-proportional manner perhaps due to ADA formation. ADAs occurred in a dose-related manner, the incidence of which ranged from 33% (low-dose males) to 100% (high-dose females). The frequency of occurrence in uterine adenocarcinomas appears incidental because the incidence is within the historical control range.

Adequate number of male rats survived to termination, but the number of female rats at study termination was <20 for placebo and LD, but was >55% at the HD. The female mortality is not dose related thus, the relation to the test article is questionable. A dose-related decrease in body weight that may be due to reduced food consumption was however observed throughout the study. The impact of weight loss on tumor incidence was not examined.

A slight increase in thyroid size was observed in females receiving the test article. Thyroid c-cell hyperplasia and tumors were also observed, which may have correlated with the increased thyroid size. Small increases in females with bone fractures and mid- and high-dose males with small adrenal glands were observed. These findings did not have microscopic correlates. A statistically significant increase in thyroid c-cell adenomas was noted for all AVE0010-treated groups. A numerical, non-statistically significant increase in c-cell carcinomas was observed for mid and high dose males and females at a low incidence (1 to 3 per group) compared with no carcinomas in either control group. A slight increase in focal c-cell hyperplasia was observed for all treated groups.

A slight increase in pancreatic islet cell adenomas was noted for high dose males The increase for the high dose was statistically significant for trend ($p = 0.0158$) and for pair-wise analysis ($p = 0.0431$) when compared with placebo. However, the increase was not statistically significant when compared with saline control and the increase was also not statistically significant versus either of the control groups when adenomas and carcinomas were added together. Moreover, hyperplasia of the islet cell was noted in all groups with non-distinguishable incidence and severity within groups.

The findings reported at the injection site, hemorrhage (all treated groups), grade 3/4 necrosis of adipose tissue (MD and HD males) were of low incidence and were not significantly different from controls. Other findings in the kidneys, such as transitional

cell hyperplasia & mineralization (all treated male groups), calculus (HD males), and/or neutrophil infiltrate (MD and HD males]), focal hyperplasia of the mammary gland (HD females), and lung hemorrhage (all male groups) did not show relations to treatment.

Dose proportionality in exposure was noted only between the low and mid dose. Exposure increased significantly at all doses by Day 86, and was slightly higher than the value on Day 359. ADAs were detected in all AVE0010-treated animals (88% to 100%) in a dose-unrelated manner. *A large percentage of control females (15/16) and a smaller percentage of control males (3/18) tested positive for ADAs. This may have been due to the apparent dosing errors in which it was suspected that control animals received test article based on body weight data.*

9. Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Insulin glargine

Combined fertility and pre and postnatal toxicity study was conducted in Wistar rats with subcutaneous doses of 0.036, 0.108 and 0.360 mg/kg HOE901. The study was controlled with human insulin (HOE36H) and vehicle. Male animals were treated 28-days prior to mating and until impregnation of either treated or untreated females. Treated female animals received the test-article 14-days prior to mating, during mating and throughout pregnancy and during 21-day lactation period.

Clinical signs of hypoglycemia were noted at the HD and 5 maternal animals were sacrificed prematurely and 3 died between days 22 and 23 of mating. Body weight and food consumption of maternal animals were not affected. Fertility, early- and embryo-developmental and parturition indices in the treated groups were not different from controls. There were no gross anatomical abnormalities in the F1 animals and sexual maturation and post-developmental landmarks were not different from controls. In general, HOE901 up to a dose of 0.39 mg/kg (2-times the high dose of 60 U/day based on mg/m^2) was not associated with remarkable reproductive toxicity in rats.

Himalayan rabbits were administered subcutaneous doses of 0.018, 0.036, 0.072 mg/kg HOE901 and 1 U of HOE36H daily during organogenesis [GD6 – GD18]. Two doe at the HD were killed on days 11 and 12 of pregnancy because of hypoglycemia associated motor attacks. Mean doe weight and food intakes were not affected by the test-article. Caesarean findings were unremarkable, except reduced number of fetuses at the HD due to higher resorptions of the embryo. There were no gross abnormalities at the low dose; one fetus exhibited asymmetry of the visceral cranium, another fetus had a bent forepaw at the mid dose. One fetus at the HD showed an apical bone between the paired nasal and frontal bones. Crossectional autopsy showed dilated ventricles of the brain in five fetuses from the HD group; there were no skeletal abnormalities in these fetuses.

In general, the HD induced intrauterine death and abortion due to hypoglycemia. The reference human insulin also induced similar effects. The top dose of HOE901 tested in the study was 1-times the highest proposed clinical dose of insulin glargine based on mg/m^2.

Lixisenatide

Doses of 0, 4, 58 & 828 µg/kg were administered twice daily via the subcutaneous route 8 hours apart to Crl:CD®(SD)IGSBR rats. Male rats were treated 4-weeks early and throughout pairing and mated with females from the same dose group. Females were treated for 2-weeks before pairing with males and throughout pairing until day 6 of gestation, inclusive. Necropsy of females was performed on day 13 of gestation for examination of uterine contents. Male animals receiving AVE0010 gained lower weight during the study. The decline in body weight was apparent within 3-days of dosing and correlated with lower food intake. Body weight gain in females was not remarkable despite lower food intake.

Fertility, conception and gestation parameters in treated animals were not different from animals receiving the vehicle.

9.2 Embryonic Fetal Development

Both the dose-range finding and pivotal embryo-fetal developmental studies have been reviewed by Dr. Tim Hummer. The following focused review of embryo-fetal developmental studies is geared towards answering the question of whether the malformations observed in both species were due to only maternal toxicity, specifically weight loss. Significant decline in food intake and weight loss was noted during test-article administration that coincided with the duration of organogenesis. Thus, weight and weight gain were calculated for these periods instead of the interval between the start of dosing and cesarean section. Since maternal animals showed adaptation to the test-article as dosing continued, the decrease in weight gain calculated during dosing is a reflection of the maximal loss to be expected.

[Study DSE 2003-1923]: Dose-range finding study in SD rats

- AVE 0010 doses of 0.14, 2.0 and 4.0 mg/kg/day injected twice subcutaneously from day 6 to 17 of pregnancy in half daily doses.
- Crown-rump length was significantly lower in LD and MD, not HD.
- Intrauterine embryo-fetal mortality was increased at doses ≥ 2.00 mg/kg/day.
- Two high dose females showed total implantation loss, and the remaining females at the HD had no implantation sites, but had corpora lutea.
- One dead fetus in 1/5 litters, five dead fetuses in 3/5 litters and one dead fetus in 1/1 litter in the LD, MD & HD, respectively. No fetal death in control group.

- The number of live fetuses and fetal development was increased as evidenced by decreased fetal body weight gains and crown rump lengths. Placental weights were decreased in all dose groups.
- No external malformations were noted in fetuses.

Table 15: Summary of body weight (g) in rat dose-range finding embryo-fetal toxicity study with lixisenatide

	Maternal				Fetal			
Dose (mg/kg/day)	0	0.14	2.0	4.0	0	0.14	2.0	4.0
Δ in BW (%)[$]	52	38	33	28	-	-	-	-
% Δ in BW gain[ϕ]	-	-28	-37	-48	-	-	-	-
Mean Crown-rump (mm)	-	-	-	-	38.1	36.9*	34.9*	31.3[&]
Mean fetal BW (g)	-	-	-	-	3.62	3.29*	2.88*	2.27[&]
Placental weight (g)					0.57	0.53	0.44*	0.34[&]

[$]*For GD16 relative to GD6 (dosed from GD6-GD17)*
[ϕ] *% Change in BW Gain = [(Group gain – control gain)/ control gain]*100 (GD6-GD20)*
[&]*Value for HD is from litter from one animal, other value from litters from 5-6 animals.*

Conclusion

The dose range finding study suggested that reduction in maternal weight gain of ≥ 37% is associated with developmental effects. These reductions were not associated with external fetal malformations. Maternal and developmental NOAEL proposed at <0.14 mg/kg/day.

[Study DSE 2004-0551]: Pivotal embryo-fetal toxicity study with SD rats

- Mean maternal weight loss of 3-10%, and decrease in weight gain of 14-27% (see **Table 16**). Food intake between GD6 and GD13 was decreased by 23% (LD), 37% (MD) and 43% (HD) relative to control dams, but was ~15% lower than controls for all treatment groups at necropsy.
- No treatment-related intrauterine deaths of fetuses in the study.
- Fetal weight and crown-rap length were statistically significantly smaller for the HD.
- There was small fetus at LD (2.6 g) and MD (1.9 g). One fetus weighing 1.6 g showed decreased skeletal ossifications in multiple bones; long bones were short

& bent. Three fetuses had short and bent scapulae, bent scapular spine, & misshapen clavicle. The pelvic griddle of the MD was bent on both sides.

- Fetal microphthalmia (unilateral) & anopthalmia (bilateral) in 1 LD and 1 MD respectively.
- The maternal and developmental NOAEL was <0.005 mg/kg/d.

Table 16: Summary of body weight (g) in embryo-fetal toxicity study with lixisenatide

	Maternal				**Fetal**			
Dose (mg/kg/day)	0	0.005	0.07	1.0	0	0.005	0.07	1.0
∆ in BW (%)[$]	42	39.2	32	35	-	-	-	-
% ∆ in BW gain[ϕ]	-	-14.0	-27.0	-19.0	-	-	-	-
Mean gravid uterus weight (g)	71.8	69	62	67	-	-	-	-
Mean Crown-rump (mm)	-	-	-	-	36.9	36.2	36.7	35*
Mean fetal BW (g)	-	-	-	-	3.41	3.28	3.35	3.06*
Placental weight (g)	-	-	-	-	0.48	0.47	0.48	0.45

[$]For GD16 relative to GD6 (dosed from GD6-GD17)

*[ϕ] % Change in BW Gain = [(Group gain – control gain)/ control gain]*100 (GD6-GD20)*

Conclusion

There is a trend in fetal weight reduction, and statistical significance at the HD. Average fetal weight in treated animals was between 2.5 and 3.0 g. Skeletal developmental defects were commonly observed in fetuses that lost significant weight. Weight gain in maternal animals was decreased up to 27% at a critical time of development. This is in contrast to the effect on weight of rats in the 13-wk rat study (0, 5, 20, 200, 1,000 and 2,000 ug/kg BID) body gain was decreased at all doses (-11% to -19%); feed consumption was not reduced in general, and was not affected by treatment in females. The discrepancy is a reflection of adaptation to the test-article.

[Study DSE 2003-1924]: Dose-range embryo-fetal toxicity study in Rabbits

- Dose 0.08, 0.4, 2.0 mg/kg from GD6-GD18 (two divided daily doses).
- Two high dose females were prematurely euthanized after premature delivery (day 24). These animals (# 121 and 122) had dead fetuses and embryos that were undergoing early and late resorption.
- Mean litter size was not different between groups with live fetuses at termination.

- One fetus at 80 µg/kg (78X the 20 µg dose in mg/m^2) [dam#110, fetus L01) exhibited thoracogastroschisis, including ventral diaphragmatic hernia and related multiple skeletal malformations.

Table 17: Summary of body weight (g) in rabbit range-finding embryo-fetal toxicity study with lixisenatide

	Maternal				Fetal			
Dose (mg/kg/day)	0	0.08	0.4	2.0	0	0.08	0.4	2.0
∆ in BW (%)[$]	6	4	5	11	-	-	-	-
% ∆ in BW gain[ϕ]	-	-172	-190	-289	-	-	-	-
Av. gravid uterus weight (g)	360	352	398	341	-	-	-	-
Mean Crown-rump (mm)	-	-	-	-	94.5	96.1	95.0	89.7
Mean fetal BW (g)	-	-	-	-	41	39	39	37
Placental weight (g)	-	-	-	-	4.86	4.48	5.23	4.93

[$]For GD19 relative to GD6 (dosed from GD6-GD17)
*[ϕ] % Change in BW Gain = [(Group gain – control gain)/ control gain]*100 (GD6-GD20)*

[Study Numbers: 2004-0552 and DSE 2005-1086]: Pivotal embryo-fetal toxicity study in rabbits (Chbb:HM (SPF) (b) (4)

Two studies were conducted, The first study at doses 5, 50 & 500 µg/kg (study 2004-0552) and the second one at lower dose of 0.3, 2 and 5 µg/kg (study 2005-1086).

Findings from study 2004-0552

- Body weight lagged for the treated animals compared to controls during organogenesis; and was statistically different on GD10 for the HD, GD13 (MD) and GD16 (LD). Mean BW was 3%, 5% and 10% less on JD19 for LD, MD, and HD, respectively.
- Food intake during GD6-GD10 was 65.1%, 84.8% and 94% lower than control animals.
- Dose-dependent increase in post-implantation loss was noted with statistical significance at HD
- Only one animal at MD aborted

- Statistically significant post-implantation loss at the HD (4.4%, 8.8%, 7.1 & 19% of implantations for control, LD, MD and HD, respectively). Also a slight decrease in the number of fetuses per dam.
- No effect on mean fetal weight, crown-rump length and placental weight. Fetal weight ranged from 20-30 g. There were single live fetuses in 5 and 50 µg/kg that weighed < 20 g.
- Major malformations, some of them multiple malformations in a single fetus were noted at all doses. The sponsor has concluded that the malformations were rare and concluded that they were treatment related.
- One notable finding is thoracogastroschisis, noted in two fetuses at the low and mid dose (4- and 40-times the proposed human dose). This malformation associated with ventral diaphragmatic hernia and multiple skeletal malformations were also reported in one fetus in the rabbit dose-range finding study at 80 µg/kg dose. Diaphragmatic hernia has been also reported in the second rabbit embryo-fetal study with lower doses at the clinical exposure. These findings indicate that lixisenatide can cause defects in midline closure at lower exposure multiple of the proposed human dose. These visceral and closure defects may not be solely explained by the maternal body weight loss.
- No NOAEL in the study
- The following is reproduced, verbatim from the study report:

Morphological examination showed multiple malformations in five fetuses consisting mainly of impaired formation or occlusion of cavities of the trunk [two cases of thoracogastroschisis with short partially rudimentary trunk (one 0.005 and one 0.05 mg/kg group fetus), lack of the entire trunk, viscera partly present (one 0.05 mg/kg group fetus), omphalocele (one 0.5 mg/kg group fetus) and as closure defect of the neural tube a spina bifida (one 0.005 mg/kg group fetus)]. There was also a cardiac ventricular septum defect (one 0.5 mg/kg group fetus) and lack of gall bladder (one 0.05 and two 0.5 mg/kg group fetuses). Four of the above described multiple malformations can be regarded as closure defects of cavities of the trunk, where in one case no trunk i.e. no abdominal walls were formed, in two cases part of the trunk including walls of body cavities were rudimentary and there was one case of omphalocele. Also the spina bifida represents a closure defect. Though similar multiple malformations are known to occur spontaneously as single events in the rabbit strain used, the relative frequency of the five cases of closure defects in the present study is considered treatment related.

Table 18: Summary of body weight (g) in rabbit embryo-fetal toxicity study with lixisenatide **(***Study 2004-0552*)

	Maternal				Fetal			
Dose (µg/kg/day)	0	5	50	500	0	5	50	500
∆ BW (%)[$]	4	-2	-3	-8	-	-	-	-
% ∆ in BW gain[ϕ]	-	-154	-179	-315	-	-	-	-
Av. gravid uterus weight (g)	423	363	360	371	-	-	-	-
Mean Crown-rump (mm)	-	-	-	-	94.4	95.6	95.2	95.8
Mean fetal BW (g)	-	-	-	-	40.0	41	39	40
Placental weight (g)	4.95	5.31	5.02	5.11	-	-	-	-

[$]*For GD19 relative to GD6 (dosed from GD6-GD19)*

[ϕ] *% Change in BW Gain = [(Group gain – control gain)/ control gain]*100 (GD6-GD29)*

Findings from study 2005-1086

- Maternal animals lost relatively small weight during the period of dosing. The weight gain in the animals was decreased in the MD and HD animals.
- Food consumption was 17%, 55% and 69% lower in LD, MD and HD animals compared to control.
- Litter size and number of live fetuses was not affected by treatment.
- Fetal malformation was reported for one fetus at the high dose, weighing 34.9 g. The findings were "bradydactyly on the left forepaw with lack of the middle and distal phalangeal bones of the 2nd to 4th digits; one fetus, weighing 34.9 g had a scoliosis to the right of the thoracic vertebral column with underlying absence of the 13th thoracic vertebral centrum and arch; another fetus, 42.2 g had a shortened 12th rib and in fetus that is 42.7 g, the gallbladder was absent. One fetus from the 0.3 µg/kg group, weighing 36 g showed a diaphragmatic hernia and a small lung.
- The sponsor has proposed the 0.3 µg/kg as NOAEL for maternal toxicity and (b) (4) µg/kg for developmental toxicity. Dr. Tim Hummer concluded that the maternal and fetal NOAEL is 0.3 µg/kg.

Table 19: Summary of body weight (g) in rabbit embryo-fetal toxicity study with lixisenatide *(Study 2005-1086)*

	Maternal				Fetal			
Dose (µg/kg/day)	0	0.3	2	5	0	0.3	2	5
∆ in BW (%)[$]	5	5	-3	-5	-	-	-	-
% ∆ in BW gain[ϕ]	-	4	-166	-202	-	-	-	-
Av. gravid uterus weight (g)	350	365	331	344	-	-	-	-
Mean Crown-rump (mm)	-	-	-	-	96.9	97.6	95.5	97.6
Mean fetal BW (g)	-	-	-	-	41	41	38	41
Placental weight (g)	5.1	5.2	5.1	5.3	-	-	-	-

[$]For GD19 relative to GD6 (dosed from GD6-GD19)
*[ϕ] % Change in BW Gain = [(Group gain – control gain)/ control gain]*100 (GD6-GD29)*

9.3 Prenatal and Postnatal Development

Lixisenatide

Pregnant Sprague-Dawley rats received subcutaneous doses of 0.004, 0.04 & 0.4 mg/kg/day AVE0010 starting GD6 through lactation. Maternal animals lost weight, consumed less food, and had decreased motor activity.

A slight increase in pup mortality was reported from PND0 to PND21 at the HD (11.9% vs. 3.8% for control) in the F1 generation. A slight decrease in suckling of male pups was noted at ≥0.04 mg/kg/day (≥20X proposed dose in mg/m^2) during the middle of lactation period, resulting in a slight downward trend in mean body weight for males. Abnormal tails were noted in treated juvenile animals. The tail abnormality included, part of tail missing (PND0), tail shortened (PND3) tail tip necrotic (PND9 to PND20) in one pup each at 0.004 mg/kg/day. No tail abnormalities were observed at 0.04 mg/kg/day. Seven juvenile animals receiving 0.4 mg/kg/day showed abnormal tails such as necrotic or shortened tail tips, or wavy, bent or deformed tails. Two cases of multiple skeletal malformations of long bones and ribs in two growth retarded dead pups were reported at 0.4 mg/kg/d. A slight delay in hair coat development was observed at ≥0.04 mg/kg/d and a slight, statistically significant decrease in the time to vaginal opening was observed, for MD (34.5 days) and HD (34.3 days) females compared with control females (37.2 days). All of the above findings were judged to be related to the administration of the test-article. Reproductive functions of the F1 animals were not affected.

The NOAEL for F0 maternal rats was considered to be <0.004 mg/kg/d based on adverse effects on body weight. The NOAEL for the F1 animals is considered to be 0.04 mg/kg/d based on the effects on mortality and skeletal and tail defects noted at 0.4 mg/kg/d.

Conclusion on reproductive risks of insulin glargine and lixisenatide

Administration of lixisenatide to pregnant rat and rabbits at the time of organogenesis is associated with impaired fetal growth and increased incidences of skeletal and visceral malformations. The embryo-fetal effects were noted in rats at the clinical exposure and rabbits at 6-times the clinical exposure. Even though dose-relations were not apparent for the malformations, increased severity can be concluded at the highest doses tested in both rat and rabbits. The sponsor has concluded that the malformations were treatment related and the incidences of the noted malformations exceeded the historical range and were not observed in control animals.

Maternal food intake declined during lixisenatide administration, which coincided with the period of organogenesis in both species, but had an upward trend as treatment was continued or drug withdrawal. The maternal weight gain decline approximated the food intake pattern during dosing. The question that was raised during this review is whether the malformations were due to only maternal toxicity, specifically weight loss.

Based on literature, the following Caesarean and fetal effects would be associated in general with maternal toxicity: post implantation loss, decreased litter size and viability, resorptions, supernumerary ribs, delayed or impaired ossifications that usually parallel reduced fetal body weight, and abortion in rabbits. Even though maternal food consumption and weight gain in both species were low at the time of organogenesis, there are no reports that implicate maternal weight loss to visceral malformations and closure defects. Moreover, no dose-related general retardation of fetal growth was noted in the study although maternal weight loss was dose-related. Malformations were apparent at the high dose in the rat study, although the decrease in dam weight gain was marginal relative to what is reported in literature in food-deprived animals (see below).

Feed-deprivation studies in rats and rabbits during organogenesis (Cappon *et al,* 2005; Fleeman, *et a*l, 2005) have suggested that feed restrictions that reduced maternal rat body weight by up to 15% had no effect on fetal viability, and external, visceral or skeletal malformations. However, feed restriction that reduced maternal rat weight gain by 51% was associated with fetal body weight loss and skeletal development defects. In the lixisenatide study, maternal food intake in rats was significantly reduced, but the decline in weight gain was between -14 to -27% of the control gain during dose administration. In the rabbit study, feed restriction to ≤ 75 g/day was associated with increased incidences of unossified sternebrae, metatarsals, metacarpals or caudal

vertebrae, and feed restriction to 15 g/day during organogenesis increased abortion, but no malformations.

In the lixisenatide embryo-fetal development study in rabbits (Study 2004-0552), the lowest food intake was noted at the high dose (500 µg/kg) and on GD6 to GD10; average weight was lowest on GD10. The estimated average feed consumption at the high dose on GD10 was about 5 g/day, and the average maternal weight gain was decreased by 315% in this group. **Table 20** below compares weight gain of individual rabbits with fetus that presented multiple malformations (closure defects, omphalocele, others as described above). The mean weight gain values for the study were -154%, -179% and -315% for LD, MD and HD group. These data suggest a potential relationship between a decline in maternal weight gain and malformations. However, this reviewer is uncertain if the cavity closure defects would be explained solely by the decline in weight gain.

Table 20: Weight gain of individual rabbits with fetal malformations

Dose (µg/kg)	Doe #	% Δ in BW gain[ϕ]
5	202	-100
	213	-213
50	229	-173
	235	-277
500	252	-621

*[ϕ] % Change in BW Gain = [(doe gain – control gain)/ control gain]*100 (GD6-GD19). Mean weight gain (0.096 kg) was used for control.*

Marginal adverse effects were reported for insulin glargine in rats when high doses of up to 0.36 mg/kg/day (2-times the maximum recommended dose based on mg/m^2) were evaluated. However, the top dose of 0.072 mg/kg/day (1-times the maximum recommended dose in mg/m^2), administered to rabbits was associated with maternal toxicity, increased incidence of resorptions and fetal loss. These effects are likely due to hypoglycemia. Lixisenatide decreased body weight gain in animals, and the premixed combination of insulin glargine and lixisenatide appears to have additive pharmacodynamic effect (see **page 32**). Since the weight loss and hypoglycemia were observed at low multiple of the proposed clinical doses, the lixisenatide and insulin combination would have liabilities to the fetus.

10 Local Tolerance

Reports from two local tolerance studies in rabbits (Tol1122 & Tol1145) with the premixed combination were submitted in this NDA. Study #Tol1122 was conducted with clinical formulation (lots AVE0010_08_838/AVE0010_08_846).

Table 21: Premixed formulation for local tolerance study

Lot Composition (mg/mL):	AVE0010_08_838	AVE0010_08_846
AVE0010	(b) (4)	
Insulin glargine		
Zn (b) (4)		
m-Cresol		
Glycerol (b) (4) %	20.0	20.0
Methionine	3.0	3.0
(b) (4)	(b) (4)	
HCl, NaOH		

Data according to appended CoAs (AVE0010, insulin glargine, m-cresol, (b) (4) and Pharmaceutical Sciences Department information (further additives, included in raw data)

The test solution and saline control were administered once parenterally [SC, PV (0.1 mL), IV, IM (0.5 mL)]. A 20% glucose solution (~20 mL/rabbit) was also injected via the subcutaneous route to prevent hypoglycemic shock. Animals were observed for clinical signs, changes in body weight and for injection site reactions. Two rabbits per administration route were sacrificed 24 hrs and two were sacrificed 120 hrs after administration to assess injection site reactions macroscopically and by histopathological examinations. In-life and histopathological findings at the injection sites showed good tolerability in rabbits.

Another study (Tol1145) evaluated ready to use aqueous solution of 0.8 mg/mL AVE0010 and 3.6378 mg/mL insulin glargine via the same routes used in the earlier study. The formulation used in the study is shown in the sponsor provided **Table 22** below:

Table 22: Test solution for second local tolerance study in rabbits

Composition:	Nominal specifications	According to CoA*
AVE0010	0.8 mg/mL	(b) (4)
Insulin glargine	3.6378 mg/mL	
Zn (b) (4)	0.030 mg/mL	
m-Cresol	2.7 mg/mL	
Glycerol (b) (4) %	20.0 mg/mL	
(b) (4) Methionine	3.0 mg/mL	
HCl, NaOH	(b) (4)	

* Analytical results ranged in (b) (4) acceptance criteria. Nominal specifications are used for reporting.

The following volumes of solution were injected IV, IM (0.5 mL), SC, PAV (0.1 mL) with normal saline as control. No mortality or adverse clinical signs were noted in the study. Minimally reddened and hemorrhagic appearances were reported equally in control and test-item injected sites. Microscopic examination at the SC injection site showed minimal to mild necrosis of the panniculus muscle that regenerated after 120 hrs. This study also suggested good tolerability in rabbits.

Local tolerance study conducted with lixisenatide in histidine and EDTA containing formulation, and in the absence of these chemicals in the formulation has indicated that, EDTA and histidine containing formulations are not tolerated via the intramuscular and paravenous routes. In subsequent study (# DSE 2005-0771), AVE0010 solution (lot # BR477/38) was injected SC, IM (0.5 mL) or PAV (0.1 mL) with normal saline as control.

Table 23: Lixisenatide formulation in local tolerance study

Ingredients	Amount [mg/mL]
AVE0010	(b) (4)
Sodium acetate trihydrate	
Meta-cresol	
Glycerol 85%	
(b) (4) Methionine	
Hydrochloric acid	
Sodium hydroxide	
Water for injection	

Clinical signs, body weight and macro- and microscopic examinations of the injection sites were performed at 0, 4, 24 hrs at the time of necropsy (24 or 120 hrs after injection). No abnormalities were noted at SC and IM injection sites. At AVE0010-treated sides, a minimal reddening in the PAV injection area was visible up to 120 hrs after injection in 3 out of 4 rabbits. The fourth rabbit showed hemorrhages at the AVE0010 injection site up to 120 hrs after injection. Note that injection of higher amount

of AVE0010 (0.1 mL of 0.8 mg/mL) through PAV as a combination was reported to have no tolerability issues as discussed above in the same strain of rabbits (New Zealand White).

A local tolerance study was conducted in New Zealand White rabbits in NDA 206,538 with 300 IU HOE901/mL (Toujeo®), and marketed formulation containing 100 U HOE901/mL (Lantus®). The test solutions and saline were injected SC, PAV (0.1 mL) or IV, IM, PAV (0.5 mL) with normal saline (9 mg/mL) as control. 20% glucose was injected SC to prevent hypoglycemia. Similar microscopic findings, minimal to moderate hemorrhage and/or reddening were observed in all groups, suggesting that the reactions were only injection related. Thus, both formulations of insulin glargine were tolerated.

11 Immunotoxicity

New Zealand white rabbits and Guinea pigs were injected subcutaneously with 40 µg and 20 µg, respectively to test for antibody formation. Immunization was performed for a total of 26-weeks, weekly for 13-weeks and biweekly for the rest of the experiment. The level of antibody production in rabbits was the lowest for HOE901 compared to human, porcine or bovine insulins. Guinea pigs on the other hand developed antibody against all insulins (Guinea pigs are known to produce antibodies to human insulin). These studies suggested that the immunogenic potential of HOE901 is not different from human insulin.

Lymphocyte (B-cell) response to AVE0010 was assessed after oral (~20 mg/kg) and subcutaneous (~2 µg/kg) administration to BALB/cJ mice. Antibody formation was tested by ELISA before dosing of AVE0010 as well as 5 and 9 weeks after initiation of dosing. Antibodies were formed in both cases, with an increased titer in mice receiving the subcutaneous dose. For T-cell response, BALB/cJ mice were treated with AVE0010 and Freund's complete adjuvant (FCA) or AVE0010 and PBS for 10 days and lymph nodes were removed and the cells stimulated with AVE0010, GLP-1, tuberculin purified peptide or PBS. Both AVE0010 and GLP-1 stimulated T-cell proliferation from mice primed with AVE0010 in FCA, but not T-cells primed with AVE0010 in PBS or FCA alone. At very high concentrations of AVE0010 (>5 µM) and GLP-1 (>45 µM) both peptides induced a decrease in the proliferative T-cell response.

As discussed in **page 41,** antibodies against AVE0010 form in rodents, dogs and humans, and are responsible for accumulation of total AVE0010 after repeat dosing **(Table 24).** In the chronic dog study, a 500-fold increase in dose between 2 and 1,000 µg/kg BID resulted in 1,500-fold increase in exposure at ≥ 6-months.

Table 24: Anti-AVE0010 antibody formation and systemic exposure

Species	Dose (µg/kg BID)	$AUC_{0\text{-}24\ h}$ (ng.h/mL)		Fold accumulation
		Initial exposure	Terminal exposure	
Mouse[§]	200	169	344	2
	1000	857	8251	10
	2000	1540	16695	11
Rat[£]	40	68.2	7455	110
	200	255	35550	139
	1000	843	39250	47
Human[¥]	Dose (QD)	$AUC_{0\text{-}4.5h}$ (ng.h/mL)		Fold
		AB^{-ve}	AB^{+ve}	
	5 µg	0.09	1.097	12
	10 µg	0.175	2.402	14
	20 µg	0.298	0.949	3
	30 µg	0.406	4.032	10

[§]Mean TK data for male and female mice from a 13-week study, days 2/1 AUC was compared with days 93/92 (Study # 2005-0443).
[£] Mean TK data from male and female rats from carcinogenicity study (day 4 and day 359 were compared).
[¥]Geometric mean of AUC values obtained from antibody negative and positive subjects in 13-week study with T2DM patient (Study DRI6012); Table 30, Module 2.7.2).

12 Special Toxicology Studies

12.1 Mechanistic studies in thyroid cells and tissues

There are warnings for long-acting GLP-1 receptor agonists concerning thyroid C-cell hyperplasia and neoplasia in rodents with undetermined relevance to humans. Increases in the incidence of thyroid C-cell adenomas were noted in mice and rats with lixisenatide, and there was a numerical increase in thyroid C-cell carcinomas at high multiples of the highest lixisenatide daily dose of 20 µg in the fixed dose combination.

In vitro mechanistic studies have been conducted to further characterize the potential neoplastic effects of lixisenatide. These studies examined GLP-R expression in various tissues and species, as well as effects on proliferative genes in calcitonin-expressing thyroid cells. Many of these studies have been reviewed by Dr. Tim Hummer in NDA 204,961 for lixisenatide; there are also brief discussions of these studied in **page 70**. Studies that examined the relative expression of GLP-1R and calcitonin in rat thyroid c-cells vs. thyroid follicular cells; and measurement of the relative activation of rodent (6-23) and human thyroid c-cell lines(C-cell line TT) with GLP-1R agonists, or GIP receptor agonist have been reviewed.

GLP-1 receptor mRNA expression was observed in C-cells but may not be expressed in follicular cells although there was no definitive answer. No difference in mRNA expression was noted for calcitonin or GLP-1R between 4-month and 12-month old rats in either the C-cells or follicular cells.

Receptor activation was assessed through measurement of cAMP formation in 6-23 and C-cell line TT with concentrations of 10 pM to 1µM of lixisenatide, exendin-4, liraglutide, or a GIP receptor agonist. The E_{max}% for the GLP-1R agonists was the same, but the relative potency (relative EC_{50}) in decreasing order for 6-23 cell line was: exendin-4~ lixisenatide ~ GLP-1(7-36) >> liraglutide >GIP. The E_{max}% for the TT cell line was lower for all GLP-1R agonists, and relative potency was not calculated because of poor curve fitting. Nonetheless, it appears that GLP-1R activation appears to favor the rat cell line.

Study reports addressing similar issues have been submitted for the insulin glargine and lixisenatide combination.

[Study # divt0064]: In vitro potency of insulin glargine, lixisenatide, as well as mixtures thereof in the rat thyroid carcinoma cell line RTC 6-23 (clone 6)

[Study # divt0065]: Influence of the combination treatment with lixisenatide and insulin glargine on the process of apoptosis in rat thyroid carcinoma cell line RTC 6-23.

The potential interaction of the two compounds at the GLP-1R was compared by measuring test-article induced cAMP formation in the rat thyroid carcinoma cell line RTC 6-23 (clone 6). Dose-response relationship of AVE0010 was measured in the presence of fixed concentrations of Lantus U100 (1 µM, 1 nM, 1 pM, 1 fM or 1 aM) and the resulting data compared with the corresponding data from dose response analysis of AVE0010, as well as insulin glargine alone (See sponsor provided **Table 25**). Geometric mean EC_{50} values for AVE0010 in the presence of different concentrations of insulin glargine were remarkably similar to EC_{50} value obtained for AVE0010 alone, except at the highest insulin glargine concentration, where a drop in EC_{50} by approximately 25% occurred. This concentration would not be achieved at the highest proposed clinical dose of insulin glargine.

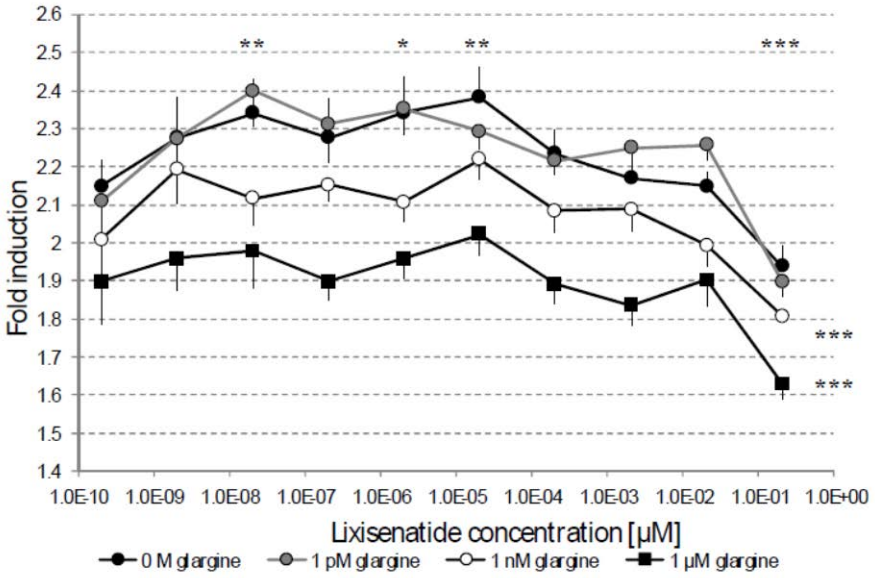
Table 25: Effect of insulin glargine on cAMP formation induced by lixisenatide in rat thyroid carcinoma cell line

Combination	Reference	Ratio of EC50 values (combination vs. reference)	
		Estimate	90% confidence interval
AVE0010 + 1 µM fc insulin glargine	AVE0010	0.748	[0.694; 0.807]
AVE0010 + 1 nM fc insulin glargine	AVE0010	0.965	[0.895; 1.040]
AVE0010 + 1 pM fc insulin glargine	AVE0010	0.930	[0.863; 1.003]
AVE0010 + 1 fM fc insulin glargine	AVE0010	0.958	[0.888; 1.033]
AVE0010 + 1 aM fc insulin glargine	AVE0010	0.883	[0.819; 0.953]

Mixed model on log-transformed EC50 with experiment considered as random effect

The sponsor also evaluated the modulatory effect of lixisenatide in TNF-α mediated induction of apoptosis in RTC 6-23 cells by measuring caspase 3/7 activation. Lixisenatide did not alter the TNF-α effect when tested up to 206 nM. The addition of medium concentration (1nM) and high concentration (1µM) of insulin glargine resulted in a weak but dose dependent and statistical significant alterations in apoptosis.

Table 26: Effect of insulin glargine and lixisenatide on apoptosis of thyroid cell line



* p<0.05, ** p<0.01, *** p<0.001:Two-way ANOVA with factors glargine dose and lixisenatide dose applied on parameter fold induction followed by Dunnett's test of glargine dose groups versus control (no glargine applied) and followed by Dunnett's test of lixisenatide dose groups versus control (smallest lixisenatide dose of 0.206 fM).

Notice: The indicated significances reflect the differences of the main effect for glargine and lixisenatide respectively because the interaction between glargine and lixisenatide is not significant (p=0.9979).

[Study # divt0071]: Influence of the combination treatment with lixisenatide and insulin glargine on the process of apoptosis in the human pancreatic beta cell line 1.1 B4.

This study tested whether exposure of human 1.1B4 pancreatic beta cell line to insulin glargine and lixisenatide would modulate apoptosis induced by the proinflammatory cytokine combination of TNF-α / IL-β and INF-γ. Three concentrations of insulin glargine (1pM, 1nM and 1µM) in combination with 8-9 concentrations of lixisenatide were analyzed. Apoptosis was measured and quantified by caspase 3/7 activation.

Lixisenatide caused dose-dependent reduction in apoptosis (IC_{50} = 3.81 pM) when added alone, or in combination with insulin glargine. However, insulin glargine alone did not alter capsase activity induced by the pro-inflammatory cytokines. The findings suggest the absence of interaction between the two drugs on anti-apoptotic effect in 1.1B4 pancreatic beta cells following treatment with pro-inflammatory cytokines.

Figure 21: Inhibition of apoptosis by lixisenatide in pancreatic cell line (sponsor figure; data from 1 out of 3 experiments)

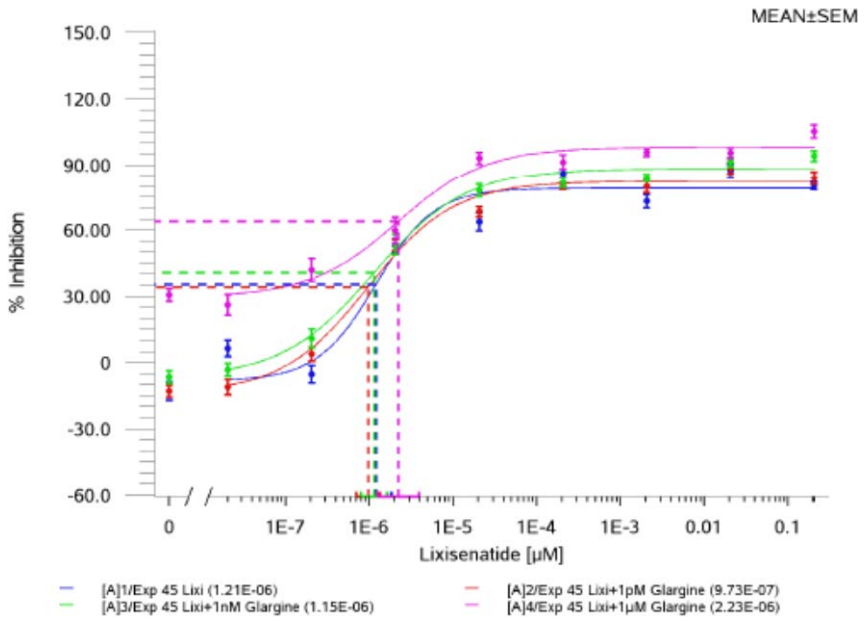


Table 27: Absence of insulin glargine effect on the apoptosis of pancreatic cell line by lixisenatide

Combination:	Reference:	Ratio of IC_{50} values (combination vs. reference)	
	Ratio	90% confidence interval	
Lixisenatide + 1 pM insulin Glargine	Lixisenatide	1.124	[0.871; 1.451]
Lixisenatide + 1 nM insulin Glargine	Lixisenatide	1.093	[0.847; 1.411]
Lixisenatide + 1 µM insulin Glargine	Lixisenatide	0.906	[0.702; 1.170]

Mixed model on log-transformed IC50 with plate considered as random effect.

[Study # divt0072]: Influence of the combination treatment with lixisenatide and insulin glargine on proliferation in human pancreatic beta cell line 1.1 B4

The mitogenic potential of lixisenatide alone and in combination with insulin glargine was tested in the human pancreatic beta cell line 1.1B4, a cell line expressing both GLP-1 and insulin receptors. ^{14}C-Thymidine incorporation was measured for the test-articles and recombinant human insulin and recombinant human IGF1 as references. Lixisenatide alone did not increase thymidine incorporation, but insulin glargine, IGF1 and human insulin promoted thymidine incorporation in a dose-related manner. The mitogenic potential of insulin glargine was not significantly altered by lixisenatide. Note that this study and others have indicated that the mitogenic potency of insulin glargine is greater than that of the human insulin.

Compound	EC_{50} geometric mean [nM]	95% confidence interval [nM]
Human insulin	9.41	[6.38; 13.9] (n = 4)
IGF1	0.192	[0.175; 2.12] (n = 4)
lixisenatide	-	- (n = 4)
Insulin glargine	0.996	[0.808; 1.23] (n = 4)
Insulin glargine + 10 nM lixisenatide	0.775	[0.630; 0.953] (n = 4)

Mixed model on log-transformed EC50.

Toxicological end points and the expression of proliferative genes, calcitonin and GLP-1R were evaluated in a mouse (CD-1) study (TXC 1492). A 2,000 µg/kg/d dose of AVE0010 was continuously infused subcutaneously (20 mg/mL; 0.10 µL/h) via osmotic minipumps for 12-weeks. Body weight of animals was reduced, but no macroscopic and microscopic changes were noted in the thyroid gland. AVE0010 treatment did not alter the expression of 7 growth regulatory genes (cyclin D1, D2 and D3, c-myc, Bcl-XL, CDKN1A, CDKN1B) in thyroid cells. The expression of GLP-1 receptor and calcitonin in thyroid was increased 5-fold in males and 2- to 3-fold in females receiving the test-

article. Immunohistochemical assessment of thyroid tissue using calcitonin and Ki-67 double staining did not reveal an increase calcitonin positive (C-cells) that were undergoing replication (Ki-67 positive).

Another 12-week infusion mouse study (Study # TXC 1505) was conducted subcutaneously with AVE0010 or exenatide, both given at the same dose and infusion rate (75 ug/kg/d; 28.41 mg/mL/0.1 µL/h). The parameters evaluated included mortality, clinical signs, body weight, food consumption and serum calcitonin. Plasma samples for toxicokinetic determinations were obtained on days 10 and 84. Additionally, one lobe of the thyroid gland from 15 or 14 animals per group/sex was used for expression analysis of proliferation markers, GLP-1R and calcitonin genes by PCR.

Marginal body weight loss associated with loss in food intake was noted in animals receiving both agents in the first week. Female animals gained weight comparably to controls, while male weight gain was only partially recovered by day 71. On day 84, mean serum calcitonin levels were increased by approximately 3-fold for groups receiving AVE0010 or exenatide. No changes in C-cell histology or cell number were detected for treated groups when evaluated microscopically by H&E staining or calcitonin immunohistochemistry, or when assessed histomorphometrically. Gene expression analysis showed a treatment-related increase in the number calcitonin and GLP-1R mRNA transcripts in thyroid cells. No meaningful differences in the expression of proliferative genes were observed between treated and control groups, which is consistent with an apparent lack of C-cell proliferation based on histopathology.

Exposure of both AVE0010 and exenatide increased between day 10 and day 84. It is uncertain whether the development of anti-drug antibodies may have been partially responsible for the apparent drug accumulation.

Thus, in mouse calcitonin secretion and GLP-1R are upregulated after sustained infusion of lixisenatide and exenatide for longer time. However, there are no indications that the prolonged activation of the GLP-1R initiates a proliferative effect.

12.2. Gene expression and receptor profiling studies

These studies were submitted in NDA 208,471 listed as “other studies, under eCTD section 4.2.3.4.3. Some of these studies have been reviewed under NDA 204,961, and under section 13.1 above. The summaries below are from selected study reports and the reviews by Dr. Hummer.

GLP1-receptor expression was assessed in various human organs and tissues. The results show that the receptor was detected at low to very low levels in most peripheral tissues. It was undetectable in aorta, skeletal muscle and vas deferens. The highest expression was found in the heart and pancreas. In brain, GLP-1R mRNA was undetectable in thalamus, low in caudate nucleus, corpus callosum, nucleus basalis, hypothalamus, nucleus accumbens, globus pallidus and putamen, and was very low in all other regions (see Dr. Hummer’s review for details).

GLP-1R and calcitonin expression testing in human thyroid tissue (Study # DIV1498) has suggested that calcitonin mRNA was expressed in C-cell fractions but may not be expressed in follicular cell fractions. GLP-1R mRNA could not be detected in C-cell fractions or follicular cell fractions of the human thyroid gland samples evaluated in this study. This may be due to low level of expression or due to methodological limitations. Another study (# DIV 14126) with frozen thyroid tissue also showed calcitonin expression in the C-cell fractions, but not in follicular fractions. GLP-1R expression was low, of the total of 5 thyroid glands, 1/5 of follicular and 2/5 of C-cell fractions showed marginal expression of GLP-1R mRNA.

The potential co-expression of GLP-1R with INSR-A, INSR-B, and IGF-1R was also assessed in normal pancreatic, thyroid, lung, stomach and tumors from the same tissues of humans (study # DIV 1404). A total of 140 diseased tissue samples, including 1 pancreatitis sample and 139 cancer samples derived from kidney (8), pancreas (21), stomach (9), lung (57), thyroid (13), and colon (32) were tested. A concomitant increase in both GLP1-R expressions together with INSR-A, INSR-B, or IGF-1R was only detected in one kidney carcinoma (increased expression of INSR-A and GLP-1R). In this tumor, GLP-1R expression was in the low to very low range. Overall it was concluded that there was no evidence of a general concomitant increase in GLP1-R together with IGF1R or one of the two insulin receptors in the tumor tissues examined.

GLP1-R was found to be expressed at the same or a lower level than in corresponding normal tissue in lung cancers, colon cancers, pancreatic adenocarcinomas and carcinomas, in a pancreatitis sample, in stomach adenocarcinomas, and in thyroid adenomas. Significantly higher expression of GLP1-R than in normal tissue was detected in 1 untyped pancreatic tumor (medium level, 1/20 tumors), in 3/8 thyroid carcinomas (low, low, and medium level), and in 1/8 kidney carcinomas (very low expression level). Overall, there was no apparent correlation between GLP1-R expression levels and neoplasia.

12.3. Impurity qualification and container closure systems

12.3.1 Qualifications of Impurities in Insulin Glargine

Study title: HOE901: 13-week subcutaneous toxicity study in rats for impurity qualification

Study no.:	8305094
Study report location:	Testing site
Conducting laboratory and location:	(b) (4)
Date of study initiation:	04-Jul-2014
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Standard/stressed HOE901 [batch 2F005 (C1021143)] and 98.3%/93.5%

Key Study Findings

This study compared the toxicity profiles of diluted sample of the marketed product (HOE901-U300 formulation) and stressed formulation of HOE901-U300 enriched in impurities/degradation products. The same doses (8 U/kg/day) were administered subcutaneously daily to Sprague-Dawley rats for 13-weeks. An additional group received the vehicle only and served as negative controls. The toxicological profile of the stressed product with total impurity of (b) (4)% and purity of 93.5% was not significantly different from that of the product in the market ((b) (4)% total impurity, 98.3% pure).

Methods

Doses:	8 U/kg/day
Frequency of dosing:	One daily
Route of administration:	Subcutaneous
Dose volume:	1 mL/kg
Formulation/Vehicle:	
Species/Strain:	Rat Crl:SD(SD) (b) (4)
Number/Sex/Group:	10
Age:	6-7 weeks
Weight:	180-259 g (♂) 134-195 g (♀)
Satellite groups:	No
Unique study design:	No
Deviation from study protocol:	None

Observations and Results

Table 28: Test Articles in Impurity Qualification Study with Insulin Glargine)

			Animal /Cage numbers			
			Male n=10		Female n=10	
Group number	Group description	Dose level (U/kg/day)	Animal Numbers	Cage Numbers	Animal Numbers	Cage Numbers
1	Control	0	1-10	1-2	101-110	7-8
2	Standard HOE901	8	11-20	3-4	111-120	9-10
3	HOE901 stressed formulation[a]	8	21-30	5-6	121-130	11-12

(b) (4)

Table 29: Insulin Glargine Placebo Solution in Impurity Qualification study

Components:	Concentration (mg/mL):
Glycerol	20.0
M-Cresol	2.7
Water for injection	-
Hydrochloric acid (for pH adjustment)	-
Sodium hydroxide (for pH adjustment)	-

Mortality

Mortality was not reported.

Clinical Signs

Clinical examination was done after 2 hrs of dosing, and in cage observation was made twice daily. Red staining on the necks of animals, with increased incidence in female animals was noted. This finding is judged as non-treatment related by the sponsor since it was also noted in one control male animal.

Body Weights

Body weight was recorded before treatment, on the first day of dosing, and weekly until week 13; weight was recorded twice weekly after week-13. No test-article effect on male animals, but body weight gain was marginally decreased in females receiving the standard HOE901 relative to control. This appears to be due to reduced feed consumption.

Feed Consumption

Food intake was recorded weekly. Male animals consumed comparable amounts of feed to controls, but females on the standard HOE901 had marginal reduction (-11%) in food intake compared to control.

Ophthalmoscopy

Indirect ophthalmoscopy and slit lamp examinations were performed prior to treatment and week 13. No finding.

Hematology and Coagulation tests

Samples for hematology, coagulation and clinical chemistry were collected 2 hrs post dose on day 92 or 93 with urine collection commencing on the previous day. Food and water were not withheld prior to obtaining blood samples or before urine collection. All the required erytrocytic parameters, platelet count and volume, and total leucocyte and differential counts were measured. Prothrombin time, activated partial thromboplastin time and fibrinogen were measured as coagulation parameters. Bone marrow smears were prepared, but were not evaluated.

Total and differential leukocyte numbers were low in female animals receiving both the standard and stressed insulin glargine doses. Reductions were noted in male animas in the number of neutrophils and monocytes (**Table 30** below). The applicant has concluded that the average values in these animals are within the historical ranges without providing the data. There were no test article-related effects on coagulation parameters.

These findings were not reported up to 1.455 mg/kg HOE901 dose in the 3-month rat study submitted in NDA 21,081. Clotting time and reticulocyte counts were however decreased in male animals in a dos-related manner in the chronic rat study that tested

doses of up to 0.73 mg/kg HOE901. Erythrocyte count and hematocrit were low in females at the HD.

Table 30: Hematological changes in rats in impurity qualification study for Insulin Glargine *(% change compared to controls)*

Gender	Male		Female	
Test item	**Standard HOE901**	**Stressed HOE901**	**Standard HOE901**	**Stressed HOE901**
Hemoglobin	2	NC	1	4*
WBC	-11	-22	-42**	-34*
Neutrophils	11	5	-54	-31
Eosinophils	-8	-22	NC	-27
Lymphocyte	-18	-29	-41	-36
Monocytes	33	17	-10	-6
Basophils	-24	-21	-45	-64
Large Unstained cells	-5	-37**	-46	-65

NC = no change. ** P≤0.01; * P≤0.05 are statistically significant relative to control mean.

Clinical Chemistry

The clinical chemistry parameters listed below were measured.

Aspartate aminotransferase	Total protein
Alanine aminotransferase	Albumin
Alkaline phosphatase	Globulin
Creatine kinase	Albumin/globulin ratio
Sodium	Total cholesterol
Potassium	Glucose
Calcium	Blood urea nitrogen
Inorganic phosphorus	Total bilirubin
Chloride	Creatinine
Triglycerides	Gamma Glutamyl Transferase

Parameters that showed difference from samples obtained from control animals are shown in **Table 31** below. In general, similar results were reported from animals dosed with the standard and stressed samples. The sponsor has submitted that the magnitudes of changes, except for lower glucose are within the historical control values. The conclusion about the absence of differential effect on clinical chemistry parameters between the stressed and market product is upheld in this review. It is to be noted that similar results were not reported in toxicology studies conducted in rats with insulin glargine, perhaps because of the relatively lower dose evaluated in the studies supporting NDA 21,081(Review by Dr. Herman Rhee, January 19, 2000). The observed decline in glucose and triglycerides are expected PD effects. Changes in the concentration of serum electrolytes are very small, thus may not have toxicological significance. Total bilirubin is significantly decreased in females, but the finding may not have clinical significance in light of the absence of clinically significant changes in

transaminases. Comparable data were reported for the two samples for other clinical chemistry parameters.

Table 31: Clinical Chemistry Changes in Rats in Impurity Qualification study for Insulin Glargine *(% change compared to controls)*

Gender	Male		Female	
Test item	**Standard HOE901**	**Stressed HOE901**	**Standard HOE901**	**Stressed HOE901**
Glucose	-66***	-65***	-58**	-73***
Triglycerides	-32	-32	-46*	-55**
ALT	14	9	-24	-29
AST	11	15	-16	-28
ALP	1	1	NC	3
Urea	22**	23**	14*	23**
T bilirubin	-20	-8	-31***	-41***
T protein	3	-2	-7**	-3
Globulin	2	-3	-11**	-1**
Creatinine	-6	-10**	-2	-7
Sodium	2***	1*	2***	3***
Potassium	-5	-4	-7	-15
Chloride	1	1	2*	2**
Calcium	-2	-3	-4	-6
Phosphorous	-11	-9	-23	-25

NC= no change; ***P≤0.05; ** P≤0.01; * P≤0.05

Urinalysis

Urine parameters listed below were determined:

Volume	pH[1]
Glucose[1]	Blood[1]
Colour	Protein
Osmolality	Appearance (Clarity)
Bilirubin[1]	Urobilinogen[1]

1 determined semi-quantitatively.

There was decrease in urine volume in male animals receiving the standard (-34%) and stressed (-11%) product, corresponding increase (+34%) in urine osmolality was noted in males receiving the standard. Similar changes were not reported for female animals. Decline in urinal protein concentration was noted in both groups in females.

Gross Pathology

On days 92 and 93 animals were weighed before terminal sacrifice. A necropsy was performed and organ weights were recorded, tissues were fixed and microscopically examined as provided in the sponsor **Table 32** below.

The only macroscopic finding was increased incidence of uterus distension in females receiving the test articles. However, there were no corroborative microscopic findings.

Table 32: List of tissues processed and examined postmortem

Tissue / organ				Tissue / organ			
Adrenals (x2)	P	W	E	Nasal cavity (x1) (inc Zymbal glands)	P		
Aorta (x1)	P		E	Oesophagus (x1)	P		E
Brain (x6)	P	W	E	Optic nerves (x2)	P		E
Caecum (x1)	P		E	Ovaries (inc. oviducts) (x2)	P	W	E
Cervix (x1)	P		E	Pancreas (x1)	P		E
Colon (x1)	P		E	Pituitary (x1)	P	W	E
Duodenum (x1)[6]	P		E	Prostate (x1)	P	W[2]	E
Epididymides (x2)	P[1]	W	E	Rectum (x1)	P		E
Eyes (x2)	P[1]		E	Salivary glands - mandibular (x2), sublingual (x2), parotid (x2)	P		E
Femur with bone marrow	P		E	Sciatic nerve (x2)	P		E
Gut-associated lymphoid tissue (GALT/Peyer's patch) (x1)	P[3]		E	Seminal vesicles (x2)	P	W[2]	E
Harderian glands (x2)	P		E	Skin/subcutis (x1)	P		E
Head (not processed)	P			Spinal cord cervical (x1)	P		E
Heart (x1)	P	W	E	Spinal cord thoracic (x1)	P		E
Ileum (x1)	P		E	Spinal cord lumbar (x1)	P		E
Injection sites (variable)[4]	P		E	Spleen (x1)	P	W	E
Jejunum (x1)	P		E	Sternum with bone marrow (x1)	P		E
Joint, Femoro-tibial (x1)	P		E	Stomach (x1)	P		E
Kidney (x2)	P	W	E	Testes (x2)	P[1]	W	E
Lacrimal glands (x2)	P		E	Thymus (x1)	P	W	E
Larynx (x1)	P			Thyroids (x2) + parathyroids (x2)[7]	P	W[5]	E
Liver (x 3)	P	W	E	Tongue (x1)	P		E
Lungs, with bronchus (x2)	P	W	E	Trachea (x1)	P		E
Mammary gland (x 1)	P		E	Ureters (x2)	P		E
Mandibular lymph nodes (x1)	P		E	Urinary bladder (x1)	P		E
Mesenteric lymph nodes (x1)	P		E	Uterus: body (x1) and horns (x2)	P		E
Muscle (diaphragm) (x1)	P		E	Vagina (x1)	P		E
Muscle (quadriceps) (x1)	P		E	Animal identification	P		
				Gross lesions (variable)	P		E
Legend:							
P = Tissues preserved; W = Organs weighed; E = Tissues processed and examined microscopically							
(xN) = number of sections for histopathology							
Footnotes:							
1: Tissue taken into Davidson's fixative							
2: Prostate gland and seminal vesicle gland weighed together							
3: Gut associated lymphoid tissue was included in section of ileum or jejunum							
4: Last dose site preserved in all animals plus site(s) with macroscopic abnormalities.							
5: Weighed after fixation							
6: Including pylorus							
7: Only one side required on slide							
Additional information:							
Left and right organs will be weighed together							
Lungs to include mainstem bronchi and bronchioles							
Bone tissue designated for histopathological examination will be decalcified using Kristenson's fluid							

Organ Weights

The weight of liver in female animals was statistically significantly decreased; -18% and -23% reduction occurred in HOE901 and stressed HOE901 dosed female animals, not in males.

Histopathology

Adequate Battery

Yes

Peer Review

Yes, by another pathologist in (b) (4)

Histological Findings

The sponsor has reported liver vacuolation in males that it considered are due to glycogen depletion (see **Table 33** provided by the sponsor). This may not be related to the test articles since the incidence is equally higher in vehicle receiving animals, and because this finding is not consistent with the pharmacodynamic actions of insulin analogs. Another microscopic finding in all groups was the lesions at the injection sites. These are related to injection, instead of the reaction due to the agents since it was noted in all groups. The small increase in the incidence of hemorrhage, inflammation and necrosis with the stressed HOE901 in male animals may not be toxicologically significant in light of the finding occurring in all groups.

Table 33: Microscopic findings in rat impurity qualification study with insulin glargine *(top glycogen vacuolation in liver, bottom incidences at the injection site)*

Sex	Males			Females		
Treatment	Vehicle	Stressed HOE901	HOE901 stressed batch	Vehicle	Stressed HOE901	HOE901 stressed batch
Dosage (U/kg/day)	0	8	8	0	8	8
Animals per group	10	10	10	10	10	10
Not present	1	2	0	4	10	9
Present	9	8	10	6	-	1
Grade 1	4	5	6	3	-	1
Grade 2	3	3	4	3	-	-
Grade 3	2	-	-	-	-	-

- = Not observed; Figures denotes number of affected animals per group.
Grades: 1= minimal, 2= slight, 3 = moderate.

Sex	Males			Females		
Treatment	Vehicle	Stressed HOE901	HOE901 stressed batch	Vehicle	Stressed HOE901	HOE901 stressed batch
Dosage (U/kg/day)	0	8	8	0	8	8
Animals per group	10	10	10	10	10	10
Hemorrhage	5	6	8	6	3	5
	(2-3)	(1-3)	(1-4)	(2-3)	(1-3)	(2-3)
Inflammation	6	7	9	7	5	4
	(1-2)	(1-3)	(1-2)	(1-3)	(1-2)	(1-3)
Myopathy	2	-	1	2	1	-
	(1)		(3)	(2-3)	(1)	
Necrosis	5	5	6	1	-	2
	(2-3)	(2)	(2)	(3)		(1-2)
Fibrosis	3	4	3	1	2	1
	(2)	(1-2)	(1-2)	(2)	(1)	(2)

Figures denotes number of affected animals per group; Numbers in ( ) = range of severity grades.
Grades: 1= minimal, 2= slight, 3 = moderate, 4= marked.

Special Evaluation

None

Toxicokinetics

Not applicable

Dosing Solution Analysis

Stock solutions were supplied by the sponsor, and the stability testing and concentration verification was done by the sponsor. The certificate of analysis shows that the Standard HOE901 was 10.72 mg/mL, corresponding to 294.8 U/mL (98.3%) and the HOE901 stressed formulation was 10.20 mg/mL, corresponding to 280.4 U/mL (93.5%).

12.3.2 Qualifications of Impurities/Degradation Products in Lixisenatide

Two toxicology studies of 2-week duration were conducted in rats with forced degradation products (studies TSA1242 and TSA1331 in NDA 208,471). Moreover, a subcutaneous impurity qualification toxicology study of 3-month duration was conducted in rats with a stressed formulation of AVE0010 at 200 µg/kg BID (study TXC 1482). For this study, a lixisenatide solution for injection with the dosage strength (b) (4) µg/mL was stored (b) (4) to obtain elevated levels of the degradation products. The solution (batch AVE0010_10_1232) contained the following amounts of individual AVE0010-related degradation products:

(b) (4)

Dr. Tim Hummer has reviewed the data and concurred with the sponsor after risk assessment relative to the 20 µg daily clinical dose (see **Table 34** below) in NDA 204,961.

Table 34: Qualifications of Drug AVE0010-related degradation Products and Impurities (reproduced from nonclinical review for NDA 204,961)

AVE0010 Batch Number	Area % Total Impurities	(b) (4)						Tested in:
1022/3330	(b) (4)	-	-	-	-	-	(b) (4)	Seg 2 genetox
PPL-AVE-100401		-	-	-	-	-		Seg 1
PPL-AVE100402A		-	-	-	-	-		Ames
PPL-AVE100404A		-	-	-	-	-		12 month dog
PPL-AVE100404B		-	-	-	-	-	-	6 month rat
PPL-AVE100501A		(b) (4)	-	-	-	-	-	Mouse & rat carc
B002			-	-	-	-	-	Mouse & rat carc
B004			-	-	-	-	-	Mouse & rat carc Juvenile rat Juvenile dog
AVE0010_10_1209		-	(b) (4)					3 month rat***
AVE0010_10_1232*		-						3 month rat***
Amount tested in toxicology studies							(b) (4)	
Shelf life criteria								
Maximum amount of impurity at clinical dose**								

* Forced degradation batch of AVE0010_10_1209.
** Based on shelf life criteria.
***Study TXC1482 (13-week SC toxicity study in rats for impurity qualification).
† Calculated based on the percentage of this impurity in the batches used in the rat carcinogenicity study, which utilized a high dose of (b) (4)
‡ Calculated based on the percentage of this impurity in the batch used in the 3-month rat study using the forced degradation batch, which utilized a high dose of (b) (4).
# Calculated based on the percentage of this impurity in the batch used in the 12-month dog study, which utilized a high dose of (b) (4).

The sponsor has set the limit for release of total lixisenatide-related impurities and degradation products at (b) (4) % (b) (4) The shelf life limit for total degradation products is set to (b) (4) % (b) (4)

12.3.3. Container closure system - Disposable pen-injector

The two different strength of the fixed dose combination of insulin glargine and lixisenatide are to be delivered by prefilled, disposable, manual pen-injectors. A 3 mL colorless cartridge made of type I glass with rubber cap on both sides is in contact with the drug product. So are (b) (4) (b) (4) I rubber (b) (4) plunger stopper. (b) (4) . The applicant has submitted that the pen-injector is based on the already marketed SoloStar® pen injector, which is combined with insulin glargine 100 U/mL solutions for injection (Lantus®, NDA 21-081). The modifications are reported to be colors of the bodies, caps, injection buttons, dose selectors and the scaling on the number sleeves. Extractable studies of the packaging material were performed by (b) (4) .

(b) (4) . The study report references a 1- and 2-year rat study with a proposed NOEL of (b) (4) mg/kg/day. (b) (4) is also negative in the in vitro and in vivo mutagenicity studies.

(b) (4)

Table 35: Methods for extractable study of packaging

Material	Solvent	Treatment	Analyses
(b) (4)			Atomic absorption
			GC-MS, HPLC-MS, ion chromatography

Table 36: Solvents and conditions for packaging extractable study (reproduced from the sponsor)

Solvent	Conditions	Concentration
(b) (4)		

Table 37: Summary of NVOC compounds detected in extractables

(b) (4)

Accelerated studies were conducted to identify leachables in the primary packaging system (b) (4)

(b) (4)

The (30-60 U) prefilled disposable pen-injectors will be used to deliver 0.6 mL per day for the highest proposed daily dose of insulin glargine (60 U) and lixisenatide (20 µg). The highest leachable reported in the study is (b) (4) µg/mL; the 0.6 mL drug product is

estimated to contain a maximum amount [redacted] µg/day. This level is [redacted] µg/day dose that is considered to be of negligible life-time carcinogenic risk.

Table 38: Summary of detected compounds identified as leachables*

Leachable	Concentration		Safety considerations
Compound	t = 0 [µg/L]	t = [redacted]	
[redacted]	n.d.	[redacted]	[redacted]
	n.d.		
	n.d.		
	n.d.		
	n.d.		
Unidentified	n.d.		Not considered a safety risk to the patients based the safety threshold of [redacted] µg/day
Unidentified	n.d.		
Unidentified	n.d.		
Unidentified	n.d.		
Unidentified	n.d.		
Unidentified	n.d.		

[redacted]

Summary and conclusions on impurity qualifications

Even though the method used to stress the standard formulation was not described in the study report, the ratios of total degradation products and high molecular weight proteins (HMWP) reported were [redacted] in the stressed formulation with HOE901 as shown in below compared to the empirically determined acceptance values. Diluted standard formulation of HOE901-U300 was compared with a stressed formulation of HOE901-U300 from the same batch in a 3-month rat study, administering 8 U/kg/d. Dose selection for this study was made based on previous subcutaneous studies that showed mortality due to hypoglycemia at ≥ 12.5 U/kg/d, but with a NOAEL of 6.3 U/kg/d.

Table 39: Degradation Products in Insulin Glargine

Insulin glargine Batch	Largest single	Total	High Mol.	pH

Number	degradation product	degradation product	Proteins	
2F005 (Unstressed)	(b) (4)			
2F005 (Stressed)				
Acceptance values				

The toxicological profile of the two samples was comparable, despite the increased level of product related impurities/degradants in the stressed formulation.

The stability of several batches of both strengths of insulin glargine/lixisenatide, covering in-use storage at +30°C up to 14 days was tested. Two batches filled in 3 mL cartridges were tested after 5- and 11-month of storage. In addition, for both strengths each, one batch was assembled in the commercial pen and tested for in-use stability after 5-month storage. Statistical evaluation of the results obtained confirms a mean increase of (b) (4)% for both formulations to be considered for the calculation of the shelf life limit. The extent of degradation and release limits are summarized in the sponsor provided **Table 40** and **Table 41** below.

Table 40: Degradation Products for Insulin Glargine during Manufacturing and Storage

(b) (4)

Table 41: Lixisenatide-Related Degradation Products during Manufacturing and Storage

(b) (4)

After stressed degradation of both strengths of insulin/lixisenatide solution for injection (b) (4), total impurities increased by about (b) (4)%. (b) (4) degradation products (b) (4) 1%) related to insulin glargine were found in

each of the formulations. Stability testing of insulin glargine/lixisenatide under normal use, and in stressful conditions, such as [redacted] produced qualitatively similar degradation products.

The insulin glargine-related products, [redacted] are produced by [redacted] (see sponsor provided **Table 42**). The drug substance related products [redacted] and individual degradation products were reported below [redacted]% in the formulations stored [redacted]

Table 42: Structural proposals for the degradation products/ product-related substances, storage for [redacted]

RRT	Mass	Structure
[redacted]		

Total lixisenatide-related impurities/degradation products were increased up to [redacted]%, in the stressed sample used [redacted] impurity qualification study. The sponsor has used acceptance criteria of [redacted]% for the total degradation products. The proposed release limit for individual lixisenatide-related products is [redacted]%, but the toxicological qualification level for individual degradation products was [redacted]%.

Table 43: Drug-related Degradation Products stored [redacted]

Degradation product	Acceptance criteria	Insulin glargine/ lixisenatide 100 U/mL with 33 µg/mL Amount [corrected area%, HPLC]	Insulin glargine/ lixisenatide 100 U/mL with 50 µg/mL Amount [corrected area%, HPLC]
[redacted]			

Other components of potential concern present in the daily dose formulation were [redacted] has been previously used [redacted] in subcutaneously administered marketed products. [redacted]

13 Integrated Summary and Safety Evaluation

Sanofi Aventis has submitted an application for the marketing of a fixed dose combination of insulin glargine and lixisenatide for type 2 diabetes mellitus as an adjunct to diet and exercise. Sanofi cross-referenced the nonclinical information from the approved insulin glargine (Lantus®) and lixisenatide (NDA 208,471 under review) to support the marketing application. The sponsor also submitted limited number of studies addressing cross-receptor activation, testing for potential exacerbation of known toxicities by GLP1-R agonists or insulins, and qualification for potential impurities and degradants for the drug substances.

Insulin glargine (HOE901, Lantus®) is a human insulin analogue with primary structure modification by addition of two arginines in the C-terminal of the B-chain and replacement of asparagine at position 21of the A-chain with glycine. These modifications alter the isoelectric point of the molecule towards neutral pH resulting in a prolonged absorption from the injection site.

Insulin glargine exhibited about two-fold lower affinity for the insulin receptor compared to human insulin, but acted as a full agonist at the insulin receptor. However, the *in vitro* binding affinity of insulin glargine to the IGF-1 receptor can be up to 12-fold depending on the cell line used compared to human insulin. The glucose lowering activity of insulin glargine at therapeutic dose and its catecholamine releasing properties at high dose were not different from other marketed insulins. Intravenous clinical testing has shown that the glucose-lowering effect of insulin glargine on a molar basis was similar to that of human insulin. Nonclinical findings have suggested that the effects of insulin and insulin glargine were additive, and there is no evidence for interference of insulin glargine with the actions of human insulin.

Lixisenatide (AVE0010) is a DPP-4 resistant GLP-1 receptor agonist that is structurally related to exendin-4, a 39 amino acid peptide. Lixisenatide is a synthetic 44 amino acid (b) (4) 6 lysine residues at the C-terminus, the 44 lysine is amidated. Marketing application for lixisenatide as monotherapy (NDA 208,471) is currently under review for T2DM indication for subcutaneous daily administration of 10 µg for 14-days as initiation dose and 20 µg as maintenance dose.

In vitro receptor binding studies with lixisenatide have suggested that lixisenatide has about 4-times greater affinity than human GLP-1. Lixisenatide induced glucose-stimulated insulin secretion, promoting insulin secretion at glucose concentration of > 10 mM, but not 5.6 mM. Lixisenatide improved oral glucose tolerance in normoglycemic and animal models of diabetes, and decreased blood glucose & HbA1C in diabetic animals.

The applicant also compared the acute effects of lixisenatide to that of liraglutide, using blood glucose, glucagon, and insulin and C-peptide levels as endpoints. Lixisenatide showed superior effect on oral glucose tolerance although 50-100-times dose of liraglutide was used. Both compounds exerted comparable reduction in glucose surge & glucagon, but had no effect of insulin or C-peptide levels in blood.

In the studies addressing potential cross-receptor binding and activation, the binding of insulin glargine to the GLP-1 receptor was evaluated *in vitro* in an insulin-secreting cell line derived from pancreatic tumor (βTC6 cells). Insulin glargine at 100 nM (606 ng/mL) did not inhibit the specific radioligand binding of 25 pM [$I^{125}I$]GLP-1(7-36) to the GLP-1 receptor. Likewise, lixisenatide at 100 nM did not activate the insulin receptor (INSR), nor did it modulate the actions of insulin glargine on the receptor in CHO cells that overexpress the human INSR. Another study was conducted to test the affinity of lixisenatide to the human INSR-B, and whether it affects the affinity of insulin glargine to the receptor in CHO cell line stably transfected with human INSR-B. Lixisenatide did not show binding to the receptor and the ratio of mean IC_{50} values of insulin glargine alone and in the presence of 1 µM lixisenatide was close to 1.

Furthermore, the potential activation of Akt (PKC-B) by lixisenatide and in combination was compared in human pancreatic 1.1B4 and the rat thyroid (RTC6-23) C-cell lines,

using human insulin, insulin glargine and insulin glargine-M1 as reference. Autophosphorylation of the INSR starts a signaling cascade that activates Akt through phosphorylation at Ser^{473} and Thr^{308}. The activated AKT is known to facilitate glucose uptake in adipocytes & muscle cells by enabling translocation of GLUT4 to the plasma membrane, and inhibits PKA activation. Lixisenatide alone was not able to phosphorylate Akt in both cell lines, and did not affect the EC_{50} values for Akt phosphorylation by insulins. These studies suggested that cross receptor binding and activation by insulin glargine and lixisenatide and the potential for interactions at the canonical receptors may be low.

The lixisenatide and insulin glargine combination improved glucose tolerance in db/db mice and normoglycemic dogs better than monotherapy. Thus, it can be concluded that the premixed combination had better pharmacodynamic profile after single dose. The effect in the absorptive phase (0.5 – 1 hr) appears to be mainly due to lixisenatide, perhaps through its effect on GI motility. An effect that appears to be due to exogenous insulin was noted in the post-absorptive phase. Thus, the enhanced PD effects in the post-absorptive phase in the combination may be a reflection of the additive effect of exogenous and endogenous insulin.

Dose-dependent effects were seen during oral glucose tolerance test (OGTT) in dogs, where the return to baseline was marginally delayed for the AVE0010-treated dogs in both the absorptive and post-absorptive phases. The surge in blood glucose was suppressed in a dose-related manner when the dose of AVE0010 was escalated from 0.15-1.5 µg/kg keeping the insulin glargine at 0.3 U/kg, lasting up to 5 hrs after glucose challenge. Further improvement in glucose tolerance was noted when the insulin glargine dose in the combination was increased to 1.0 U/mL. Serum insulin was not significantly different in animals receiving AVE0010 alone compared to controls during the absorption phase, but was lower in the animals receiving the combination. Post absorptive insulin concentration was lowest in animals receiving AVE0010 & insulin glargine doses of 1.0 µg/kg/0.3 U/kg and 1.5 µg /0.3 U/kg, but was higher than the control level at 1.0 µg/kg/1.0 U/kg. Serum C-peptide mirrored the insulin values in the groups that received insulin glargine alone or in combination. However, C-peptide was higher than controls in the post-absorptive phase in the groups that received AVE0010 alone, except at the lowest dose tested (0.15 µg/kg).

Another OGGT study in normoglycemic male Beagle dogs compared the individual compounds to equal combined doses of lixisenatide (0.15 µg/kg) and insulin glargine (0.3 U/kg) injected at two different strengths (100 U/mL and 300 U/mL). The effects of the combination on blood glucose were similar to that of lixisenatide alone during the absorptive phase and to that of insulin glargine in the post-absorptive phase.

Given the C-peptide data discussed above, and based on what is known about GLP-1 receptor agonists and GI motility, it appears that both the suppression of glucose surge and maintenance of glucose level appear to be dependent on AVE0010 at the lower dose of insulin glargine. The post-absorptive effect appears to be a reflection of the additive effect of endogenous insulin due to AVE0010 and insulin glargine injection; this

is reflected in enhanced complementary effects of on blood glucose (**Figure 10**), and increased insulin in the 1.0 µg/kg/1.0 U/kg group. Since insulin level was higher in this group, the suppression of C-peptide is a reflection of the relatively higher dose of insulin glargine in this group.

In a single dose intravenous CV safety study conducted with a premixed combination of insulin glargine and lixisenatide in anesthetized dogs, serum potassium was lowered and heart rate was increased after administration of single dose (<1-times MRHD based on mg/m^2) of insulin glargine. No hemodynamic or electrical activity changes in the heart were reported in the previously reviewed toxicological studies with insulin glargine.

Insulin glargine shares similar PK profile with human insulin injected subcutaneously, except the rate of release from the injection site. Insulin glargine related activity was measured at the injection site up to 24 hrs. Metabolic studies in multiple species, including humans have suggested that insulin glargine may undergo similar biodegradation as human insulin. The main route is the successive removal of three amino acids at the carboxy-end of the B-chain of insulin glargine. The intermediate compound 21^{A}-Gly-30^{B}-L-Arg-insulin and the biologically active metabolites 21-$^{A-}$Gly-insulin (M1) and 21^{A}-Gly-des-30^{B}-Thr-insulin (M2) were recovered from human plasma and at the injection site. There is evidence from human studies that about 50% of the subcutaneous dose in the depot may be biodegraded insulin glargine.

Lixisenatide showed relatively comparable plasma protein binding in all species tested (55%, humans 49% dogs and 62% rats). After subcutaneous administration, tissue distribution was limited early to the injection site and increased within 0.25 hrs in the pancreas, renal cortex, lungs and glandular tissues. Distribution to adipose tissue, skeletal muscle, testis and the spinal cord was negligible. Lixisenatide does not seem to enter the brain. Placental transfer studies in rat and rabbits indicate a very low placental transfer (0.1% in rat and < 0.01% to 0.3% in rabbit), and a small amount of lixisenatide is secreted in milk.

Lixisenatide was extensively metabolized in all species, mainly to peptide degradants. Terminal t½ of lixisenatide ranged between 0.5 and 6.5 hrs after IV administration in animal species (mouse, rat, rabbit, dog, and pig). Terminal plasma t½ after subcutaneous dosing tended to be shorter in the smaller animals (mouse, rat) and longer in the larger species (rabbit, dog, pig), perhaps a reflection of allometric differences in absorption.

Anti-lixisenatide antibodies were formed in rodents and dogs in a dose and duration-dependent manner and resulted in accumulation of the test-article in the systemic circulation up on multiple dosing. However, results of cell-based receptor activation assays have indicated that the antibodies are not neutralizing.

Single dose PK interaction was evaluated in dogs after co-administration of 0.15 µg/kg lixisenatide with and without the application of 0.3 U/kg Lantus (HOE901) as U100 or U300 formulation. Lixisenatide exposure in terms of both C_{max} and AUC was

comparable, but the t½ was reduced after single dose administration of a premixed formulation. The reason for this change is unclear in light of the absence of differences in T_{max} values. In the second testing, the concentrations of insulin glargine and its metabolites (insulin glargine-M1 and insulin glargine–M2) were assessed in dogs after the same subcutaneous doses. Mean exposure, in terms of C_{max} and AUC was however comparable for insulin glargine-M1 after administration of insulin glargine as U100 formulation with or without co-administration of lixisenatide. The T_{max} for insulin glargine-M however varied between 0.5-8 hrs.

Toxicological studies of various durations conducted with insulin glargine in rats and dogs have suggested that its untoward effects are due to exaggerated pharmacology. Clinical signs related to hypoglycemia, such as irregular respiration, tremor, unsteady or ataxic gait, convulsions and coma have been reported. Reversible degranulation of the β-cells of the islets of Langerhans was noted in both rats and dogs, indicating a decline in insulin production due to exogenous supply. Marginal and sporadic changes in hematological parameters were also reported in rodents.

In local tolerability studies, injection site reaction and inflammation were hinted in the original review for Lantus®, but was not observed in the subsequent studies with injection of a more concentrated product of insulin glargine (Toujeo®). Antibodies were formed in rats against insulin glargine, but not in dogs. This may not be surprising given the similarity of the primary sequence of dog and human insulin compared to that of rat.

Local tolerance studies have been conducted with the pre-mixed clinical formulation and by injection of individual drug formulations on different sides of rabbits via various parenteral routes (SC, PV, IV, and IM). One of the studies evaluated a ready to use aqueous solution of 0.8 mg/mL AVE0010 and 3.6 mg/mL insulin glargine. No mortality or adverse clinical signs were noted in the study. Minimally reddened and hemorrhagic appearances were reported equally in control and test-item injected sites. Microscopic examination at the SC injection site showed minimal to mild necrosis of the panniculus muscle that regenerated after 120 hrs.

The level of antibody production was the lowest for HOE901 compared to human, porcine or bovine insulins in rabbits. Guinea pigs on the other hand developed antibody against all insulins. These studies suggested that the immunogenic potential of HOE901 is not different from human insulin. As discussed in **page 41,** antibodies against AVE0010 form in rodents, dogs and humans, and are responsible for accumulation of AVE0010 after repeat dosing **(Table 24).** In the chronic dog study, a 500-fold increase in dose between 2 and 1,000 µg/kg BID dose in females resulted in 1,500-fold increase in exposure at ≥ 6-months.

Mitogenicity assays in cell lines have suggested that insulin glargine might have a mitogenic potential through binding to the IGF-1 receptor. Testing was done in a human pancreatic beta cell line to evaluate whether lixisenatide would alter the mitogenic activity of insulin glargine, IGF1 and human insulin. AVE0010 alone did not increase thymidine incorporation, but insulin glargine, IGF1 and human insulin promoted

thymidine incorporation in a dose-related manner. The mitogenic potential of insulin glargine is greater than that of the human insulin, and was not altered by AVE0010.

AVE0010 has been evaluated for potential toxicity in rodents and dogs for various study durations. The main findings in these studies were decreased body weight gain and food consumption during the period of test-article administration in all species and gastrointestinal disturbances in dogs. The testes and epididymis in rats and dogs were also targeted at high doses of AVE0010 after repeat dosing. Moderate to severe hypo-spermatogenesis in seminiferous tubules; epididymal dilation, degeneration, oligospermia or aspermia in testis were noted at ≥200 µg/kg BID (> 5800-times exposure at the proposed clinical dose). These effects were treatment duration-dependent and reversed after 1-month of drug withdrawal. A clinical investigation has been conducted in normal subjects at the recommended therapeutic dose of 20 µg to evaluate sperm parameters (count, motility, morphology) and the level of reproductive hormones. There were no significant effects on the parameters. Dogs may be more sensitive to the testicular effects of lixisenatide because the GLP-1R is expressed 3-fold in dogs compared to humans and about 100-times fold in rats.

Minimal increase in the incidence of subdermal fibrosis/inflammation was noted at the injection site of animals in the 13-week mouse study. There were also a slightly increased incidence of hemorrhage, fibroblastic reaction, and abscess at the injection site for male rats receiving ≥100 µg/kg BID. The incidence of increased red focus/area at injection sites, as well as dose-related increase in inflammation and fibrosis was reported at ≥200 µg/kg BID in the chronic dog study.

Administration of lixisenatide to pregnant rat and rabbits at the time of organogenesis is associated with impaired fetal growth and increased incidences of skeletal and visceral malformations. The embryo-fetal defects were noted in rats at the clinical exposure and in rabbits at 6-times the clinical exposure. Even though dose-relations were not apparent for the malformations, increased severity was noted at the highest doses in both rat and rabbits. The sponsor has concluded that all malformations were treatment related and the incidences of the noted malformations exceeded the historical incidences and were not observed in control animals.

Maternal food intake declined during lixisenatide administration, which coincided with the period of organogenesis in both species, but had an upward trend as treatment was continued or after drug withdrawal. Maternal weight gain decline approximated the food intake pattern during dosing. Even though maternal food consumption and weight gain in both species were low at the time of organogenesis the relationship to the reported visceral malformations and closure defects is unclear. Moreover, there were no dose-related general retardations of fetal growth in the studies despite a dose-related maternal weight loss. Malformations were apparent at the high dose in the rat study, although the decrease in dam weight gain was marginal relative to what is reported in literature for food-deprived animals (Cappon *et al,* 2005; Fleeman, *et a*l, 2005).

Marginal adverse effects were reported for insulin glargine in rats when high doses of up to 0.36 mg/kg/day (2-times the maximum recommended dose based on mg/m^2) were evaluated. However, the top dose of 0.072 mg/kg/day (1-times the maximum recommended dose in mg/m^2), administered to rabbits was associated with maternal toxicity, increased incidence of resorptions and fetal loss. These effects are likely due to hypoglycemia. Lixisenatide decreased body weight gain in animals, and the premixed combination of insulin glargine and lixisenatide appears to have additive pharmacodynamic effect (see **page 32**). Since the weight loss and hypoglycemia observed at low multiples of the proposed clinical doses can harm the fetus, the lixisenatide and insulin combination can pose potential risk to the fetus during pregnancy.

Carcinogenicity studies of 2-year durations were conducted in CD-1 mice and Sprague-Dawley rats with twice daily subcutaneous doses of 40, 200, or 1,000 µg/kg. Statistically significant increase in thyroid C-cell adenomas were observed in male mice at 2,000 µg/kg/day, resulting in systemic exposures that are >190-times the human AUC achieved at 20 mcg/day in antibody positive patients[13]. Increased incidence of endometrial adenocarcinoma of the uterus was observed in mice at mid- and high-dose, with statistically significance at the mid-dose group only (at 161-times exposure ratio to the recommended dose).

Statistically significant increases in thyroid C-cell adenomas were seen at all doses in rats receiving AVE0010, resulting in systemic exposures that are ≥15-times the human exposure achieved at 20 µg based on AUC. A numerical increase in thyroid C-cell carcinomas was observed at ≥400 mcg/kg/day (≥58-times AUC of 20 µg dose) in males and females.

In vitro mechanistic studies have been conducted to further characterize the potential neoplastic effects of lixisenatide. These studies examined GLP-1R expression in various tissues and species, as well as effects on proliferative genes in calcitonin-expressing thyroid cells. The GLP-1R was expressed in C-cells but may not be expressed in follicular cells although definitive determination could not be made due to methodological issues.

The applicant also compared GLP-1R activation by lixisenatide and other marketed GLP-1R agonists in rat vs. human thyroid C-cell lines. The E_{max}% for cAMP for all the GLP-1R agonists was the same, but the relative EC_{50} values of exendin-4, lixisenatide and GLP-1(7-36) were comparable, but was much greater than that of liraglutide and GIP. The E_{max}% for the human thyroid C-cell line was lower for all GLP-1R agonists;

[13]The margins compare AUC values from Day 4 of rat carcinogenicity study and a 3-month mouse study (DSE 2005-0443) to ADA positive human in study ACT6011.

relative potency was not calculated because of poor curve fitting. Nonetheless, it appears that GLP-1R activation appears to favor the rat cell line.

A 2,000 ug/kg/day dose subcutaneous dose of AVE0010 was continuously infused for 13-weeks in mice to assess the expression of growth regulatory genes in thyroid cells, and immunohistochemical and microscopic examination of thyroid tissue with H & E and, calcitonin and Ki-67 double staining. The expression of GLP-1R and calcitonin in the medullary thyroid was increased 5-fold in males and 2- to 3-fold in females receiving the test-article. Immunohistochemical assessment of thyroid tissue using calcitonin and Ki-67 double staining did not reveal an increase C-cell replication.

In another 12-week infusion study, AVE0010 or exenatide were infused at the same dose and rate. Serum calcitonin levels were increased by approximately 3-fold for groups receiving AVE0010 or exenatide. No changes in C-cell histology or cell number were detected for treated groups when evaluated microscopically by H&E staining or for calcitonin immunohistochemistry, or when assessed histomorphometrically. Gene expression analyses showed a treatment-related increase in the number calcitonin and GLP-1R mRNA transcripts in thyroid C-cells. No meaningful differences in the expression of proliferative genes were observed between treated and control groups, which is consistent with an apparent lack of C-cell proliferation based on histopathology.

Thus, in mouse calcitonin secretion and GLP-1R are upregulated after sustained infusion of lixisenatide and exenatide. However, there are no indications that the prolonged activation of the GLP-1R initiates a proliferative effect.

There are warnings for long-acting GLP-1 receptor agonists concerning thyroid c-cell hyperplasia and neoplasia in rodents with unknown relevance to humans. Incidences of thyroid C-cell adenomas were increased in mice and rats with lixisenatide. However, the neoplastic effects were noted at high multiples of the clinical exposure. Moreover, lixisenatide has a short half-life, and there are no data in many of the mechanistic studies that implicate lixisenatide in thyroid C-cell proliferation. These data support the notion that the carcinogenicity profile of lixisenatide is similar to the twice daily formulation of exenatide, although it is developed as a once daily formulation.

Post-marketing reports implicate GLP-1 agonist in pancreatitis. Consistent with other investigational and marketed GLP-1 mimetics, there were no findings of adverse histological changes or signs of drug-related neoplasms in pancreatic tissue in mice or rats administered lixisenatide for up to 2 years, or in dogs for up to 1 year.

In conclusion, since lixisenatide did not affect the pharmacokinetics and mitogenic effects of insulin glargine, and in light of the absence of the potential for cross-receptor activation between the two drug substances, the safety profile of the fixed dose combination was comparable with that of the individual components. However, there may be additional risk of hypoglycemia at the highest proposed clinical doses.

14 Proposed Label

8. USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Risk Summary

(b) (4)

(b) (4)

Lixisenatide administered to pregnant rats and rabbits during organogenesis was associated with (b) (4) closure (b) (4) skeletal (b) (4)s at systemic exposures that are 1-times and 6-times higher than the 20 mcg/day clinical dose, (b) (4), respectively, based on plasma AUC [see Data]. (b) (4)

The estimated background risk of (b) (4) miscarriage for the indicated population (b) (4) is unknown. In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2-4% and 15-20%, respectively.

Clinical Considerations

Disease-associated maternal and/or embryo/fetal risk

(b) (4)

DATA

Human Data

(b) (4)

Animal Data

(b) (4)

Insulin glargine

Lixisenatide
In pregnant rats receiving twice daily subcutaneous doses of 2.5, 35, or 500 mcg/kg during organogenesis (gestation day 6 to 17), fetuses were present with visceral closure defects (e.g., microphthalmia, bilateral anophthalmia, diaphragmatic hernia) and stunted growth. Impaired ossification associated with skeletal malformations (e.g., bent limbs, scapula, clavicle, and pelvis) were observed at ≥2.5 mcg/kg/dose, resulting in systemic exposure that is 1-time the 20 mcg/day clinical dose, based on plasma AUC. Decreases in maternal body weight, food consumption, and motor activity were observed concurrent with the adverse fetal findings, which confounds the interpretation of relevance of these malformations to the human risk assessment. Placental transfer of lixisenatide to developing rat fetuses is low with a concentration ratio in fetal/maternal plasma of 0.1%.

In pregnant rabbits receiving twice daily subcutaneous doses of 2.5, 25, 250 mcg/kg during organogenesis (gestation day 6 to 18), fetuses were present with multiple visceral and skeletal malformations, including closure defects, at ≥5 mcg/kg/day or systemic exposures that are 6-times the 20 mcg/day clinical dose, based on plasma AUC. Decreases in maternal body weight, food consumption, and motor activity were observed concurrent with the fetal findings, which confounds the interpretation of relevance of these malformations to the human risk assessment. Placental transfer of lixisenatide to developing rabbit fetuses is low with a concentration ratio in fetal/maternal plasma of ≤0.3%. In a second study in pregnant rabbits, no drug-related malformations were observed from twice daily subcutaneous doses of 0.15, 1.0, and 2.5 mcg/kg administered during organogenesis, resulting in systemic exposures up to 9-times the clinical exposure at 20 mcg/day, based on plasma AUC.

In pregnant rats given twice daily subcutaneous doses of 2, 20, or 200 mcg/kg from gestation day 6 through lactation, decreases in maternal body weight, food consumption, motor activity were observed at all doses. Skeletal malformations and increased pup mortality were observed at 400 mcg/kg/day, which is approximately 200-times the 20 mcg/day clinical dose, based on mcg/m^2.

8.2 Lactation

Risk Summary

(b) (4)

(b) (4) Lixisenatide is present in rat milk, (b) (4) . The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for TRADENAME and any potential adverse effects on the breastfed child from TRADENAME or from the underlying maternal condition.

Data

(b) (4)

(b) (4)

(b) (4)

Lixisenatide

A study in lactating rats showed low (9.4%) transfer of lixisenatide and its metabolites into milk and negligible (0.01%) levels of unchanged lixisenatide peptide in the gastric contents of weaning offspring.

DATA

A study in lactating rats showed low (9.4%) transfer of lixisenatide and its metabolites into milk and negligible (0.01%) levels of unchanged lixisenatide peptide in the gastric contents of weaning offspring. (b) (4)

(b) (4)

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

TRADENAME

No animal studies have been conducted with the combination of insulin glargine and lixisenatide to evaluate carcinogenesis, mutagenesis, or impairment of fertility..

Insulin glargine

In mice and rats, standard two-year carcinogenicity studies with insulin glargine were performed at doses up to 0.455 mg/kg, which was for the rat approximately 2 times and for the mouse approximately 1 times the recommended human subcutaneous high dose of 60 Units/day (0.0364 mg/kg/day), based on mg/m^2. The findings in female mice were not conclusive due to excessive mortality in all dose groups during the study. Histiocytomas were found at injection sites in male rats (statistically significant) and male mice (not statistically significant) in acid vehicle containing groups. These tumors were not found in female animals, in saline control, or insulin comparator groups using a different vehicle. The relevance of these findings to humans is unknown.

Insulin glargine was not mutagenic in tests for detection of gene mutations in bacteria and mammalian cells (Ames- and HGPRT-test) and in tests for detection of chromosomal aberrations (cytogenetics *in vitro* in V79 cells and *in vivo* in Chinese hamsters).

In a combined fertility and prenatal and postnatal study with insulin glargine in male and female rats at subcutaneous doses up to 0.36 mg/kg/day, which was approximately 2 times the recommended human subcutaneous ,maximum dose of 60 Units/day (0.0364 mg/kg/day), based on mg/m^2, maternal toxicity due to dose-dependent hypoglycemia, including some deaths, was observed. Consequently, a reduction of the rearing rate occurred in the high-dose group only.

Lixisenatide

Carcinogenicity studies of 2-year durations were conducted in CD-1 mice and Sprague-Dawley rats with twice daily subcutaneous doses of 40, 200, or 1,000 mcg/kg. A statistically significant increase in thyroid C-cell adenomas was observed in male mice at 2,000 mcg/kg/day, resulting in systemic exposures that are ≥180-times the human exposure achieved at 20 mcg/day based on plasma AUC^{14}.

Statistically significant increases in thyroid C-cell adenomas were seen at all doses in rats, resulting in systemic exposures that are (≥15 times) the human exposure achieved at 20 mcg/day based on plasma AUC. A numerical increase in thyroid C-cell carcinomas was observed at ≥ 400 mcg/kg/day, resulting in systemic exposures that are ≥56-times times) the human exposure achieved at 20 mcg/day based on plasma AUC.

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Lixisenatide was not mutagenic or clastogenic in a standard battery of genotoxicity tests (bacterial mutagenicity (Ames), human lymphocyte chromosome aberration, mouse bone marrow micronucleus).

Impairment of Fertility

Studies in which male and female rats received twice daily subcutaneous doses lixisenatide of 2, 29, or 414 mcg/kg/dose prior to pairing through gestation day 6 did not indicate any adverse effects on male or female fertility in rats up to the highest dose tested, 414 mcg/kg/dose, or approximately (b) (4) times the clinical systemic exposure at 20 mcg/day based on plasma AUC.

Animal (b) (4)

Insulin glargine

(b) (4)

Subcutaneous reproduction and teratology studies have been performed with insulin glargine and regular human insulin in rats and Himalayan rabbits. Insulin glargine was given to female rats before mating, during mating, and throughout pregnancy at doses up to 0.36 mg/kg/day, which is approximately 2-times the recommended human subcutaneous high dose of 60 units/day (0.0.0364 mg/kg/day), based on mg/m^2. In rabbits, doses up to 0.072 mg/kg/day, which is approximately 1-times the maximum recommended human subcutaneous dose of 60 units/day (0.0364 mg/kg/day), based on mg/m^2, were administered during organogenesis. The effects of insulin glargine did not generally differ from those observed with regular human insulin in rats or rabbits. However, in rabbits, five fetuses from two litters of the high-dose group exhibited dilation of the cerebral ventricles. Fertility and early embryonic development appeared normal.

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/s/

FELEKE ESHETE
07/18/2016

TODD M BOURCIER
07/19/2016
Concur with reviewer; nonclinical supports approval